Cucurbitaceae 2012

Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae

October 15-18, 2012, Antalya-Turkey

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EUCARPIA

EUROPEAN ASSOCIATION FOR RESEARCH ON PLANT BREEDING
EUROPÄISCHE GESELLSCHAFT FÜR ZÜCHTUNGSFORSCHUNG
ASSOCIATION EUROPÉENNE POUR L'AMÉLIORATION DES PLANTES

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PREFACE

On behalf of the Organizing Committee of EUCARPIA Cucurbitaceae 2012, it gives me great pleasure to welcome you in Antalya, Turkey. The first EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae was organized by INRA, France 24 years ago. The following meetings were held in Poland (1992), Spain (1996), Israel (2000), Czech Republic (2004) and France (2008). It is a great honor and privilege for us to host the 10th EUCARPIA Cucurbitaceae Meeting in Antalya.

Turkey is an important country in agricultural production and very rich in Cucurbit genetic resources. You will have the opportunity to see our genetic diversity in all Cucurbit species during our technical excursion.

We expect more than 220 participants from 33 countries at the meeting in Rixos Downtown Hotel in Antalya. About 70% of the participants are from public research institutions and 30% are from private seed companies. We are pleased to welcome the international scientific community on cucurbit genetics, breeding and genomics.

The proceeding contains 35 full text papers presented in oral sessions and 96 papers presented as posters at the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. The authors should be thanked for their willingness to share their results.

Our special thanks to scientific committee members for their contribution in evaluating oral and poster presentation and reviewing all the papers. We also thank the keynote speakers for their informative presentations.

This meeting is vital for transferring results, stimulating new ideas for research topics, and in fostering co-operation possibilities. We would like to thank EUCARPIA, our public (Çukurova University, Ministry of Food, Agriculture and Livestock and Turkish Scientific and Technological Council) and private sponsors (Antalya Tarim, Manier Seed, Yuksel Seed, Syngenta, AG Seed, Fito Seed, Multi Seed, Nunhems and Rijk Zwaan) for accepting financial support of the meeting. We are grateful to the public institutions and private companies taking place in the technical trip.

We also thank Bilkon Tourism and Travel Agency for their excellent service and organization.

We will do our best to make Cucurbitaceae 2012 a most informative and enjoyable experience for all participants. We hope you enjoy this meeting and your stay in Antalya, Turkey.

Antalya, October 2012

Prof. Dr. Nebahat SARI
On behalf of the Organizing Committee

The organization of this meeting has been possible by the financial support of:

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ORAL PRESENTATIONS

Domestication and diversification of melon

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Keywords: Germplasm, *Cucumis melo*, genetic control

Abstract

As for many crops, cultivated melons present a very large phenotypic polymorphism compared with the low phenotypic polymorphism of wild melons. Domestication has not been intensively studied and the genetic control of domestication traits is still poorly understood. The results of the subsequent diversification and selection processes are the present day types of melons. Genetic control of a majority of the diversification traits is under recessive genetic control: sex expression, fruit shape, sutures, number of placentas, gelatinous sheath around the seeds, white flesh colour… Other phenotypic traits are dominant (orange flesh colour, netting, yellow colour of mature fruit in the Amarillo type…) as are most of the disease resistances. Presence of the same traits in very different botanical groups can be the result of parallel evolution but also of intercrossing between groups and selection of preferred alleles. New results (genome sequencing) and methods will allow a better understanding of the genetic control of domestication and diversification.

INTRODUCTION

Melon (*Cucumis melo*) is a polymorphic species cultivated in many countries with a hot season. Even if leaves are consumed in some places, it is mainly cultivated for its fruits. Wild melons can be found in Africa or Asia. But what is a "wild" melon? The word "wild" can be used as the opposite of "domesticated" or of "cultivated" which are not synonymous. Domestication is the status of co-evolution or co-adaptation or co-dependence of a plant and human beings. In other words, since the Neolithic revolution and the development of agriculture, man is dependent from domesticated plants for his food and the plant is dependent from man; without agriculture, a domesticated plant cannot survive in competition with weeds. A "domestication syndrome" (Hammer 1984) has been defined and studied in a few crops, mainly cereals (rice, barley, wheat and maize): seed dormancy, fragile or tough rachis (shaterring), plant structure, inflorescence structure can be cited

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along with larger leaves or fruits or seeds, change in reproduction mode (autogamy *versus* allogamy)… All cultivated plants are not domesticated: for instance many forage grasses can survive in natural meadows. But melon, like most other fruits and vegetables has a low fitness to survive without man care and good horticultural practices and can be considered as domesticated.

Diversification or crop improvement corresponds to the different cultivars, cultigroups, botanical varieties which have been selected since the beginning of agriculture until now in different places in the world. In the case of melon, which is a plant domesticated since a long time, a large phenotypic diversity can be observed today, even if some types cultivated in some places many centuries ago are now extinct.

There is no clear limit between "domestication" and "diversification" but instead a continuum. Indeed many traits found in present day cultivars can be found in present day wild melons; for instance green flesh, monoecious or 3 placentas are observed wild melons and in some cultivars and the opposite traits (orange flesh, andromonoecious or 5 placentas) cannot be considered as domestication traits.

In some cases, escape from the "domesticated" status and return to the "wild" status can be observed. These populations are called "feral" and can be observed for instance in places which are not the centre of origin of the species. In the case of melon, the wild melon growing in Central America can be considered as feral. Their study in comparison with the wild types can bring interesting complementary data.

The wild types observed today are not the ancestors of the cultivated types. Indeed what we observe today is, for the wild melons, the result of natural selection from the ancestor and, for the cultivated melons, the result of artificial or man-driven selection (= domestication and diversification) from the ancestor.

In the case of vegetables, there are almost no archaeological data. Some pictures are available but are only a few centuries old (mainly from the European Renaissance) and do not represent the world diversity.

In this paper, I will try to identify some traits of domestication or diversification of melon and what is known on their genetic control.

WILD MELONS

As for many crops, there is a low phenotypic diversity in "wild" melons compared with the large phenotypic diversity of "cultivated" melons. Typical wild melons can be described as plants with small leaves. They are monoecious, with small flowers and small smooth oval-shaped fruits often light green with dark green dots or stripes, sometimes uniform light green. The flesh is very thin, light green often bitter with three placentas. Seeds are small, embedded in a gelatinous sheath and there is no seed cavity. Maturation can be climacteric or non-climacteric.

Several species such as *Cucumis pubescens*, *Cucumis trigonus* or *Cucumis*

turbinatus are now considered as wild types (and synonym) of *C. melo*.

Wild melons can grow close to field of cultivated melons in Africa or in India for instance. As they are allogamous, pollination can occur between both of them. This gene flow results in intermediate or recombinant types for instance the presence of sutures or light orange flesh.

In Central America and the Caribbean Islands, one can find small melons round or slightly oval with a yellow skin. The flesh is thin, light green or white and there is no seed cavity. Seeds are small and embedded in a gelatinous sheath. Plants are monoecious. In short, except for the yellow colour of the fruit exocarp, they look like "wild" and have been described under the name *chito* (Naudin 1859). America is not the centre of origin of melon. These genotypes could result from gene exchange between wild types brought from Africa (by slaves?) or Asia and cultivated melons. They could also represent feral accessions i.e a return to a wild status from the cultivated melons introduced in the New World.

Two sub-species have been described in *C. melo*: subsp. *melo* is characterized by long and spreading hairs on the ovary or the young fruit, subsp. *agrestis* by short and appressed hairs (Kirkbride 1993). The word "agrestis" had been used earlier to describe the tribe of "wild" melons (Naudin 1859). A lot of confusion has resulted from these two meanings as the wild melons ("agrestis" *sensu* Naudin) can belong to the subsp *agrestis sensu* Kirkbride with short hairs or to the subsp *melo* with long hairs on the ovary and the young fruit.

DOMESTICATION TRAITS

Phenotype

It is surprising to observe that, in the vicinity of fields where melons are cultivated, wild types remain quite typical despite repeated pollination (back-crosses!) by cultivars. For instance in Sudan, where *flexuosus* type is commonly cultivated, the wild melons have no elongated fruits.

As mentioned above, domestication traits have not been studied in vegetables and particularly in melon. A character often observed in many plants is the seed dormancy in wild types opposed to the absence of dormancy in cultivars. It can be easily understood that a negative selection has been applied by farmers for seed dormancy because they want that all the seeds germinate at the same time and not for several years in one field. Seed dormancy of wild melons has not been clearly described but it is a common observation in seed banks that wild accessions have a low germination rate.

Compared with wild melons, modern cultivars are characterized by large, non-bitter fruits with a thick flesh and large seeds. Most of the other traits of wild melons like sex expression, fruit shape, fruit colour, flesh colour, sugar, aroma… have been conserved in some cultivars and must be considered as diversification

and not as domestication characters.

The most typical trait of domestication i.e. the loss of fitness or competitiveness towards weeds has not been analysed in any plant and is poorly understood.

Domestication events

There is no definitive answer to the number of domestication events. The existence of two sub-species and the fact that wild types can be found in the two sub-species are strong arguments in favour of two independent domestication events. The presence of the same phenotypic traits in both sub-species, for instance, sex expression, fruit size and shape, fruit flesh colour, sugar, some disease resistance like *Fusarium oxysporum* f.sp. *melonis* race 1 or race 2 can be explained by the appearance and the selection of the same trait in independent lines or by gene exchange between lines. The general picture is that sub-species *agrestis* is found from India to Far-East with the types *momordica*, *acidulus*, *conomon*, *momordica*, *chinensis*, and the sub-species *melo* in India and west of India (central and western Asia, Africa, Europe and America) with the types *cantalupensis*, *inodorus*, *reticulatus*, *ameri*, *flexuosus*, *chate*, *dudaim*… The exceptions are the *chito* in America and the *tibish* in Africa. The *chito* case has been discussed above and in the case of *tibish*, it could correspond to a third domestication event.

Where and when the domestication event(s) occur(s) is not known. Data on the existence of cultivated melon in ancient time are scarce (Moldenke and Moldenke 1952; Andrews 1956; Keng 1974; Vishnu-Mittre 1974; Stol 1987; Manniche 1989; Walters 1989; Schoske et al. 1992; Decker-Walters 1999; Meirano 2000). The main conclusion is that melon was probably cultivated more than 2000 years BCE.

DIVERSIFICATION TRAITS

The large phenotypic diversity of modern cultivars is the result of positive or negative selection by farmers during the millennia of domestication and diversification. A non exhaustive list of the characters follows.

Fruit size and shape

Fruit shape can vary from flat to very long (up to 1.8 m) in the *flexuosus* group. Fruit weight is commonly between 1 and 2 Kg but smaller (200-300 g) to larger (more than 5 Kg) fruits can be observed, compared with 20-50 g of wild melons.

Fruit exocarp

Primary and secondary fruit colours are white, bright yellow, orange, brown, light green, dark green. The pattern can be uniform (honeydew, Amarillo, Yuva), with dots (Piel de sapo, Kırkağaç) or stripes (*dudaim*). Sutures can be present (Charentais, Ogen) or not. Exocarp can be netted (*reticulatus*) or wrinkled (Tendral).

Fruit flesh

Flesh colour can be white, green or orange. It can be noted that no red-flesh melon with high lycopene content (like in watermelon) has been selected. Melon fruits can be sweet (*cantalupensis, reticulatus, inodorus*…) or not (*acidulus, flexuosus*, *conomon*…). Texture varies from mealy (*momordica*) to juicy (Ananas) to firm and crispy (*acidulus*). Many volatile compounds have been identified: *dudaim* represents a highly aromatic type and *acidulus* or *conomon* a non-aromatic type. Some types are characterized by 5 placentas (*dudaim*, *conomon*, *chinensis*) instead of 3 (*cantalupensis, inodorus*).

Seeds

Seed size can be up to 10-12 seeds, compared to 290 seeds per gram for wild types, a more than 20-fold increase. Seed colour can be white, yellow or brown. Seeds can be embedded or not in a gelatinous sheath.

Sex expression

A majority of cultivars is andromonoecious and a few accessions from China are hermaphrodite.

Leaves and roots

A large variability exists in leaves shape, size and colour from light green to dark green, in stem diameter and also in root architecture from many superficial small roots to a few very large roots.

Adaptation to climactic conditions

Cultivars have been selected in area with different soil and climatic conditions. They can be adapted to dry, sandy soil and high temperature (warm desert) to lower temperature and more humid conditions (semi-continental).

Disease and pest resistance

Wild accessions are generally susceptible to most of the viruses, bacteria, fungi, insects, nematodes that can attack melons. May be there is a bias because few wild accessions maintained in the collections have been evaluated. Among the resistances identified in wild accessions, resistance to PRSV-W (in PI 180280 and PI 180283) and to *Dacus cucurbitae* (in *Cucumis callosus*), one of several sources of resistance to *Didymella bryoniae* (PI 140471) or to *Acidovorax avenae* subsp *citrulli* (PI 536473) can be cited. In contrast, in some cultigroups resistance to many diseases has been identified, for instance to powdery and downy mildew in the *momordica* and *acidulus* types from India, to *Cucumber mosaic virus* (CMV) and *F. oxysporum* f.sp. *melonis* race 1.2 in the *conomon* and *makuwa* types from Far-East.

Of course many mutations with "negative" effect which are now maintained in gene banks have not been selected by farmers: chlorophyll deficiency, male-sterility, nectarless…

GENETIC CONTROL

What is the genetic control of these different traits? To study the inheritance of quantitative traits such as sugar content, disease resistance, fruit shape and weight, earliness… homozygous "immortalized" genotypes such as doubled haploid lines, recombinant inbred lines or introgression lines are very powerful tools. One inconvenient of this approach is that the dominance or recessivity of each detected QTL cannot be estimated.

Recessive genes and QTLs

Absence of bitterness in the fruit is maybe one domestication trait and the absence of cucurbitacins in the plant and/or the fruit is controlled by recessive alleles at several loci (*Bi*$^+$, *Bif-1*$^+$, *Bif-2*$^+$, *Bif-3*$^+$).

Andromonoecy is controlled by the recessive allele *a* and hermaphroditism by the two recessive alleles *a* and *g*. Regarding some fruit characters, presence of sutures (allele *s*), uniform colour (alleles *Mt*$^+$, *spk*, *st*), white colour of mature fruits (allele *w*), white flesh colour (allele *wf*), absence of gelatinous sheath around the seeds (allele *Gs*$^+$) are recessive genes.

Resistance to *Melon necrotic spot virus* (MNSV, allele *nsv*), to *Cucumber vein yellowing virus* (CVYV, allele *cvy-2*), to *Cucurbit aphid borne yellows virus* (alleles *cab-1* and *cab-2*) or polygenic resistance to CMV or *F. oxysporum* f.sp. *melonis* race 1.2 are also controlled by recessive genes.

Dominant genes and QTLs

There are also some dominant genes or QTLs present in cultivated types.

Orange flesh colour (allele *gf*$^+$), yellow colour of mature fruit (allele Y), netted fruit exocarp or andromonoecy in *tibish* type are controlled by dominant genes. Many pest and disease resistance are also controlled by dominant genes: *F. oxysporum* f.sp. *melonis* races 1 or 2 (allele *Fom-1* and *Fom-2*), *Aphis gossypii* (allele *Vat*), powdery mildew (several loci and alleles), *Alternaria cucumerina* (allele *Ac*), *D. bryoniae* (several loci), *Papaya ringspot virus watermelon type* (PRSV-W, locus *Prv*), *Zucchini yellow mosaic virus* (allele *Zym*), CVYV (locus *Cvy-1*)…

Recessive *versus* dominant

Compared with the phenotype of a typical wild melon, most of the genes and QTLs controlling traits in cultivars are recessive, except disease resistance (Lester 1989). Recessive genetic control corresponds often to non-functional

proteins whatever the cause: point mutation, stop codon, transposon insertion… Nevertheless, the loss or decrease in function does not indicate a reduced trait but can correspond to an increase or a hypertrophy. For instance the decrease of a hormone level can result in larger fruits resulting from an increase of cell numbers or of cell sizes. When an ACC synthase gene (*CmACS-7*) is active with production of ethylene, there are no stamens in the female flowers; when this enzyme is inactive, corresponding to the allele *a* (andromonoecy), stamens are present in female flowers (Boualem et al. 2008).

In contrast, more resistances under dominant genetic control compared to the wild type than under recessive control have been identified. A dominant resistance is usually an active mechanism induced in the interaction plant * pathogen while a recessive one is usually constitutive and corresponds to a lack of function necessary for the pathogen. The cost in term of fitness for a permanent loss corresponding to a recessive control could be higher than the temporary induction of a mechanism only at the time of contact with a pathogen.

Parallel evolution?

For most of the phenotypes listed above, the mutant trait, compared with the wild type, is observed in both sub-species; for instance andromonoecy in *cantalupensis*, *reticulatus* or *dudaim* in sub-species *melo* and in *conomon* or *makuwa* in sub-species *agrestis*; Fusarium wilt race 1 or race 2 or MNSV resistance in both sub-species… Did this phenotypic trait appear only one time and spread in the different present-day cultigroups by intercrossing or did this trait appear independently several times in the cultigroups? For a monogenic trait both hypothesis can be true. For polygenic traits such as high sugar content, fruit size, some resistance (CMV, *F. oxysporum* f.sp. *melonis* race 1.2) the first hypothesis is more probable.

Polygenic recessive traits are more probably the result of domestication (long term selection by man) while monogenic traits can appear in hot-spots of diversity: for instance many genes for pest and disease resistance are found in different types, mainly *momordica* and *acidulus*, in India.

MOLECULAR DATA

There are few data on the allelic diversity of genes with a phenotypic effect controlling domestication or diversification traits.

Wild melons are monoecious while about 2/3 or cultivars are andromonoecious. This last phenotype is controlled by a single point mutation which codes for a deficient ACC synthase. Eleven haplotypes for the allele a^+ have been described and only one haplotype for the allele *a* controlling andromonoecy. This last allele is present in many melon types from different geographical origins. The different haplotypes of the a^+ allele are not clearly related with geographical origin or with

botanical groups (Boualem et al. 2008).

Wild melons are susceptible to MNSV and resistance has been described in several accessions from Far-East or USA. The *nsv* locus encodes for the translation factor eIF4E and resistance corresponds to a single point mutation (Nieto et al. 2006). Six haplotypes have been described but there was no general relationship with geographical origin (Nieto et al. 2007).

CONCLUSION

Wild and cultivated melons differ by many traits but very little is known on the genetic control of the domestication characters. It is also the case of other vegetables compared with cereals. Absence of fruit bitterness and large fruit size are two traits which have been studied but there are no data on other traits probably involved in domestication such as seed dormancy or fitness in non-horticultural conditions. It is not clear how many independent domestication events give way to the present day cultivated melons. Based on the two sub-species and the isolated position of *tibish* group, one can speculate on three domestications; the *chito* type could correspond to a feral melon.

Concerning the diversification characters, most of them are under recessive control which does not mean a loss of a trait. Many traits are present in different botanical groups such as flesh colour, sutures, sex expression, some disease resistances. It corresponds to parallel evolution i.e. the appearance of one phenotypic trait in genetically independent lineages. But it could also result from intercrossing and migration.

As soon as a gene with a phenotype involved in domestication and/or diversification is identified, the allelic diversity of this gene could be studied by eco-TILLING or by resequencing. In the next years, we will have a better understanding of the evolution of melon.

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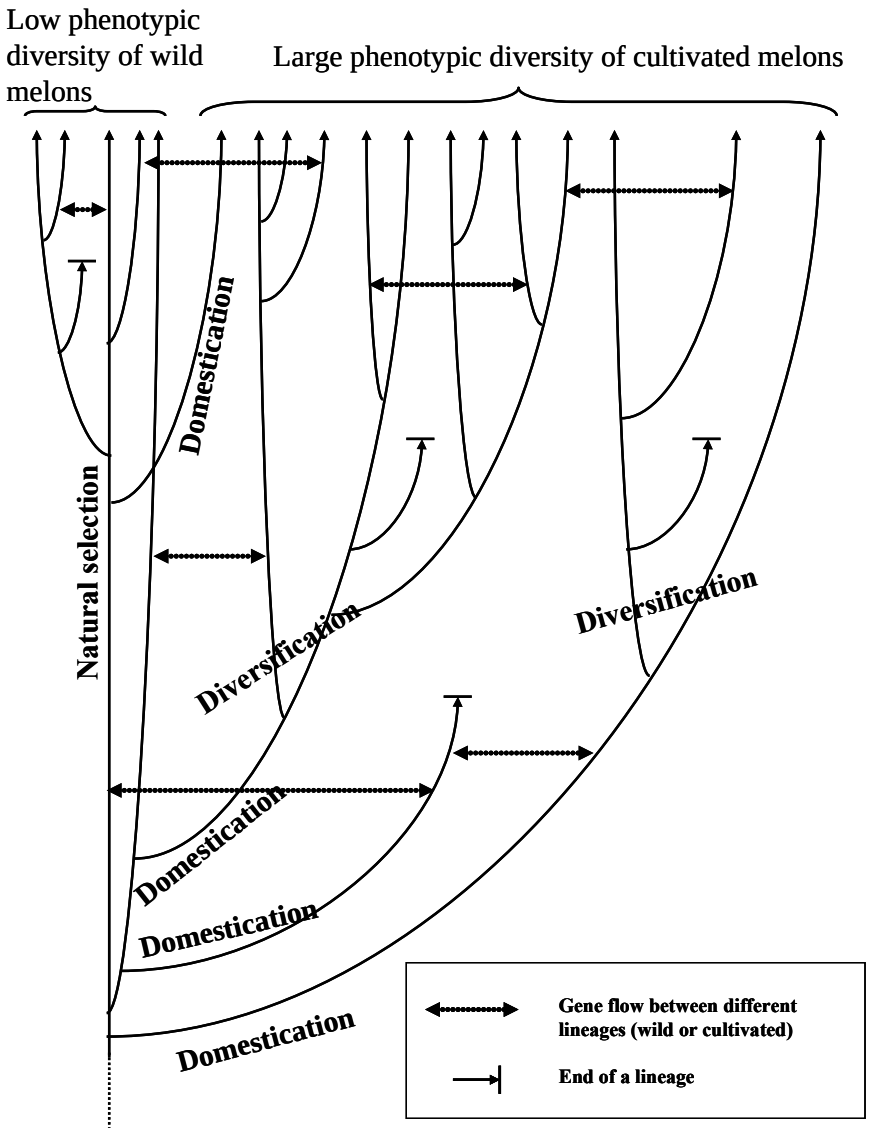

Fig. 1. Schematic representation of the effect of natural selection (left) and domestication/diversification (right). The bottom to top represents time from the first domestication to present day. Four independent domestication events are represented but one ends before producing modern varieties. Gene flow results from intercrossing between wild and/or cultivated types at places where different types (wild and/or cultivated) are present. Domestication represents the first step from the wild status and is followed by diversification or selection of different cultivated types. Domestication and diversification are not clearly separated but represent a continuum.

Exploiting genetic diversity in *Citrullus* spp. to enhance watermelon cultivars

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Keywords: *Citrullus*, genotyping, HFO-TAG, SNP, markers, genome

Abstract

There is a continuous need to enhance watermelon cultivars for disease and pest resistance. U.S. Plant Introductions (PIs) representing the different groups of watermelon (*Citrullus* spp.), including the desert watermelon *Citrullus colocynthis* (L.) Schrader (*CC*), the "cow" or Tsamma watermelon *Citrullus lanatus* subsp. *lanatus* var. *lanatus* [also known as *C. lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb.] (*CLC*), and *Citrullus lanatus* subsp. *lanatus* (*CLL*) are considered a useful source for enhancing disease or pest resistance in watermelon cultivars. However, the genetic relationships among these species and subspecies need further investigation. In this study, we examined genetic diversity among *Citrullus* spp. accessions representing *CC*, *CLC*, and *CLL* groups collected in their center of origin in Africa. High frequency oligonucleotides - targeting active gene (HFO-TAG) primers were used in polymerase chain reaction (PCR) experiments to produce over 560 polymorphic markers among the *Citrullus* genotypes. Cluster and multidimensional scaling plot analysis produced distinct groups of *CLC*, *CLL*, and *CC* PIs. Several PIs that were designated as *CLC* or *CLL* were in transitional positions, indicating that they are the result of gene flow between the major *Citrullus* groups or subgroups. Population structure analysis

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indicated that *CLC* comprises two subgroups; each containing a set of unique alleles. Also, unique alleles exist in the *CLL* and the *CC* genotypes. The results here are consistent with our single nucleotide polymorphism (SNP) results developed in our recent genotyping-by-sequencing (GBS) study and our recent findings using fluorescence in situ hybridization (FISH) technology showing major differences in configuration of ribosomal DNA markers between the *CLC* and the *CLL* or *CC* genotypes. Overall, a wide genetic diversity exists among *Citrullus* spp. and different sources should be useful for enhancing disease or pest resistance in elite watermelon cultivars.

INTRODUCTION

Watermelon belongs to the xerophytes genus *Citrullus* Schrad. ex Eckl. et Zeyh. that thrive in the Old World tropics (Wehner 2008). Diverse populations of *Citrullus* spp. grow freely throughout the southern African continent (Jarret et al. 1997; Mujaju et al. 2010, 2011; Mujaju and Fatei 2011) which is considered the center of origin of *Citrullus* spp. (Dane and Lang 2004).

As a result of many years of cultivation and selection for a desired fruit quality a narrow genetic base exists among watermelon cultivars (Levi et al. 2001). This narrow genetic base is reflected in the low number of single nucleotide polymorphism (SNP) markers (one SNP for every 1,430 bp) identified between the sequenced genomes of the elite Chinese watermelon line "97103" (small globular fruit with dark rind and red flesh) and the American heirloom "Charleston Gray" (elongated fruit with light green-gray rind and a pink flesh). There is a continual need to enhance disease or pest resistance in watermelon cultivars using available sources of wild type watermelon (*Citrullus* spp.) that have shown to contain resistance.

The genus *Citrullus* comprises four known diploid (n = 11) species (Jarret et al. 1997; Dane and Lang 2004). Among them is the annual *Citrullus lanatus* (Thunb.) Matsum et Nakai, which is indigenous to the arid sandy regions of southern Africa (Bates and Robinson 1995; Wehner 2008). According to Jeffery (2001), this species includes *C. lanatus* subsp. *lanatus* (comprising the Tsamma watermelon and the cultivated citron type) (*CLL*), and *C. lanatus* subsp. *vulgaris* (representing the sweet-dessert watermelon group) (*CLV*).

The *C. lanatus* subsp. *lanatus* var. *lanatus* (often reported as "*C. lanatus* var. *citroides*") (*CLC*) is considered a group of ancient cultigens derived from the 'Tsamma' melon that thrive in southern Africa. It is also known as the 'Citron' melon (Jarret et al. 1997). The *CLC* is adapted to semidry conditions and is an essential source of water and nutrients for animals which, in return, disseminate the seeds throughout the African desert.

In contrast with the commonly known sweet red watermelon varieties (*CLV*), the *CLC* watermelons have white or green flesh with a wide range of flavors, have

high pectin levels, and are rich in dietary fibers and are commonly consumed by the native people in southern and central Africa (Dahl Jensen et al. 2011). The USDA, ARS, Plant Genetic Resources Conservation Unit (PGRCU), Griffin, GA, maintains over 1,800 U.S. Plant Introductions (PIs) of the genus *Citrullus*. Among these PIs, 130 are designated by the USDA-ARS as *C. lanatus* var. *citroides* (*CLC*) (www.ars-grin.gov) and about 1,600 PIs designated as *C. lanatus* var. *lanatus* (*CLL*). Different PIs that are classified as *CLC* have been shown to possess resistance to root-knot nematodes (Thies and Levi 2007), Fusarium wilt race 2 (Martyn and Netzer 1991; Wechter et al. 2012), gummy stem blight (Gusmini et al. 2005), anthracnose (races 1, 2 or 3) (Boyhan et al. 1994), powdery mildew (Davis et al. 2007; Tetteh et al. 2010), or potyviruses (Harris et al. 2009a, 2009b). However, our previous studies with molecular DNA markers indicated that a considerable number of PIs that are classified as *CLC* or *CLV* maybe misclassified or are in transitional position among the *Citrullus* spp. groups. There is a need to clarify genetic diversity and genetic relationships within and among *Citrullus* spp. and identify germplasm sources that can be useful for enhancing disease or pest resistance in watermelon cultivars.

In this study we used robust marker systems that include HFO-TAG and SNP markers to determine the depth of genetic diversity within and among *Citrullus* spp. Also, we employed florescent in situ hybridization (FISH) technology to clarify the genetic relationships among the *Citrullus* spp.

MATERIALS AND METHODS

Plant material and isolation of DNA. 190 PIs were selected for analysis (Table 1). Seedlings of these PIs were grown in the greenhouse at 26/20 ˚C (day/night temperatures). Young leaves were collected from three plants (two week-old) representing each PI or cultivar and stored at -80 ˚C for later DNA isolation. The DNA was isolated from the frozen leaves using the method described by Levi and Thomas (1999).

PCR amplification and analysis using HFO-TAG primers: Twenty-three HFO-TAG primers (Table 2) were used in this study as described by Levi et al. (2010).

DNA amplification conditions and HFO-TAG marker analyses: The PCR reaction and thermal-cycling conditions for HFO-TAG primers selected were as described by Levi et al. (2012).

Genotyping by Sequencing: The Cornell Institute for Genomic Diversity (IGD) has developed a high-throughput "Genotyping-by-Sequencing (GBS)" method for analyzing SNPs along with informatics pipelines for handling the large amounts of DNA sequence data produced by next-generation sequencers (Elshire et al. 2011). GBS focuses the power of next-generation sequencing (Illumina platform) to the ends of short restriction fragments, preferentially located in low-copy genomic

regions. To construct GBS libraries, total genomic DNA is digested to completion with a methylation-sensitive restriction enzyme, which facilitates the exclusion of repetitive regions while targeting the low copy genomic regions. In the next step, adapters containing unique barcodes are attached to the DNA fragments produced by the methylation-sensitive restriction enzyme(s). Then, 96 or 384 DNA samples are pooled and amplified using a PCR procedure and sequenced in a single lane on the Illumina HiSeq 2000 (Fig. 1). In collaboration with the Cornell team we have optimized the GBS procedure for watermelon using genomic DNA samples of the cultivars Charleston Gray, Black Diamond and PI 595203. We have tested several restriction enzymes to identify those that can be most useful in reducing genome complexity and constructing GBS libraries. Among the restriction enzymes, *ApeKI*, *PstI*, and *EcoT22I* produced the largest number of fragments in the low copy fraction, and for this reason are considered most suitable for GBS and SNP analysis of the watermelon genome. Then, we performed GBS with 95 watermelon accessions (PIs and cultivars) as described in Fig. 1.

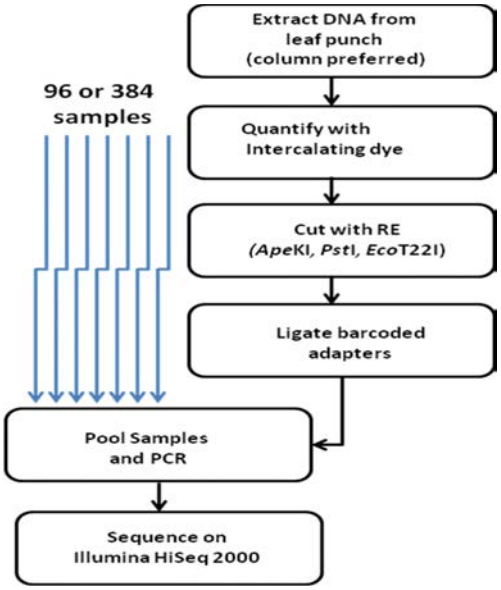

Fig. 1. A chart describing the Genotyping-by-Sequencing procedure for the development of single nucleotide polymorphism (SNP) markers that cover large part of the watermelon genome.

RESULTS AND DISCUSSION

In this study, we first employed HFO-TAG markers to examine phylogenetic relationships and allele frequency within and among the *CLC*, *CLL*, and *CC* PI groups, particularly among PIs possessing resistance to diseases or pests that could be useful in watermelon breeding programs. The phylogenetic analysis and

multidimensional scaling plot differentiated the *Citrullus* spp. in this study into three distinct groups that include *CLC*, *CLL* or *CC* genotypes and transitional groups representing genotypes that fall between the major groups (Fig. 2 and 3). The *CLC* group consists of two distinct subgroups and a transitional group between them (Fig. 3). In addition, there are two distinct groups representing the *CLL* and *CC* PIs. Several PIs that were designated as *CLC* or *CLL* were in a transitional position between the major groups, indicating that they might be a product of gene flow between the major *Citrullus* groups (Fig. 2 and 3).

The population structure analysis, which examines gene frequency in genotypes (Falush et al. 2007), identified two major sets of alleles among the *CLC* PIs (data not shown). A recent study (Levi et al. 2012) indicated that *CLC* PIs may possess alleles that do not exist in the sweet-red watermelon cultivars. The population structure analysis also identified a set of alleles unique to the *CLL* PIs and a set of alleles unique to *CC* PIs (data not shown). Overall, wide genetic diversity exists between the *CLC*, *CLL* and *CC* groups examined in this study. The population structure analysis (Falush et al. 2007) proved useful in identifying genotypes that represent each of the phylogenetic groups and in identifying those genotypes that are transitional, resulting from gene flow between groups (as shown in Fig. 2 and 3). The *CC* PIs are distinct, but share a large number of alleles with the *CLL* accessions, mostly with the watermelon cultivars Black Diamond and Charleston Gray (*CLV*). These results indicate possible evolutionary association of the desert watermelon *CC*, which thrives in the deserts of North Africa, the Middle East, and Central Asia, with the cultivated watermelon (*CLV*).

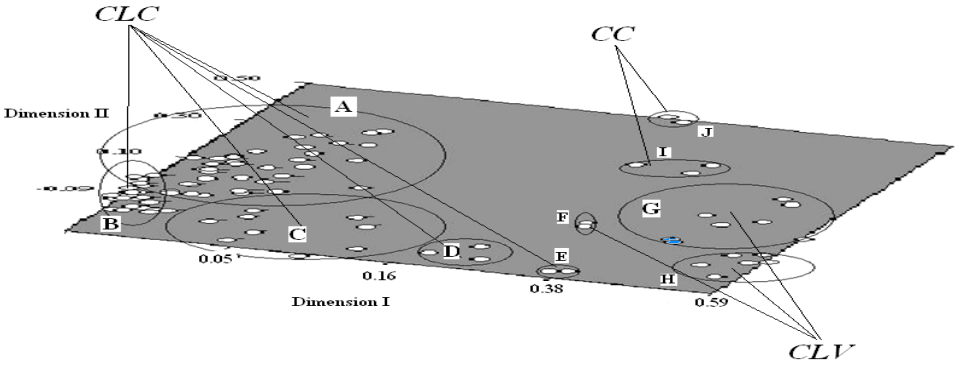

Fig. 2. Two-dimensional plot of watermelon accessions using multi-dimensional scaling based on the 536 polymorphic HFO-TAG marker data points. PI's in groups A, B, C, D and E are *Citrullus lanatus* subsp. *lanatus* var. *lanatus* [also known as *C. lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb.] (*CLC*). PI's in groups F, G and H are *Citrullus lanatus* var. *lanatus* or *Citrullus lanatus* var. *vulgaris* (*CLV*), while PI's in groups I and J are *Citrullus colocynthis* (*CC*).

The HFO-TAG primers produced an average of 24 polymorphic markers among the genotypes examined (Table 2). The HFO-TAG markers were scored with high confidence using the CEQ 8800 capillary system (Beckman Coulter, Fullerton, CA), and ranged in molecular weight from 63 to 436 bp. Some of these markers differed in molecular size by only one nucleotide and may represent alleles of the same gene loci, as has been indicated in our recent genetic mapping study for watermelon (Levi et al. 2011). Overall, the HFO-TAG markers in this study identify polymorphism at a higher rate, and have shown greater utilization, than RAPD or SSR markers in targeting genomic regions associated with active genes (Levi et al. 2010).

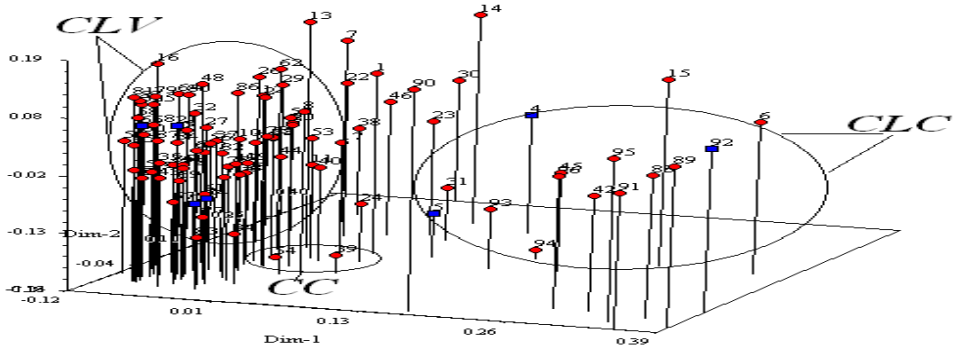

Fig. 3. Three-dimensional plot of watermelon accessions using multi-dimensional scaling based on 360 polymorphic HFO-TAG marker data points. PI's in *Citrullus lanatus* var. *citroides* group are designated as *CLC*, while PI's in the sweet watermelon group *Citrullus lanatus* var. *vulgaris* are designated *CLV* and *Citrullus colocynthis* PI's are designated as *CC*.

Genotyping-by-Sequencing: The annotated watermelon genome sequence (Guo et al. 2012) has been a useful reference for assembly and positioning of the SNPs identified by GBS (Table 1). The GBS procedure produced 449,517 polymorphic SNP markers among 76 PIs designated as *CLL*, 16 PIs designated as *CLC,* and 3 PIs designated as *CC* (Table 1). The SNP markers are present on all linkage groups of the watermelon reference genome with an average frequency of 1 SNP per 1.27 Kb. Our preliminary analysis based on the SNP data shows overall close genetic relationships among the *CLL* genotypes, including red sweet and white bitter flesh genotypes. Overall, the cluster analyses based on SNP data produced a dendogram similar to that produced by the HFO-TAG markers. The data produced in this study will be used for creating a public genotyping/informatics platform for watermelon based on next-generation sequencing. Also, the data will be used for genome wide association mapping study that requires a large number of SNPs within the genome.

Table 1. Shows the number of single nucleotide polymorphism (SNP) markers produce by Genotyping-by-Sequencing (GBS) procedure, and their frequency in the reference genome of watermelon.

Chromosome	Chromosome size in reference genome (bp)	Number of SNPs per Chromosome	SNP Frequency (per Kb) in the Reference Watermelon Genome
1	34.083.085	46.245	1.36
2	34.414.252	43.806	1.27
3	28.939.167	34.407	1.19
4	24.315.960	28.705	1.18
5	33.714.806	52.017	1.54
6	27.018.480	36.922	1.37
7	31.477.646	35.887	1.14
8	26.149.438	33.064	1.26
9	34.986.854	44.703	1.28
10	28.419.553	36.865	1.3
11	27.106.780	37.089	1.37
100	24.621.398	19.807	0.8
Total	355.247.419	449.517	1.27

General remark: Various watermelon types and landraces exist throughout Africa (Mujaju et al. 2010; Dahl Jensen et al. 2011). Extensive research is needed with respect to phylogenetic relationships and genetic diversity among PIs that represent the *Citrullus* subspecies and landraces. Additional studies to explore phylogenetic relationships among *Citrullus* spp. should be useful in identifying and maintaining germplasm valuable for enhancing watermelon cultivars for biotic and abiotic stress and for improvement of watermelon fruit quality and nutrient content.

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Association mapping of some selected watermelon traits using molecular markers

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Keywords: *Citrullus lanatus*, watermelon, morphological character, association mapping, linkage disequilibrium

Abstract

Association mapping of qualitative and quantitative characters is useful for finding gene markers and have not been reported in watermelon. The objective of this study was to detect molecular markers associating with 19 plant and fruit related traits such as number of branches and seed coat color. Ninety-six watermelon lines were selected from 259 accessions selfed 4 to 6 times available in germplasm resources. Sequence related amplified polymorphism (SRAP) primer combinations selected from 208 combinations were used to study 259 watermelon lines available in the Turkish genetic resources. The number of watermelon lines was reduced to 96 to eliminate highly identical ones based on Dice coefficient and UPGMA tree nested in NTSYS software PC version 2.1. This result indicated that most watermelons available in this germplasm were highly related. Morphological evaluations were performed at the University of Cukurova in Adana. Population structure data were obtained using "STRUCTURE" software. Association analyses are being performed using the "TASSEL" software to identify molecular markers associated with 19 important watermelon traits. Using a SAS program, regression analysis is being performed to exclude redundant markers at the alpha 0.10 level. Our preliminary results indicate that association mapping to identify molecular markers associated with phenotypic qualities is possible with watermelon lines.

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Early viability assessment in mutant populations of *Cucurbita pepo*

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Keywords: *Cucurbita pepo*, mutagenesis, pollen viability, fruit set

Abstract

To produce mutant populations for *Cucurbita pepo* zucchini TILLING platforms you must consider any loss of plant fertility due to the proper mutagenesis process. Even high germination rates in mutant plants, do not necessary lead to a high number of mutant lines once extended periods from sowing to seed production have passed. Therefore, in the present research we have evaluated the relationship between the pollen viability and the final fruit production, once we had established that there was no relationship between flower abnormalities rates and population fruit yield. Plants were treated with 1% and 0.4% of Ethyl methane sulfonate (EMS) as a mutagenic agent and then classified into one of three separate groups: plants without fruits, plants with fruits containing seeds, and plants with seedless fruits. Pollen viability, which is one of the essential factors of plant fertility, was analyzed by the Fluorochromatic reaction in each of these 3 groups. Both treatments with EMS gave as a result a substantial decrease in male fertility compared to non-treated plants. Sixty per cent of plants treated with 1% of EMS and which showed pollen viability rates between 45 to 90% produced fruit with seed. Such fruits were obtained in 70% of plants treated with the 0.4% EMS. All plants with pollen viability above 90% did produce fruits, and most plants with pollen viability below 45% produced no fruit. Plants without fruits were pollinated with viable pollen to evaluate other factors of fertility and only 30 and 45% of these plants produced fruits after treatment with 1% and 0.4% of EMS, respectively. Although the accumulation of mutations can seriously compromise fertility of mutant lines of zucchini, in our experimental conditions, plants that showed pollen viability higher than 45% lead to seed production in at least 60% of the mutant population.

INTRODUCTION

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In recent decades, methods for high throughout selection in populations of plants with induced mutations, such as TILLING (Targeting Induced Local Lesions In Genomes) (Henikoff et al. 2004) has unveiled new plants gene variation in short periods of time. These resources are established in large mutant populations with big rates of mutations to cover as much genome as it can. In the case of zucchini, *Cucurbita pepo*, there are a sharply reduction of the fertility of plants in the mutant populations that prevent its consecution. Higher rates of mutations reduce drastically the number of mutant lines (Martin et al. 2009), although the population reach elevated levels of germination. This requires a larger area of cultivation and also a prolonged period of time from planting until obtaining the seed. Therefore, an early analysis of the population feasibility take a particular interest since the traditional analysis does not allow to know the viability until the end of the cycle of the plant. In this work, we have evaluated the use of pollen viability from male flowers as a possible early analysis of the viability of the population, analyzing their repercussion in the production of fruit with seeds.

MATERIALS AND METHODS

Seeds of MU-CU16 zucchini cultivar were treated with 1% and 0.4% of EMS (Ethyl methane sulfonate) as the mutagenic agent shaked for 16 hours. Approximately 50%-80% of these seeds were viable respectively for each treatment. M1 plants were transplanted to the greenhouse to self-pollination and to generate the M2 seed bank. To study the mutants populations viability we have classified plants into one of 3 separate groups: plants with fruits containing seeds, plants with seedless fruits and plants that didn't produce fruit. Pollen viability, fruit set and seed production were determined to study the fertility of the plants setting 3 blocks of randomly arranged plants. Pollen viability was analyzed by the Fluorochromatic reaction (FCR test, Heslop-Harrison and Heslop Harrison 1970). Flowers were collected early in the morning and kept in a humid chamber for at least 2 hours to rehydrate the pollen. Subsequently, pollen grains were immersed in a solution of fluorescein diacetate, made up in a concentration of about 10^{-6} M in sucrose 0.5 M. Few minutes later pollen grains were observed in a fluorescence microscope (Olympus BX40F4). Viability was expressed as percentage of viable grains, counting at least 200 pollen grains per sample and considering fluorescent pollen grains viable. We also make a new pollination of plants that didn't produce fruits of selfing, applying pollen of male flower with high viability to determine percentage of fruit and seed production.

RESULTS AND DISCUSSION

The quality of a plant mutant population is determined by mutation frequency. Reaching saturation of mutations in genome implies a higher probability of finding

more diversity using a higher concentration of mutant agent, but it also matches more instability in lines generated due to lethal mutations or fertility. 3000 zucchini M1 mutants have resulted from a population with low fertility after a treatment of 1% EMS. The consequences of the high level of genetic instability have been fifty per cent of plants without fruits, and approximately fifty per cent of fruits without seeds. Other population originated with 0.4% EMS, rate currently used in this specie (Whitwood and Weigle 1978), also showed problems of fertility because only half of the population produced fruits, 87% of these with seeds.

To test if observed mutations explained the decrease in fertility, the analysis of mutations of plant development were made up. In this population the largest number of mutations altered the vegetative growth, while mutations that were affecting the reproductive development supposed a relatively low percentage (Martínez-Valdivieso et al. 2012). Mutations altering the reproductive development were grouped into different categories, including modification of general plant formation (plants without flowers or blinded in their early development), changes in sex determination (only male flowers or only female flowers) and flowers with defective organs (stamen without pollen or deformed stigma, Fig. 1). The percentage of the flower abnormalities was variable (Table 1) but the higher rates corresponded to mutations of morphology of floral organs. However, the mutation rates observed that alter the reproductive development did not explain the low fertility rates in the population, because most of the population shows male and female flowers apparently normal, but not enough to form fruits after successive pollinations.

Analyzing how mutagenesis can be conditioning fertility, we determined pollen viability, which is one of the essential factors of plant fertility, and it was performed a test for each concentration in a pattern of three randomized blocks. In each block, the self-pollinated plants were included into one of three separate groups: plants without fruits, plants with fruits containing seeds, and plants with seedless fruits. Each group was compared with untreated control plants.

The pollen viability of control plants was always higher than 90% and each plant produced fruit with seed. However, each treatment with EMS resulted in a substantial decrease in male fertility over the control plants, higher at 1%. Plants treated with 1% EMS and with pollen viability between 45-90% produced 60% fruits with seed (Fig. 2a), however plants with the same pollen viability but treated with 0.4% EMS produced 70% fruit with seeds (Fig. 2b). A high percentage of plants with less than 45% pollen viability produced no fruit, meanwhile pollen viability values ranging from 45-90% reduced this percentage.

In addition it has been studied cross pollination with viable pollen in a group of 75 mutant plants of each treatment, selected because it hasn't produced fruit with self pollination. After application of pollen of control plants, mutants pollinated with normal pollen produced 30% of plants with fruit and seeds for the dose of 1%,

contrasting to 45% of plants with seeds in case of the dose of 0.4%.

To sum up, plants with pollen viability above 45% reach 60% of seed production in the population, which was increased when the dose of the mutagenic agent decreased. In other plant species such as Arabidopsis or maize had already been shown that increase the number of mutations in the genome compromises the fertility of the population (Martin et al. 2009), compromising the transmissibility of the mutations (Naito et al. 2005). The decrease of the fertility in mutant populations of squash, seems to be not only the result of direct mutations on the flowering, but probably it results to the cumulative effects of mutations that alter, directly or indirectly, the fertility of the plant.

Early analysis in mutant populations has been comprehensively developed to select in big populations of crops (Saito et al. 2011) and to increase the potential on relevant and phenotypic characteristics (Xiao et al. 2007; Henry et al. 2008; Jiang and Ramachandran 2010). Also it could be necessary to develop early analysis that solves field performance of unstable mutant crops as in *Cucurbita pepo*. In this way analysis of early mechanisms that control the fertility of a population is essential to determine the possibilities of establishing a mutant population, even more in species such as zucchini that need four months to obtain the fruit and to value if it has or not seeds. With the analysis of pollen viability we could assess the viability of the population, and in the future even evaluate their relationship with the load of mutations per line.

ACKNOWLEDGEMENTS

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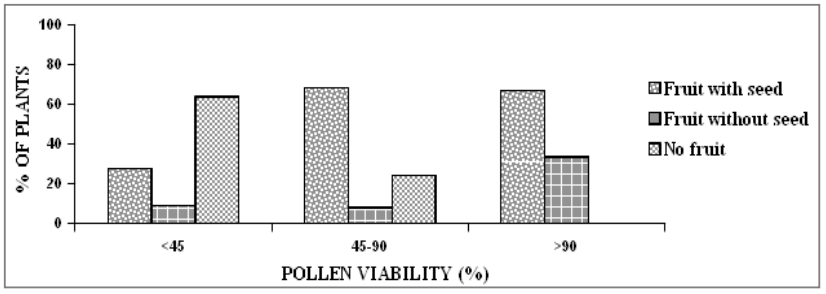
Table 1. Flower development observed in the zucchini mutant population.

Process altered	Plant Phenotype	%
General development	Plants without fruits	2
	Blind plants	5
Sex determination	Only male flowers	6,5
	Only female flowers	---
Floral morphogenesis	Male flower anomalies	25
	Female flower anomalies	7,5

Fig. 1. Differences in the morphology of male sexual organs (a) and female sexual organs (b) found in the zucchini mutant plants treated with 1% EMS. The normal phenotype of each of these organs is to the left of the images.

a.



b.

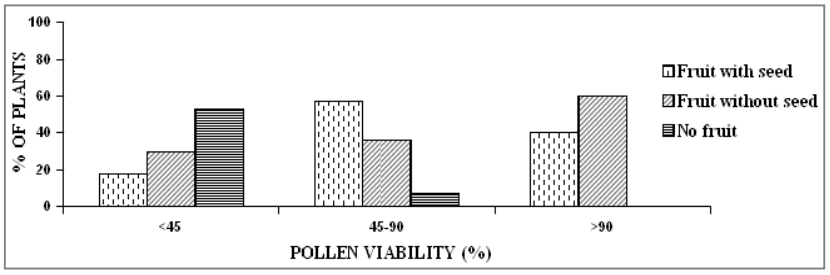


Fig. 2. Fruit production phenotype in the mutants populations. Plants were grouped by rates of pollen viability in populations of 1% (a) and 0,4% (b) EMS treatments.

Promotion of growth and health of Styrian oil pumpkins (*Cucurbita pepo* var. *styriaca*) by bacterial broad-spectrum antagonists

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Keywords: Pumpkin diseases, biocontrol, stress tolerance, field trial, fruit rot, seed treatment

Abstract

Due to increasing yield losses of Styrian oil pumpkins caused by the fungus *Didymella bryoniae* and bacterial pathogens, it was aimed to control this multi-pathogen disease by biological means. Out of 2320 microbial isolates of oil pumpkin-associated bacteria and fungi, six strains with a broad-spectrum antagonistic potential against respective oil pumpkin pathogens were selected and applied to the seeds. Effects of the bacterial inoculants, with and without additional chemical seed treatment, on plant growth and health were evaluated in three field trials. The treatments showed different effects against fungal diseases: no effect on fruit rot was observed, whereas powdery mildew was suppressed by *Paenibacillus polymyxa* PB71 and *Lysobacter gummosus* L101 in 2010. In addition, both bacteria increased seed yield in all three field trials. Application of *Serratia plymuthica* S13 resulted in increased seedling emergence, up to 109% in comparison to the control treatment in 2011. Furthermore, some treatments resulted in drought tolerance and increased seed weight. The promising bacterial inoculants will continue to be tested under field conditions, in order to develop a biological product supporting the productivity of the Styrian oil pumpkin crop.

INTRODUCTION

Due to the lack of lignification of the seed coat and the high content of bioactive ingredients in seeds, Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.; Cucurbitaceae) has become an important oil crop world-wide

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with increasing production in several Southern European and African countries, China, Russia and the USA.

During the last few years, substantial yield losses of oil pumpkin were reported in Styria due to fruit rot caused by the fungus *Didymella bryoniae* (black rot) and the bacterial pathogen *Pectobacterium carotovorum* (soft rot) (Huss et al. 2007; Huss et al. 2009). Vegetative plant parts are affected by Zucchini Yellow Mosaic Virus (ZYMV), Cucumber Mosaic Virus (CMV), *Pseudomonas* spp., *Xanthomonas cucurbitae, D. bryoniae* and powdery mildew as well (Huss 2007; J. Winkler, pers. commun. 2011; Huss and Mavridis 2007; Huss and Winkler 2009; Huss 2011). In 2011, infections of fruits as well as of vegetative plant parts with *Sclerotinia sclerotiorum* were reported in Styria (Huss et al. 2011). Additionally, symptoms of drought stress of oil pumpkin plants were observed in Styria, and these symptoms could play an even more important role in the future due to the effects of climate change (Trnka et al. 2010).

Considering the aforementioned numerous and varying negative impacts on oil pumpkin in Styria, there is demand for environmentally friendly and efficient possibilities to improve the general health of oil pumpkins in addition to the conventional agricultural practice in Austria. Biological control agents (BCAs) increase plant health by antagonizing pathogens via different modes of action like the production of antibiotics and extracellular enzymes, pathogen cell signal interference, predation and parasitism, and competition for nutrients and niches. Furthermore, they can support host immune response by induced systemic resistance (Compant et al. 2005; Berg 2009; Lugtenberg and Kamilova 2009). Plant growth promoting bacteria (PGPB) serve plants directly by supplying nutrients via the fixation of atmospheric nitrogen (N_2), phosphorous (P) solubilisation, phytohormone synthesis (e.g. indole-3-acetic acid), and by lowering host ethylene level due to 1-aminocyclopropane-1-carboxylate deaminase activity (Lugtenberg and Kamilova 2009). To date, the beneficial plant-microbe interaction has often been ignored in breeding strategies even though plant-associated microorganisms fulfil important ecosystem functions for plants and soils (reviewed in Smith et. al 1999). In comparison with chemically synthesised pesticides and fertilizers, microbial inoculants have several advantages: they (1) are more safe, (2) show reduced environmental damage and potentially smaller risk to human health, (3) show much more targeted activity, (4) are effective in small quantities, (5) multiply themselves but are controlled by the plant as well as by the indigenous microbial populations, (6) decompose more quickly than conventional chemical pesticides, (7) resistance development is reduced due to several mechanisms, and (8) can also be used in conventional or integrated pest management systems (Berg 2009).

In a previous study, endophytic microbial communities from the spermosphere (seeds), endorhiza (roots), anthosphere (flowers) and carposphere (fruits) of three

pumpkin cultivars were analysed. In all microenvironments, potential bacterial antagonists were present (Fürnkranz et al. 2012). Out of 2320 microbial isolates of oil pumpkin-associated bacteria and fungi, six phylogenetically distant broad-spectrum antagonists were selected according to their *in vitro* antagonism against the oil-pumpkin pathogens *D. bryoniae*, *P. carotovorum*, *P. viridiflava* and *X. cucurbitae* (Fig. 1), whereas five of them were active under greenhouse conditions against gummy stem blight (Fürnkranz et al. 2012).

The objective of this study was to evaluate the most active PGPB respectively BCAs regarding their effect on plant growth, health and stress tolerance of oil pumkins under field conditions. The overall performance of tested bacteria *ad planta* was evaluated and ultimately three strains were suggested as biological inoculants and are continuing to be tested under practical conditions, in order to develop a biological product supporting the productivity of the Styrian oil pumpkin crop.

MATERIALS AND METHODS

Bacterial broad-spectrum antagonists

The oil pumpkin-derived bacterial broad-spectrum antagonists evaluated in both years of field trials and continually tested were *Lysobacter gummosus* L101 (HQ163910) (isolated from the endorhiza), *Paenibacillus polymyxa* PB71 (HQ163909) (from the spermosphere), and *Serratia plymuthica* S13 (HQ163914) (from the anthosphere). The capability of *S. plymuthica* S13 to live endophytically was demonstrated under greenhouse conditions. As shown in Fig. 1, selected strains have the capacity to suppress growth of the oil-pumpkin pathogen *Didymella bryoniae* A-220-2b, and at least of two of the bacterial pathogens *Pectobacterium carotovorum* subsp. *atrosepticum* 25-2, *Pseudomonas viridiflava* 2 d1, and *Xanthomonas cucurbitae* 6 h4 *in vitro* (Fürnkranz et al. 2012). The strains are stored in the Culture Collection of Antagonistic Microorganisms (SCAM) at Graz University of Technology.

Plant material and inoculation of oil pumpkin with bacterial broad-spectrum antagonists

For all field trials, seeds of *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* 'GL Opal' were used. 'GL Opal' is a three-way cross hybrid between the F1 hybrid 'GL Diamant' and an inbred line, registered in 2008. Due to the bushy growth type, shown in Fig. 2, the spacing between rows can be reduced in the fields and mechanical processing is facilitated. 'GL Opal' is characterised by an early to mid-season onset of flowering and maturity and by a low to moderate susceptibility to fruit rot and ZYMV. Additionally, seeds can easily be removed from the surrounding placental tissue.

Seeds of this popular pumpkin variety were treated separately with suspensions

of bacteria described before. In 2010, the concentrations of cells were adjusted to 4 x 10^8 colony-forming units (CFU) mL^{-1} and seeds were primed in those suspensions for 8 h under agitation. For the control treatment, seeds were primed with sterile 0.85% NaCl. Primed seeds were sown immediately after the treatments without and with the addition of the chemical stripper Maxim® XL [2.4% (w/v) Fludioxonil, 1.0% Metalaxyl-M]. A quantity of 56 mL Sacrust® SK 76 including 2.2 mL Maxim® XL were used per one kg of seeds. Additional controls without priming and only the chemical stripper were sown.

For the field trials in 2011, seeds were immersed for 9.5 h in bacterial suspensions containing 2.6 x 10^8 CFU mL^{-1}. The control treatments were performed as described above. In contrast to the procedure for the field trial in 2010, seeds were dried after priming in a climate chamber at 25 °C until the residual moisture content was about 8%. Only *S. plymuthica* S13 treated seeds were sown without Maxim® XL as this strain led to a germination rate that was comparable to results obtained from the chemical treatments in the field trial of 2010.

Fields and field designs

All field trials were organised in a completely randomised plot design with three replicate plots per treatment. Sowing was between the end of April and mid-May, plants were grown without artificial irrigation and the fruits were harvested 18 to 22 weeks after sowing. The first field trial in 2010 was located in Gleisdorf (Styria, Austria). The soil can be described as gleyed loose brown earth, loamy silt, and covered loams on quaternary terrace, which were neutral to slightly acidic and deficient in lime. There were 24 seeds per treatment which were sown in three plots (eight seeds per plot) with a distance of 40 cm between the plants. The field was treated with herbicides (Dual Gold, Centium, Flexidor) and mineral fertilizer (DC Rot). In 2011, two field trials were conducted. One field site was located in Flöcking (Styria, Austria), exhibiting the same soil as described above. This field was treated with cow dung and mineral fertilizer. The second field trial in 2011 was located in Gabersdorf (Styria, Austria). The soil was a profound loose sediment brown earth. Only mineral fertilizer was applied here before seeding. Both fields were treated with the same herbicides used for the field trial in 2010 (as described above). Seeds were generally sown in five rows per plot and each row consisted of 30 seeds (with a distance of 20 cm between the seeds). After the assessment of germination rates, the number of plants was reduced to 15 per row (on average, 40 cm spacing between the plants) to obtain a consistent number of 75 plants per plot.

Parameters monitored during field trials and statistics

The parameters measured quantitatively were germination rates, number of fruits, number of fruits completely rotten or not harvestable due to fruit rot, yield

(dry weight of harvested seeds) and seed dry weight. For the measurement of yields in 2010, all fruits of a plot were harvested, seeds were mechanically extracted from the pumpkins, dried and weighed, whereas in 2011, only fruits of the middle 25 m^2 (comprising 45 plants) per plot underwent this procedure. The percentage of the leaf canopy with symptoms of drought stress (leaf area representing less turgor), powdery mildew infestation, necrosis, symptoms of virus infection and finally the degree of lacking leaf area in an advanced developmental stage was estimated for each plot. More, but hardly definable differences between treatments were observed, like the amount of chlorophyll (different shades of green), average height and surface area of leaves as well as their orientation and formation. Data for precipitation and maximal/minimal day temperatures were registered for each day during the oil pumpkin vegetation periods in Gleisdorf/Flöcking and provided by ZAMG (Zentralanstalt für Meteorologie und Geodynamik, http://www.zamg.ac.at/).

In order to compare mean values of evaluated parameters indicating the enhancement of plant growth and health by tested bacteria, Analysis of Variance (ANOVA) with Games-Howell and Duncan's post-hoc tests ($P < 0.1, 0.05, 0.01$) was performed, using Statistical Product and Service Solutions for Windows, Rel. 11.5.1 (SPSS Inc.).

RESULTS AND DISCUSSION

Germination rate

Germination rates of all treatments were monitored and compared with the respective control treatments. In 2010, bacterial inoculation of seeds without chemical treatment increased the germination rate by 27 to 47% compared to the non-inoculated control with NaCl-priming and could compensate for the absence of the chemical treatment. The highest increase of germination rates was obtained with chemically untreated seeds inoculated with *S. plymuthica* S13, shown in Fig. 3. The same treatment led to a considerable increase of 109% in seedling emergence compared to the non-inoculated control with NaCl-priming in the field trial in Flöcking in 2011. In Gabersdorf 2011, the effect of *S. plymuthica* S13 applied without chemical stripper could not be monitored as crows fed on sown seeds. Results from combined applications of bacteria and chemicals were similar to the results from the control, treated solely with the chemical stripper in all field trials.

General health of leaves

In 2010, a high incidence of leaf infestation with powdery mildew (*Sphaerotheca fuliginea*) was recorded after heavy rainfall in the middle of July. The lowest level of infestation was estimated for chemically treated variants of *P. polymyxa* PB71 (26.7% of infested leaf area) and *L. gummosus* L101 (53.3%)

that was statistically different to both control treatments (always 83.3%) (Duncan's multiple range test, $P < 0.01$ and 0.1 respectively). In 2011, leaf infestation was less pronounced and no effects on reduction of mildew by tested broad-spectrum antagonists were observed.

Statistically significant effects on reduction of drought stress symptoms were observed 2011 in Flöcking by the chemical variant of the *L. gummosus* L101 treatment (Duncan's multiple range test, $P < 0.05$), leading to a 100% drought stress reduction compared to both controls (0.0% in contrast to 6.7% of leaf area showed drought stress symptoms), and by the chemically untreated variant of *S. plymuthica* S13 (Duncan's multiple range test, $P < 0.1$), conferring a 75% reduction of drought stress symptoms with an affected leaf area of 1.7%. For all other parameters concerning the health of leaves, no statistical differences could be observed.

Biocontrol of fruit rot

The effect of the selected bacterial strains against black rot (by *Didymella bryoniae*) was the predominant question of our study. Although all selected broad-spectrum antagonists showed *in vitro* inhibition of *D. bryoniae* (Fürnkranz et al. 2012), no biocontrol effect on fruit rot was observed in the field experiments. The degree of black rot depends mainly on the climatic conditions, the oil pumpkin cultivar, and field management (Huss et al. 2007, Babadoost and Zitter 2009, Huss 2011). Provoked by heavy rainfall in June 2011, an infestation of oil pumpkins with *Sclerotinia sclerotiorum* was reported for the first time in Styria (Huss et al. 2011). Nevertheless, the amount of rotten pumpkins was relatively low in comparison to other vegetation periods (Huss et al. 2007). The occurrence of affected fruits in the fields was irregular within the replicated plots, indicating a high influence of soil characteristics on fruit rot: the lowest percentage of rotten fruits from one plot was measured in 2011 in Gabersdorf (0.6%), the highest value was 2010 in Gleisdorf (15.2%).

Dry weight of harvested seeds (seed yield/treatment) and seed weight

Combined treatments with bacteria and chemicals resulted in similar or higher yields compared to the controls throughout the field trials (Fig. 4). For plants inoculated with *L. gummosus* L101, increases of yields by 26% and 20% compared to the NaCl-primed control were observed in the field trials of 2011. The increment was statistically significant for the field trial in Gabersdorf (Duncan's multiple range test, $P < 0.1$). Inoculation with *P. polymyxa* PB71 resulted in higher yields in all field trials of 2010 and 2011 (+ 12%, 20%, and 15% respectively). In the case of the chemically untreated variants, the greatest increase in yield was obtained for *S. plymuthica* S13 by 40.3% at the field trial in Flöcking 2011, in comparison to the respective chemically untreated control. The effect was statistically significant (at P

< 0.1, Games-Howell test).

In field trial of 2010, the chemical and non-chemical variants of the *P. polymyxa* PB71 treatment revealed increases of seed weight by 8.7% and 5.0% respectively, compared to the NaCl-primed control. Besides this, no marked increases (> 5%) in seed weight were observed for other inoculants in 2010 and 2011.

General discussion

The selection of antagonists by *in vitro* assays is usually a straightforward procedure. Obtaining BCAs that succeed under practical conditions in agriculture is actually much more complex and represents one of the main hurdles in the commercialisation process (Berg 2009). Discrepancies between the antagonistic effects under *in vitro* conditions and the corresponding *in situ* efficacies are frequently reported (Weller and Cook 1983, Reddy et al. 1993). We observed that three bacterial strains, selected in a comprehensive, ecology-based study including greenhouse trials (Fürnkranz et al. 2012), were active under field conditions but showed differential activities. *Serratia plymuthica* S13 acted as a PGPB and increased germination percentage of oil-pumpkin seeds whilst *Paenibacillus polymyxa* PB71 acted as a BCA against powdery mildew infection. Furthermore, *S. plymuthica* S13 and *Lysobacter gummosus* L101 acted as agents against drought stress. Although differences in the efficacies of tested bacteria were found in the different field trials, overall the oil-pumpkin plants responded to bacterial inoculation with increased yields of seeds. Increased yields in separate trials were obtained with *L. gummosus* L101 and *P. polymyxa* PB71 in 2011. A similar increase was found with *S. plymuthica* S13, without the addition of a chemical stripper, in Flöcking, as compared with the chemically untreated control.

Further investigations are necessary to develop a formulation with a high shelf-life for the three promising bacteria. For *P. polymyxa* PB71, spore-based formulations could be developed as our preliminary results indicated a high shelf-life of encapsulated spores. No decrease of the inoculum occurred even after five months of storage at 4°C. As seed priming led to partial removal of the green cuticle of seeds in 2011, further variants of application of bacteria were tested in 2012. Although all strains belong to risk groups 1 (without risk for humans and environments), further risk assessment is important (Berg et al. 2009).

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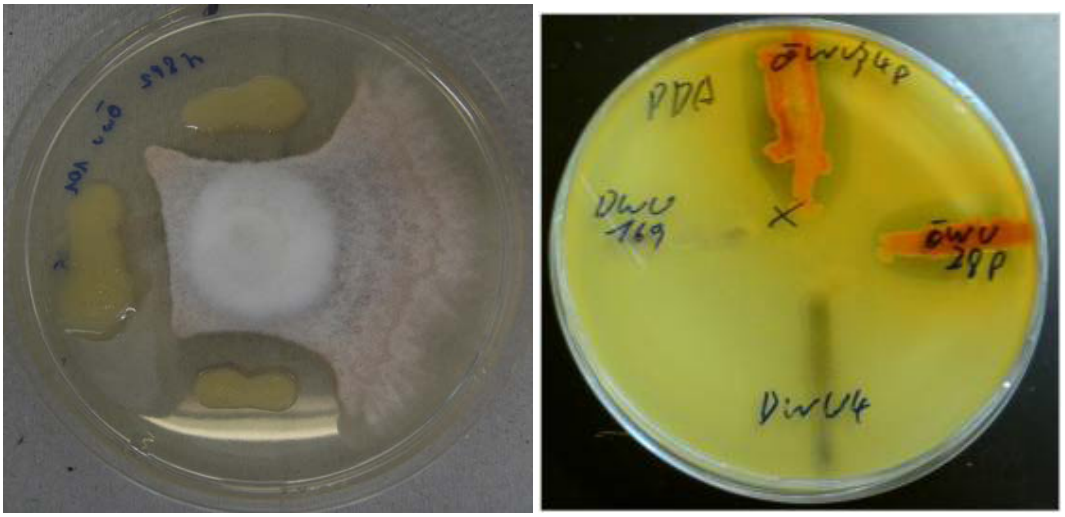

Fig. 1. *In vitro* screening for broad-spectrum antagonists. Left picture: inhibition of *Didymella bryoniae* A-220-2b by *Lysobacter gummosus* L101; right picture: four isolates from the endorhiza against *Xanthomonas cucurbitae* 6 h4.

Fig. 2. Oil pumpkin variety ‘GL Opal’: bushy growth type of plants 44 days after sowing (field trial 2012 in Gleisdorf, left photo) and fruits two weeks before harvesting (field trial 2011 in Flöcking, right photo).

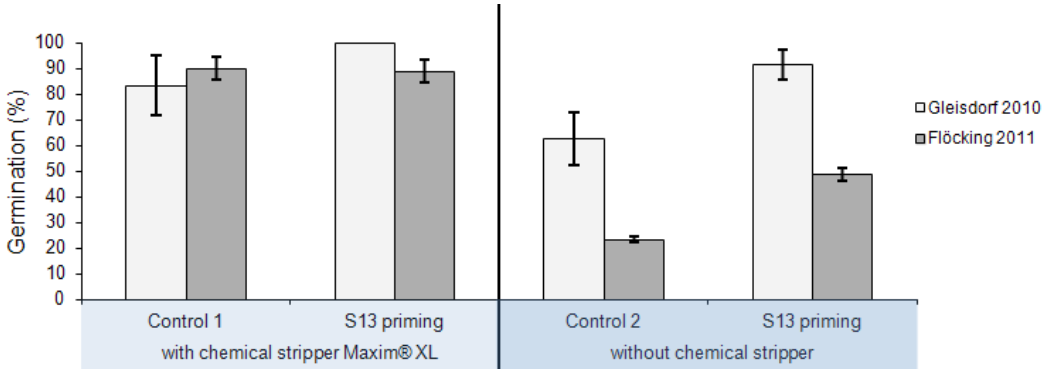

Fig. 3. Germination percentage of Styrian oil pumpkins after seed priming with *Serratia plymuthica* S13 with and without the addition of a chemical stripper at different field sites in comparison to the respective control treatment. Control 1: with the addition of Maxim® XL as chemical stripper without any priming; Control 2: without the addition of a chemical stripper but with NaCl-priming. Error bars indicate standard deviations.

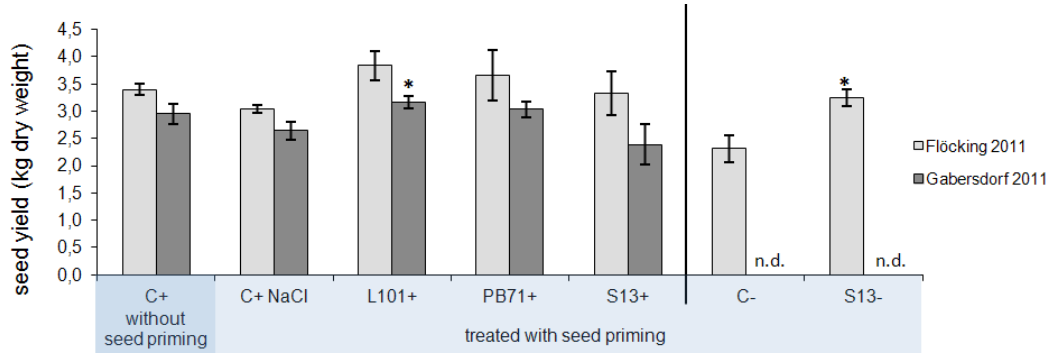

Fig. 4. Yields, after seed-priming treatments with broad-spectrum antagonists *Lysobacter gummosus* L101, *Paenibacillus polymyxa* PB71 and *Serratia plymuthica* S13 (C = control), with (+) and without (-) the addition of a chemical stripper at different field sites. Error bars indicate standard deviation. *Significant differences compared to respective control treatments (Games-Howell and Duncan's post-hoc tests, $P < 0.1$).

Variation for bitterness and other fruit traits in bitter gourd (*Momordica charantia* L.) collections

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Keywords: Fruit bitterness, genetic diversity, *Momordica charantia*

Abstract

Thirty-eight (38) bitter gourd accessions that originated from 12 countries in South and Southeast Asia and South America were assessed for four fruit traits: bitterness, color, shape, and surface appearance. Considerable diversity for these fruit traits was observed. Variation for fruit bitterness (3 variants), fruit color (4 colors), fruit shape (2 shapes) and fruit skin surface appearance (2 patterns) were found in the accessions. Low, medium and high fruit bitterness was observed in 12, 14 and 12 of the accessions, respectively. Medium green, dark green, light green and cream fruit skin coloration were observed in 11, 15, 8 and 4 of the accessions, respectively. Twenty-eight (28) of the accessions produced fusiform fruits; the others produced elliptical fruits. Twenty-nine (29) of the accessions had warty fruit skin surface; the others had a ribbed surface. There appeared to be no association between fruit bitterness and fruit color and fruit shape, as all three categories of bitterness were observed among the various fruit colors and skin patterns, whereas accessions with ribbed fruits were either low or medium in fruit bitterness.

INTRODUCTION

Bitter gourd (*Momordica charantia* L., 2*n* = 22) is an important market vegetable in Asia, where about 340,000 hectares are devoted to its cultivation annually. Local cultivars from Asia are also cultivated in Africa, Australia and the southern part of the United States. Bitter gourd is believed to have been domesticated in India and southern China. It has become naturalized into almost all tropical and subtropical regions of the world (Reyes et al. 1994). Immature fruits and sometimes leafy shoots are consumed in Asia. It is also used as a folk medicine to manage type-2 diabetes, a non-communicable disease currently affecting 346 million people worldwide, with 80% living in low-income and middle-income countries (WHO 2011). The hypoglycemic activity of bitter gourd fruit extract has been confirmed in

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cell culture and animals (Cummings et al. 2004, Shih et al. 2008). It is also used for its antiviral, antimalarial, antimicrobial and antitumor activity (Cheng et al. 2004; Hu et al. 2004). Bitter gourd fruits provide important nutrients including β carotene, folic acid, vitamin C, iron and potassium.

Bitter gourd breeders agree that a huge amount of untapped genetic diversity exists within this taxon as a result of domestication (Behra et al. 2010). These plant genetic resources are potential sources of novel alleles that can be exploited to improve yield and nutritional quality. The AVRDC genebank houses an extensive collection of bitter gourds collected from various parts of Asia including India, the primary center of genetic diversity. Knowledge, access and use of this available diversity are essential for broadening the genetic base of modern bitter gourd cultivars and for sustainable genetic improvement. AVRDC researchers are engaged in the comprehensive evaluation of this bitter gourd collection. The objective of this study was to evaluate 38 bitter gourd accessions that originated in 12 countries in South and Southeast Asia and South America for four fruit traits, bitterness, color, shape, and skin surface appearance.

MATERIALS AND METHODS

The 38 accessions were planted in a single replicate. Seeds were planted in soilless media in plastic seed trays during the last week of November 2010. Fifteen-day-old seedlings (three-leaf stage) were transplanted into raised beds covered with black plastic mulch in rows spaced 2.5 m apart. Plots were 7 m long and contianed 7 plants each. Vines were supported by trellises. Recommended horticultural practices were used. The five middle plants of each plot were evaluated for four fruit traits (bitterness, color, shape and skin surface) (Table 1). Fruit bitterness was evaluated by the same person throughout the evaluation process. Evaluation was done on one single day for all the entries. Fresh immature marketable fruits were washed, cut into small pieces and chewed. Three categories of bitterness were recorded: low, medium and high. The tester rinsed his mouth with water after each tasting. One fruit from each of the five plants of each entry was sampled for bitterness.

RESULTS AND DISCUSSION

Fruit characteristics of the bitter gourd accessions used in this study are provided in Table 1. Low, medium and high bitterness was observed in 12, 14 and 12 of the accessions, respectively. Four categories of fruit skin color were observed in the germplasm. Medium green, dark green, light green and cream fruit skin colorations were observed in 11, 15, 8 and 4 of the accessions, respectively. Two fruit shapes were observed in the collection. Twenty-eight accessions produced fusiform fruits, the rest produced elliptical fruits. Two types of skin surface were observed in the accessions. The majority (29) of the accessions had warty skin;

the others had a ribbed surface. No association between fruit bitterness and fruit color and fruit shape was observed, as all three classes of bitterness were found among various fruit colors and skin patterns. However, accessions with ribbed fruits were either low or medium in fruit bitterness. The bitter gourd accessions used in this study are landraces that meet the preference of local consumers; consumers in South Asia prefer bitter fruit. Fruit of the accessions originating in South Asia (India, Bangladesh, Pakistan, and Sri Lanka) have medium to high bitterness (Table 1). Saponins, momordicosides K and L, and momordicines I and II in bitter gourd fruit cause the bitter taste (Yasuda et al. 1984; Harinantenaina et al. 2006). Development of less bitter varieties is expected to lead to wider adoption of the crop in other regions. Variability for fruit bitterness exists in the bitter gourd germplasm collection. More accessions of bitter gourd need to be evaluated for bitterness intensity, which may lead to the identification of accessions with bitter-free fruits.

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Table 1. Fruit traits of bitter gourd accessions.

Accession	Origin	Bitterness			Color	Shape		Skin surface	
		L	M	H		E	F	R	W
TOT 4244	Bangladesh			+	DG		+		+
TOT 4256	Bangladesh		+		DG		+		+
TOT 4362	Bangladesh			+	G		+		+
TOT 4370-1	Bangladesh		+		LG		+		+
TOT 4409	Bangladesh			+	C		+		+
TOT 4491	Bangladesh			+	C		+		+
TOT 4531	Bangladesh			+	LG		+		+
TOT 4549	Bangladesh	+			DG		+		+
TOT 6236	Bangladesh			+	G		+		+
TOT 7308	Bangladesh			+	DG		+		+
TOT 3991	Belize	+			G		+		+
TOT 6983	Cambodia		+		LG	+		+	
TOT 7098	Cambodia	+			LG	+		+	
TOT 1756	India		+		G		+		+
TOT 2533	India		+		DG		+		+
TOT 5852	India			+	G		+		+
TOT 1843	Indonesia		+		LG	+		+	
TOT 1850	Indonesia		+		C	+		+	
TOT 4009	Lao PDR	+			C		+		+
TOT 2377	Pakistan			+	DG		+		+
TOT 1504	Philippines	+			G		+		+
TOT 1505	Philippines	+			DG	+		+	
TOT 1513	Philippines			+	DG	+			+
TOT 1567	Philippines	+			LG	+		+	
TOT 1568	Philippines	+			G	+		+	
TOT 6511	Philippines		+		DG		+		+
TOT 5869	Sri Lanka		+		LG		+		+
TOT 6093	Taiwan	+			G		+		+
TOT 6173	Taiwan		+		DG		+		+
TOT 0950	Thailand			+	G		+		+
TOT 1095	Thailand		+		DG		+		+
TOT 1140	Thailand			+	DG		+		+
TOT 1398-1	Thailand	+			G		+		+
TOT 5468	Thailand		+		MG		+		+
TOT 4131	Vietnam	+			G		+		+
TOT 4785	Vietnam		+		DG	+		+	
TOT 4786	Vietnam	+			DG	+		+	
TOT 4839	Vietnam		+		DG		+		+

Fruit bitterness: L = low; M = medium; H = high, Fruit shape: E = elliptical; F = fusiform
Fruit skin color: G = green; DG = dark green; LG = light green; C = cream
Fruit surface: R = ribbed; W = warty

Mode of inheritance of bitterness and spine colour in cucumber fruits (*Cucumis sativus* L.)

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Keywords: Cucurbitacin, bitterness trait, genetics, dominant and monogenic traits, breeding

Abstract

The mode of inheritance of bitterness and spine colour in cucumber was investigated. Two cucumber lines, namely 'IIHR-82-2' which is a cultivated bitter free advanced line and a wild species namely *Cucumis sativus* var. *hardwickii* which is highly bitter in taste were taken for the present study. The two parents, diverse with respect to spine colour (i.e. 'IIHR-82-2' is white and *C. sativus* var. *hardwickii* being brown), self pollinated for three successive generations, fixed the character under the study. Crosses were made between the parents to produce F_1, F_2, BC_{1P1} and BC_{1P2} populations. The fruits from all the F_1 plants were bitter, indicating dominant inheritance of bitterness in *C. sativus* var. *hardwickii*. The segregating F_2 plants showed ratio 3:1 (bitter : non bitter) which confirmed that bitterness trait was governed by a single dominant gene *Bt*. This interpretation was largely confirmed by segregation ratio of 1:1 (bitter : non bitter) in BC_{1P1} generation and in BC_{1P2}, where all bitter plants were obtained.. For spine colour, the F_1 generation plants bore brown spines on fruits indicating that brown spine was dominant over white spine. The F_2 generation showed segregation ratio of 3:1 (brown spine : white spine) indicating monogenic control of spine colour. This was confirmed by segregation ratio of 1:1 (brown spine : white spine) in BC_{1P1} generation and in BC_{1P2}, where all fruits obtained showed brown spine. The presence of spine colour was controlled by single dominant gene.

INTRODUCTION

Bitterness in cucumber (*Cucumis sativus* L.) is a major constrain for edible purposes in cases of salads, etc. The most important qualitative parameters are fruit quality that includes (skin and spine colour) and taste (free from bitterness), as compared to fruit yield. In general, bitter taste in cucumber is mainly due to presence

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of cucurbitacin C (Balkema-Boomstra et al. 2003). The extent of bitterness in the fruits depends on genetic character of the cultivars and on the growing conditions (Vogel 1934). This trait is inherited as single dominant gene (Anderweg and De Bruyn 1959), single dominant gene *Bt* (Barham 1953) and one or more additive intensifier gene(s) (Ponti and Garretson 1980). All of these genes have played a major role for breeders to develop non-bitter cucumber cultivars having light green to dark green stripes, brown to black or cream to white spines (Andeweg and De Bruyn 1959).

The aim of this study was to investigate the mode of inheritance of bitterness and spine colour in fruits of two diverse cucumber lines.

MATERIALS AND METHODS

Plant materials consisted of IIHR-82-2 cultivated bitter free, white spine uniform homozygous advanced line, and the wild species *Cucumis sativus* var. *hardwickii* with bitter taste and brown spine fruits, including the F_1, F_2, and backcross populations from crosses of the F_1 with either parental line. Bitterness of the fruit was recorded by tasting each fruit and spine colour was recorded by visual observation during the fruit harvest time. The population mean for each replication was calculated for 220 entries (BC_{1P1} and BC_{1P2}=40 plants each , F_1 = 20, F_2= 100 and 10 plants mean of each row per replication was calculated for P_1 and P_2. The means were used for analysis of variance by the method given by Panse and Sukhatme (1961). Data were tested for goodness-of-fit to theoretical ratios using the Chi-square tests for each of the F_2 and BC_{1P1} populations (Snedecor and Cochran 1967), separately for each trait studied.

RESULTS AND DISCUSSION

Inheritance mode of cucumber fruit bitterness

The parent IIHR-82-2 was totally free from bitterness, whereas the other parent *C. sativus* var. *hardwickii* was highly bitter in taste. All the F_1 plants developed by crossing between IIHR-82-2 and *C. sativus* var. *hardwickii* were highly bitter in taste (Table 1). In the F_2 population, 79 plants were bitter and 21 plants were bitter-free, which fitted the monogenic ration of 3:1 with a probability of 0.3556 (Chi-square=0.853). The B_{1P1} population showed segregation 18 bitter: 22 non bitter plants/fruits. These data fit a ratio 1:1 with probability of 0.5270 (Chi-square=0.4). All the plants in B_{1P2} population were bitter.

Inheritance pattern of spine colour

The IIHR-82-2 had white spine fruits, whereas the other parent *C. sativus* var. *hardwickii* bore brown spines on fruits. All the F_1 plants produced only brown spines. In the F_2 generation, spine colour segregated in a ratio of 71 : 29 (brown :

white). (Table 2) These data fit a ratio of 3 brown : 1 white with a probability of 0.3556 (Chi square=0.853). Whereas, in BC_{1P1} generation, there were 23 brown : 17 white plants. These data fit a ratio of 1 brown: 1 white with a probability of 0.3427 (Chi-square=0.90). All the plants in BC_{1P2} produced brown spined fruits.

In the cross, the inbred IIHR-82-2 (P_1) was totally free from bitter and *C. sativus* var. *hardwickii* (P_2) was highly bitter and F_1 plants were bitter indicating dominant inheritance of the bitterness. This is in total agreement with findings of Andeweg and De Bruyn (1959). This was confirmed by the segregation ratio of 1:1 (bitter : non-bitter) in BC_{1P1} and bitter plants in BC_{1P2} generation (Barham 1953). In case of spine colour, two parents showing wide diversity in respect to spine colour were studied and F_1 plants bore brown spines on the fruits, indicating that brown spine trait was dominant over white spine. This is in total agreement with the report of Wellington (1913). It is very much conspicuous, that both traits: the bitterness and brown spines are under the control of single dominant genes. Therefore, breeders can select for non-bitter plants with white spined fruits from the segregating populations, which could be utilized for hybridization programmes in order to exploit higher fruit bearing capacity, more number of lateral branches, sequential fruiting habit, and higher yield.

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Table 1. Inheritance pattern of bitterness in cucumber (IIHR-82-2 x *Cucumis sativus* var. *hardwickii*)

Generations	Number of plants			Test ratio	Chi- square	Probability
	Total	Bitter	Non-bitter	Bitter : non bitter		
IIHR-82-2 (P_1)	10	0	10			
C. sativus var. *hardwickii* (P_2)	10	10	0			
IIHR-82-2 x *C. sativus* var. *hardwickii* (F_1)	20	20	0			
F_2	100	79	21	3 : 1	0.853	0.3556
BC_{1P1}	40	18	22	1 : 1	0.4	0.5270
BC_{1P2}	40	40	0	1 : 0 All bitter		

Table 2. Inheritance pattern of fruit spine colour in cucumber (IIHR-82-2 x *Cucumis sativus* var. *hardwickii*)

Generations	Number of plants			Test ratio	Chi-square	Probability
	Total	brown	white	brown : white		
IIHR-82-2 (P_1)	10	0	10			
C.sativus var. *hardwickii* (P_2)	10	10	0			
IIHR-82-2 x *C.sativus* var. *hardwickii* (F_1)	20	10	0			
F_2	100	71	29	3 : 1	0.853	0.3556
BC_{1P1}	40	23	17	1 : 1	0.90	0.3427
BC_{1P2}	40	40	0	1 : 0 All bitter		

Development of Fusarium wilt-resistant F_1 hybrids of bottle gourd (*Lagenaria siceraria* Standl.) for watermelon rootstocks

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Keywords: Genetic resources, *Citrullus lanatus*, *F. oxysporum* f. sp. *lagenariae*, germination rate, fruit quality

Abstract

Ninety-eight bottle gourd genotypes were collected and used in the study to select accessions with resistance to *Fusarium* wilt. Based on germplasm screening procedures, nine accessions were found to be resistant to *F. oxysporum* f.sp. *lagenariae*, with less than 25% diseased plants in 1998. The resistant plants from nine accessions were again selected and have been put in advance trial from R_1 to R_9 generations on the basis of selecting resistant plants to *F. oxysporum* f. sp. *lagenariae* in each generation. As a result, thirteen inbred lines were selected for *Fusarium* wilt resistance and as parental lines to produce F_1 hybrid rootstock for watermelon. Consequently, two *Fusarium* wilt-resistant F_1 hybrid varieties, 'FR-Ganggeon' and 'FR-Sinsegye', were developed from the cross between 'FRD 22×963381-42' and 'FRD 210×963385-2', respectively. The *Fusarium* wilt resistance, germination speed, yield and quality of fruits grafted onto 'FR-Sinsegye' and 'FR-Ganggeon' rootstocks was also superior or at least equal to popular commercial bottle gourd rootstocks. The study confirmed the possibility of producing high-quality watermelons by using *Fusarium* wilt-resistant bottle gourd rootstocks.

INTRODUCTION

Vegetable production using grafted seedlings is being safely adapted for organic as well as environment-friendly crop production that minimize uptake of undesirable agrochemical residues (Lee et al. 2010). Grafting techniques are widely adapted for watermelon (*Citrullus lanatus*) cultivation in many parts of the world. Remarkable yield increases achieved by grafting watermelon onto different gourd rootstocks under protected structures attracted the interest of many vegetable growers (Lee and Oda 2003).

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The first attempt at grafting was made with pumpkin (*Cucurbita moschata*) rootstocks to control *F. oxysporum* f. sp. *niveum* and other soil-borne diseases caused by successive cropping (Ashita 1927). Various rootstocks from existing cultivars have been screened for use watermelon. Interspecific squash hybrid rootstocks (*Cucurbita maxima*× *C. moschata*) excelled resistant to soil-borne diseases and also enhanced cold tolerance level. However, fruit's texture and firmness of watermelon grafted onto squash were inferior to those of watermelon grafted to bottle gourd (*Lagenaria siceraria*) or non-grafted watermelon (Yamasaki et al. 1994; Yetisir et al. 2003; Karaca et al. 2012). The deterioration of fruit quality of watermelon on *Cucurbita* rootstocks can be attributed to too much nutritional uptake (Kato and Ogiwara 1978; Shinbori et al. 1981; Masuda et al. 1986).

Several rootstocks including *Citrullus* spp. (Huh et al. 2002) and several species *Cucumis* and *Cucurbita* (Igarashi et al. 1987; Hirai et al. 2002) were identified that possess high level of *Fusarium* resistance. Among them, bottle gourd is the most popular rootstock used for watermelon in Korea and Japan. Hence, several bottle gourd varieties have been developed and used as watermelon rootstocks. However, recently, *Fusarium* wilt caused by *F. oxysporum* f. sp. *lagenariae* in these rootstocks have occurred in some major watermelon producing areas in Japan and Korea. It has resulted in severe foliage wilting at late developmental stages of watermelon and caused severe crop losses (Huh et al. 2002; Kuniyasu 1979).

Therefore, this study aimed to develop resistant bottle gourd F_1 hybrids against *Fusarium* wilt to be used as rootstock for watermelon.

MATERIALS AND METHODS

Selection of bottle gourd with resistance to *Fusarium* wilt

Sixty fungal isolates were collected from wilted watermelon plants grafted onto bottle gourd rootstocks in some major watermelon growing areas in Korea.

Single spore isolates of *F. oxysporum* f. sp. *lagenariae* isolated from bottle gourd were cultured in Potato Dextrose Agar (PDA) media at 25°C for 7 days. The isolates grown in PDA media were then transferred to Potato Dextrose Broth (PDB) media under continuous agitation at 220 trs min^{-1} at 25°C and subcultured at 5 to 7 days intervals. The suspension was filtered with two layers of sterile cheesecloth diluted with sterile distilled water and the conidia concentration was adjusted to $5x10^6$ conidia per milliliter using a hemacytometer. The roots of 7 to 10-day-old plants with one or two true leaves were dipped into the conidia for one minute and then transplanted to pots. Disease ratings were conducted three weeks after inoculation.

Among the sixty fungal isolates, the fourteen isolates were identified as *Fusarium oxysporum.* The one isolate had pathogenicity to bottle gourd and it was used in this study.

A total of 98 bottle gourd genotypes (*Lagenaria siceraria* Standl.) was collected for the selection of bottle gourd plants resistant to Fusarium wilt.

Disease index (DI) was recorded with ratings as follows: DI = Σ(dr×n)/N, where dr=individual disease rating, n=number of diseased plants, N=number of total plants examined. Individual disease rating (dr) was arbitrarily designated as follows: 1= symptomless; 2=unclear lesions developed on the stem or leaves; 3=moderate lesions developed on the stem or leaves; 4=severe lesions developed on the stem or leaves; 5=severe lesions developed on the entire plant nearly or completely dead.

The individuals with resistant to *F. oxysporum* f. sp. *lagenariae* were selected and put in advance trial from R_1 to R_9 selfing generations. Selections were carried out in each generation for selecting resistance lines. Thirteen inbred lines were then selected as potential breeding materials to be used as rootstocks and crosses.

Germination and survival rate, fruit quality and yield

Two field trials were conducted in a plastic house during the spring and fall of 2007 at the National Institute of Horticultural and Herbal Science RDA, located in Suwon, Rep. of Korea (37°18'23''N, 126°58'40''E). In two field experiments, watermelon (*Citrullus lanatus* (Thunb) Matsum & Nakai) cultivar, 'Smabokkul' (Monsanto Korea) was grafted onto twelve Fusarium wilt-resistant F_1hybrids and two conventional bottle gourd rootstocks, 'FR-Dantos' (Dongbu Hannong, Korea) and 'Bullojangsaeng' (Syngenta Korea). Non-grafted 'Smabokkul' watermelon cultivar was use as control in the study.

A total of 100 seeds from each bottle gourd rootstock were sown in 'Partner (Nongwoo Bio, Korea)' bed soil into plug trays and seeds of the scion, 'Smabokkul' were approximately 3 days later. Seed germination was recorded three times at seven day interval. Germination speed after sowing 7 days and germination percentage after sowing 21 days were calculated based on the number of germinated seeds divided by total number of seeds.

Seven to ten days old watermelon seedlings were grafted using splice grafting technique (Lee et al. 2010). The live grafted plants were counted and the survival rate (%) was investigated 20 days after grafting, dividing the number of survived by the total number of the grafted plants. The survived grafted plants were transplanted to a plastic house.

The experimental design consisted of randomized blocks with three replicates. A total of ten plants per replication were grown horizontally at spacing of 3 m x 0.5 m (row-row x plant-plant) for each F_1 hybrid rootstocks and cultivars. Fertilizer was applied at the ratio of 130 kg N ha^{-1}, 50 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1}. Micro-nutrient fertilize was not applied. The amount of nitrogen and potassium

were divided equally into three and applied in three times; 1st dose before planting, 2nd dose at 30 days and 3rd at 45 days after planting. Water was applied by drip irrigation. Ripe fruits were harvested from 40 to 48 days after pollination, Fruit yield and fruit quality parameters were also examined.

The statistical analysis was conducted with SAS software (SAS Institute 1995). The data from all the experiments were subjected to ANOVA and Duncan's multiple range tests to determine the statistical significance of differences among treatments.

RESULTS AND DISCUSSIONS

Selection of bottle gourd with resistance to Fusarium wilt

Disease index rating of a majority of accessions tested for *Fusarium* wilt ranged between 2.6 and 5.0. Nine accessions, '963381', '963383', '963385', '963390', 'DJ-41', 'FP1', 'FR', 'PFR11', and 'FR79' were found to have disease index rating between 1.6-2.5 and performed better compared to control 'FR-Dantos' (3.5) and 'Bullojangsaeng' (4.4) in 1998 (Table 1).

The individuals without symptoms (disease index=1) were selected among nine accessions and advanced from R_1 generation to R_9 generation by self pollination.

Based on selection for *Fusarium* wilt resistance, thirteen inbred lines were again selected as potential breeding materials for rootstock (Table 1). Selections were made on the basis of finding out resistance to *F. oxysporum* f. sp. *lalgenariae* in each generation. 963381-42, DJ-41, FR-22, FR-210 and FR-271 were selected after six selfing generations, 963383, 963385-25, 963385-231, 963385-233 and 963390 after five generations, PFR11-6N after eight generations, and FP1, FR79-3 nine generations. Crosses were made between these lines in order to develop F_1 hybrids with resistance to *Fusarium* wilt.

F_1 hybrids from bottle gourd lines exhibited high level of resistance to *F. oxysporum* f. sp. *lagenariae* (Table 2). Bottle gourd rootstocks were well known for its resistance to *Fusarium* wilt but *Fusarium* spp. was first isolated from infected roots of gourd rootstocks. Which possibly due to the widespread use of bottle gourd rootstocks (Sato and Ito 1962). Later, the strain was identified as *F. oxysporum* f. sp. *lagenariae* Matsuo & Yamamoto (Sakata et al. 2007). Breeding and selection for *Fusarium* wilt resistance in bottle gourd rootstocks can prevent the reoccurrence of this resistance breakdown.

Five forma specialis in *F. oxysporum* which cause wilts of the family *Cucurbitaceae* clearly showed the host specific pathogenicity (Armstrong and Armstrong 1978; Kawai 1958). Therefore, it is presumed that the bottle gourd with resistance to *F. oxysporum* f. sp. *lagenariae* would also show resistance to other forma specialis.

Germination and survival rate, fruit quality and yield

The germination speed and germination percentage of two field experiments are in Table 2.

The germination speed after sowing 7 days is very important characteristic of the rootstock for grafting with scions in the nursery simultaneously. The germination speed of *Fusarium* wilt-resistant F_1 hybrids and two conventional bottle gourd rootstocks varied from 60% to 93%. The rootstocks 'FR-210×963385-233', 'FR-210× 963385-25' and 'FR-22×963381-42' were found to have with high germination rates than other rootstocks, and this could be considered as additional commercial value to nurseries.

Survival rate and fruit quality and yield characteristics of watermelon plants grafted onto different rootstocks are in Tables 3 and 4.

The survival rate of different graft combinations was not affected by the rootstock in two field experiments. Survival rates ranged from 93% to 100%. The total yield ($t \cdot ha^{-1}$) was significantly affected by rootstocks and fruit yield of grafted plants was significantly higher than that of non-grafted watermelon in two separate experiments.

The yield of watermelon plants grafted onto 963390×DJ-41'(52.0 $t \cdot ha^{-1}$), 'FP1×963383' (50.7 $t \cdot ha^{-1}$), '963383×963385-233' (49.8 $t \cdot ha^{-1}$), 'FR-210×963385-233' (48.1 $t \cdot ha^{-1}$), and 'FR-22×963381-42' (48.1 $t \cdot ha^{-1}$) was significantly higher than that onto the commercial rootstock, 'FR Dantos' (37.1 $t \cdot ha^{-1}$) in spring trial, 2007 (Table 3). The rootstocks 'FR-210×963385-233' (31.0 $t \cdot ha^{-1}$) and '963390×DJ-41' (30.8 $t \cdot ha^{-1}$) were significantly higher in yields than the commercial rootstocks, 'FR Dantos' (26.6 $t \cdot ha^{-1}$) and 'Bullojangsaeng' (26.0 $t\ ha^{-1}$) in fall trial, 2007 (Table 4). Marketable yield was also influenced by rootstock and more than 86% of fruits from the grafted plants were marketable. All plants grafted onto rootstocks in two field experiments were higher in marketable yield than the non-grafted watermelon. Watermelon grafted onto rootstocks produced large fruits than the control watermelon. The watermelon grafted onto 'FR-210×963385-233' (4,183 $g \cdot ea^{-1}$), '963390×DJ-41' (4,162 $g \cdot ea^{-1}$), 'FR-22×963381-42' (4,019 $g \cdot ea^{-1}$), 'FR-210×963385-25' (3,929 $g \cdot ea^{-1}$) and '963385-231×C60' (3,612 $g \cdot ea^{-1}$) performed better compared to either that grafted onto other rootstock or control, Sambokkul (2,210 $g \cdot ea^{-1}$), 'FR Dantos'(3,593 $g \cdot ea^{-1}$) and 'Bullojangsaeng' (3,510 $g \cdot ea^{-1}$).

The soluble solids contents of watermelon fruits varied among rootstocks. Watermelon fruits grafted onto F_1 hybrid rootstock, '963385-231×FR-271' and '963385-231 ×C60' had highest soluble solids contents in spring trial, 2007. Fruit of watermelon plants grafted onto '963385-25×PFR11-6N' rootstocks had higher soluble solids contents than those of non-grafted watermelon. Other rootstocks had similar soluble solids contents to those non-grafted watermelon fruits in fall trial, 2007.

It has been suggested that the type of rootstock utilized for grafting in watermelon has significant effect on total yield, marketable yield and fruit quality. Fruit size and harvest duration of watermelon cultivars significantly increased when grafted onto rootstocks having vigorous root systems compared to those of non-grafted plants (Yetişir et al. 2003). The influence of rootstocks on fruit quality can often be detrimental but some reports showed variation depending on the scion cultivars (Lee 1994; Yetişir et al. 2003).

In this study, two *Fusarium* wilt-resistant F_1 hybrids, 'FR-Ganggeon' and 'FR-Sinsegye', were developed from crosses between 'FRD 22×963381-42' and 'FRD 210×963385-2', respectively. The *Fusarium* wilt resistance, germination speed and fruits quality and yield of 'FR-Sinsegye' and 'FR-Ganggeon' rootstocks were superior or equivalent to those of commercial bottle gourd rootstocks.

Further studies are needed to exploit the genetic variation available among the existing bottle gourd genotypes to be used as rootstock for producing high-quality watermelons with resistance to *Fusarium* wilt and cold tolerance.

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Table 1. Distribution and disease index of Fusarium wilt resistance in bottle gourd by root inoculation of seedling.

Accessions	Generations	No. of seedlings tested	Number of plants in each disease rating [z]					Disease index[y]
			1	2	3	4	5	
963381-42	R_6	47	9	35	3	0	0	1.9
963383	R_7	44	10	17	12	5	0	2.3
963385-25	R_7	41	5	17	17	1	1	2.4
963385-231	R_7	44	7	30	5	2	0	2.0
963385-233	R_7	44	3	33	7	1	0	2.1
963390	R_7	50	18	25	5	2	0	1.8
DJ-41	R_6	50	12	28	7	3	0	2.0
FP1	R_9	39	3	30	5	1	0	2.1
FR-22	R_6	45	3	20	17	5	0	2.5
FR-210	R_6	45	20	22	3	0	0	1.6
FR-271	R_6	43	10	25	5	3	0	2.0
FR79-3	R_9	46	3	17	17	7	2	2.7
PFR11-6N	R_8	50	10	28	7	5	0	2.1
FR-Dantos	-	50	2	7	15	14	12	3.5
Bullojangsaeng	-	50	0	0	5	18	27	4.4

[z] Disease rating (dr) : 1=symptomless; 2=unclear lesions developed on the stem or leaves; 3=moderate lesions developed on the stem or leaves; 4=severe lesions developed on the stem or leaves; 5=severe lesions developed on the entire plant nearing death or complete death (Matsuo etc, 1985).

[y] Disease index (DI) was calculated as follows : $DI = \Sigma(dr \times n)/N$ where dr= Individual disease rating, n = number of diseased plants, N= number of total plants examined.

Table 2. Disease index, germination speed and percent germination of different rootstocks.

Rootstock	Spring 2007			Fall 2007		
	Disease index	Germination		Disease index	Germination	
		speed[z] (%)	percentage[y] (%)		speed (%)	percentage (%)
963385-231×FR-271	1.5[x]	64	94	1.8	68	96
963385-231×C60	3.2	63	98	3.5	60	94
963383×963385-233	2.0	70	92	2.2	68	94
963385-25×PFR11-6N	1.8	74	96	1.6	70	94
PFR11-6N×FR-271	1.7	68	94	2.0	73	96
963390×DJ-41	2.5	70	88	2.4	66	82
DJ-41×FR-22	1.6	73	92	1.9	78	94
FP1×963383	2.0	60	96	2.0	66	100
FR79-3×FRD-210	1.6	68	92	1.9	64	90
FR-210×963385-233	1.7	90	92	2.0	84	88
FR-210×963385-25	1.8	90	98	1.7	88	94
FR-22×963381-42	1.6	88	98	1.9	93	96
Sambokkul (own root)	-	96	100	-	93	98
FR Dantos	2.8	68	88	3.2	76	84
Bullojangsaeng	4.6	74	84	4.0	76	84

[z] Germination speed after sowing 7 days

[y] Germination percentage after sowing 21 days

[x] See footnotes in Table 1.

Table 3. Survival rate, yield and fruit quality of watermelon grafted onto different rootstocks in spring 2007.

Rootstock	Survival rate (%)	Total yield ($t \cdot ha^{-1}$)	Market-able yield (%)	Fruit weight ($g \cdot ea^{-1}$)	Rind thickness (mm)	Soluble solid contents (°Bx)
963385-231×FR-271	98	34.4 e[z]	100	4,643 e	10.0[ns]	10.8 a
963385-231×C60	100	34.5 e	95	4,662 e	11.0	10.8 a
963383×963385-233	100	49.8 ab	100	6,717 ab	11.3	10.1 bc
963385-25×PFR11-6N	100	41.1 c-e	100	5,543 c-e	11.3	10.3 bc
PFR11-6N×FR-271	98	33.7 e	86	4,545 e	9.8	10.4 ab
963390×DJ-41	95	52.0 a	100	7,014 a	11.1	10.3 bc
DJ-41×FR-22	100	39.3 de	95	5,312 de	11.2	10.1 bc
FP1×963383	98	48.1 a-c	100	6,498 a-c	11.0	9.9 bc
FR79-3×FRD-210	100	40.3 de	91	5,445 de	11.0	10.0 bc
FR-210×963385-233	100	50.7 a	90	6,848 a	11.6	9.9 c
FR-210×963385-25	98	42.6 b-d	100	5,748 b-d	11.9	10.4 ab
FR-22×963381-42	100	48.1 a-c	100	6,493 a-c	10.7	10.1 bc
Sambokkul (own root)	-	26.2 f	76	3,533 f	10.1	10.4 ab
FR Dantos	95	37.1 de	100	5,014 de	11.6	9.9 bc
Bullojangsaeng	100	49.7 ab	95	6,714 ab	11.8	10.1 bc

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

Table 4. Survival rate, yield and fruit quality of watermelon grafted onto different rootstocks in fall 2007.

Rootstock	Survival rate (%)	Total yield ($t \cdot ha^{-1}$)	Market-able yield (%)	Fruit weight ($g \cdot ea^{-1}$)	Rind thickness (mm)	Soluble solid contents (°Bx)
963385-231×FR-271	100	21.4 e[z]	100	2,893 e	7.8[ns]	10.9 a-c
963385-231×C60	100	26.7 b-d	100	3,612 b-d	8.3	11.0 a-c
963383×963385-233	93	25.8 cd	100	3,488 cd	9.0	10.7 b-d
963385-25×PFR11-6N	100	24.8 d	91	3,343 d	8.6	11.4 a
PFR11-6N×FR-271	95	25.4 d	91	3,431 d	8.7	10.7 b-d
963390×DJ-41	100	30.8 a	100	4,162 a	9.5	10.3 de
DJ-41×FR-22	98	23.4 de	95	3,162 de	8.3	10.8 b-d
FP1×963383	100	21.1 e	86	2,855 e	7.8	11.2 ab
FR79-3×FRD-210	93	20.5 e	91	2.767 e	8.1	10.9 a-c
FR-210×963385-233	100	31.0 a	95	4,183 a	8.5	10.2 e
FR-210×963385-25	100	29.1 a-c	100	3,929 a-c	8.5	10.6 c-e
FR-22×963381-42	100	29.8 ab	86	4,019 ab	9.2	10.5 c-e
Sambokkul (own root)	-	16.4 f	67	2,210 f	8.6	10.7 b-d
FR Dantos	100	26.6 b-d	91	3,593 b-d	8.5	10.3 de
Bullojangsaeng	98	26.0 cd	100	3,510 cd	8.2	10.7 b-d

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

Undulate leaf blade margin of patty pan (*Cucurbita pepo* var. *patisonina* Greb.) new spontaneous mutation

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Abstract

Quite recently, a new spontaneous mutation, denominated undulate leaf blade margin of patty pan (*Cucurbita pepo* var. *patisonina* Greb.), has been detected in the breeding material derived from the gene bank (accession EKAN-2). The plants were identified in the experimental field of Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences, Poland. The characteristic feature of the plants was their leaf blade with undulate margin, which was found in all the leaves of the mutant. The plants with undulate leaf blade margin were somewhat smaller than the typical ones. Flower buds, both male and female, were typical. In male flowers viable and fertile pollen grains were observed. After mutant female flower pollination ovaries developed and then, 5-7 days later, degenerated. The typical plants (heterozygous) self pollinated, developed fruits and seeds. In the next generation progeny segregated into typical and mutant plants in approximate ratio 3:1.

On the basis of genetic analysis it can be suggested that the trait is determined by one recessive semilethal gene. The gene was designated *ul* - undulate leaf.

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Stability of fruit yield in watermelon genotypes tested in multiple US environments

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Abstract

Watermelon (*Citrullus lanatus* [Thumb.] Matsum & Nakai) is a valuable crop grown throughout southern and western United States (US). We were interested in identifying watermelon cultivars having high stability for fruit yield and yield components over diverse environments in the US. A set of 40 genotypes was tested over 3 years (2009, 2010, and 2011) at 8 locations across the southern US in replicated, multi-harvest trials. Yield traits were summed over harvests, and measured as marketable yield, fruit count, % cull fruit, % early fruit, and fruit size. The influences of years and locations on yield performance and genotypes with high stability for yield were identified. Four genotypes including, 'Fiesta F1', 'Stars-N-Stripes F1', 'Stone Mountain' and 'Calhoun Gray' had a high trait mean performance and high phenotypic stability. These four stable genotypes had high marketable yield, average fruit count, low % cull fruit, above average early yield, and medium fruit size. Inbreds 'Big Crimson' and 'Legacy' would be good lines for breeding for high yield and stability.

INTRODUCTION

During the past century, watermelon cultivars have been developed with

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high fruit yield and quality (Gusmini and Wehner 2008). However, high yield is often associated with decreased yield stability (Padi 2007). Yield stability is important, but has not been studied in watermelon. By growing genotypes in different environments, the highest yielding and most stable genotypes can be identified (Lu'quez et al. 2002).

Genotypes tested in different locations or years often have significant fluctuation in yield due to the response of genotypes to environmental factors such as soil fertility or the presence of disease pathogens (Kang 2004). These fluctuations are often referred as genotype x environment interaction (GEI). GEI affects breeding progress because it complicates the demonstration of superiority of any genotype across environments and the selection of superior genotypes. Therefore, the magnitude and nature of GEI determine the features of a selection and testing program.

Several statistical methods have been proposed for the analysis of yield stability including linear regression coefficient (b_i), mean square deviation from regression (S^2_d), Shukla's stability statistic (σ_i^2) and Kang's stability statistic (YS_i) (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Kang 1993). Since no single method adequately explains genotype performance across environments. Therefore Stability statistics (variation) are not informative and useful in selection unless they are combined with performance (mean).

The objectives of this study were to (i) evaluate the influence of years and locations on yield of watermelon genotypes, and (ii) identify genotypes with high stability for yield.

MATERIALS AND METHODS

Data analyzed in this study were obtained from set of watermelon yield trails conducted for 3 years (2009, 2010, and 2011). Each year, 40 genotypes were evaluated at 8 locations across the United States. The genotypes were chosen to represent key watermelon genotypes that were used in breeding purpose in last one century and the locations were chosen to represent the key watermelon production regions in the US. Locations were Kinston NC, Clinton NC, Charleston SC, Cordele GA, Quincy FL, College Station TX, Lane OK and Woodland CA. At each location, a randomized complete block design with four replications was used. Watermelon seedlings were transplanted at the two-true-leaf stage on raised beds (rows).

The 40 watermelon genotypes were evaluated for marketable yield (Mg ha^{-1}), marketable fruit number or count (thousand fruit ha^{-1}), % cull fruit (100 x cull fruit weight / total fruit weight), % early fruit (100 x first harvest of marketable yield / total harvest weight) and fruit size (kg $fruit^{-1}$). Data were not collected on % cull fruit from Charleston SC in 2009, 2010, and 2011; and Quincy FL in 2009 and 2010. Data were analyzed using the SAS statistical package (SAS Institute, Cary, NC).

The stability parameters used were b_i, S^2_d, and σ_i^2. Least squared means or adjusted trait means (*M*) and their least significant difference (LSD) for each genotype were computed over the 24 environments for the traits of interest. Additionally, the Kang's yield-stability statistic (YS_i) was computed for simultaneous selection of high mean and high stability for yield and yield components. Tests for significance were derived using a t-test for each b_i and an *F* test for each S^2_d for statistical differences from unity and zero, respectively, at 0.05, 0.01 and 0.001 levels of probability

RESULTS AND DISCUSSIONS

The combined analysis of variance (ANOVA) indicated highly significant environment, genotype and genotype x environment interaction effects for all traits evaluated. These results suggested that yield and yield components were influenced by genotype x environment interaction and, thus, required separate stability analysis for each trait.

Marketable yield ranged from 80.44 to 27.43 Mg ha^{-1}. Highest marketable yield was for 'Big Crimson', but 'Stone Mountain', 'Stars-N-Stripes F1' and 'Starbrite F1' were not significantly different (Table 1). Other high yielding genotypes were 'Fiesta F1', 'Regency F1', 'Calhoun Gray', 'Legacy' and 'Mountain Hoosier'. Fruit count ranged from 3.94 to 15.59 thousand fruit ha^{-1} (Table 1). Highest fruit count was for 'Golden Midget', followed by 'Minilee' and 'King & Queen'. Genotypes with high marketable yield had intermediate fruit count, confirming the results of Gusmini and Wehner (2005). Large fruit size was correlated with high % cull fruit (Tables 1). 'NC Giant' and 'Congo' had large fruit and 'Golden Midget', 'Minilee', and 'Mickylee' had the smallest fruit size. 'Golden Midget', 'Early Canada', 'Stone Mountain', and 'Regency F1' had the highest % early fruit (Table 1).

The genotypes that recorded significantly high marketable yield in this study included four hybrids and four inbreds: 'Starbrite F1'; 'Stars-N-Stripes F1'; 'Fiesta F1'; and 'Regency F1', and 'Big Crimson'; 'Stone Mountain'; 'Calhoun Gray'; and 'Legacy', respectively. According to the Eberhart and Russell (1966), a regression coefficient (b_i) approximating unity along with a deviation from regression (S^2_d) near zero indicates stability. When this is associated with high mean yield, genotypes have general adaptability and when associated with low mean yield, genotypes are poorly adapted to all environments. Based on this definition, the best genotypes were hybrids 'Fiesta F1', 'Stars-N-Stripes F1', and 'Regency F1'; and inbreds 'Calhoun Gray' and 'Stone Mountain'. They had high marketable yield, b_i close to unity, non-significant S^2_d, and low σ_i^2; hence, they had high adaptability across wide range of environments (Table 1).

Similarly, according to Eberhart and Russell (1966) model high yielding genotypes 'Stars-N-Stripes F1', 'Regency F1', 'Calhoun Gray', and 'Stone Mountain' were desirable for fruit count (Table 1). For % cull fruit and % early fruit

genotypes ‘Stars-N-Stripes F1’ and ‘Regency F1’, and ‘Fiesta F1’ and ‘Starbrite F1’ were stable (Table 1). However, for fruit size all top eight high yielding genotypes were stable and desirable, except ‘Calhoun Gray’ (Table 1). The strong correlation between Kang’s stability statistics (YS_i) analysis used both mean (*M*) indicated that suggested these statistics measure the same aspect of stability and provided same information.

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Table 1. Means (*M*) and stability parameters (regression coefficient [b_i], deviation from regression [S^2_d] and Shukla's stability variance [σ_i^2]) for top 8 high yielder genotypes out of 40 watermelon genotypes tested in 3 years and 8 locations.

	Marketable yield (Mg ha^{-1})			
Genotype	*M*‡	b_i,	S^2_d	σ_i^2
Big Crimson	80.44	1.81*	1221.68**	1147.63
Calhoun Gray	69.36	1.06	357.14	328.05
Fiesta F1	71.25	1.33	656.44	616.62
Legacy	68.28	1.09	1406.18***	1351.33
Regency F1	70.13	0.56	614.90	566.88
Starbrite F1	80.40	2.21**	1365.50**	1221.86
Stars-N-Stripes F1	77.25	1.13	432.06	416.09
Stone Mountain	79.10	1.57	497.48	523.36
	Fruit count (thousand fruit ha^{-1})			
Big Crimson	9.21	1.24	16.01**	15.06
Calhoun Gray	8.32	1.19	4.94	4.12
Fiesta F1	7.62	0.99	16.30***	15.66
Legacy	8.36	0.31	7.14	6.92
Regency F1	9.10	1.79*	8.51	6.54
Starbrite F1	9.18	1.04	5.75	5.39
Stars-N-Stripes F1	9.06	1.13	8.14	7.17
Stone Mountain	9.21	1.24	16.01**	15.06
	% cull fruit			
Big Crimson	6.46	1.48	245.23***	194.91
Calhoun Gray	11.28	2.21*	221.95*	156.39
Fiesta F1	6.93	0.08*	130.83*	74.58
Legacy	6.27	-0.36**	84.43	37.23
Regency F1	5.26	0.43	62.42	48.18
Starbrite F1	5.82	0.11*	58.94	39.42
Stars-N-Stripes F1	6.28	0.66	45.43	44.41
Stone Mountain	7.07	2.79**	334.06**	154.14
	% early fruit			
Genotype	*M*‡	b_i,	S^2_d	σ_i^2
Big Crimson	23.24	0.85	348.66*	282.65
Calhoun Gray	35.58	1.37	615.01**	472.83
Fiesta F1	29.76	0.82	410.59	303.73
Legacy	35.80	0.63	1071.41***	804.17
Regency F1	39.10	1.42	573.47*	444.39
Starbrite F1	37.43	0.92	324.72	246.83
Stars-N-Stripes F1	35.16	0.37	700.04***	434.81
Stone Mountain	39.66	1.04	1030.07**	787.81
	Fruit size (kg fruit^{-1})			
Big Crimson	8.60	1.05	2.41	2.42
Calhoun Gray	8.31	0.69	3.08**	3.31
Fiesta F1	7.62	0.94	2.09	2.11
Legacy	8.81	1.19	3.21	3.77
Regency F1	8.09	0.77	1.94	2.09
Starbrite F1	8.54	1.13	2.83	3.15
Stars-N-Stripes F1	8.30	1.00	1.11	1.02
Stone Mountain	8.52	1.11	2.93	4.03

*, **, *** indicate significantly different from unity for the regression coefficients or slope (b_i) and from zero for the deviation from regression (S^2_d) at 0.05, 0.01 and 0.001 levels of probability, respectively

‡LSD = 7.56, 1.07, and 4.27 for marketable yield, fruit count, and %cull fruits, respectively.

‡LSD = 6.45 and 0.55 for % early fruit and fruit size, respectively.

Occurrence of androgynous inflorescence in *Luffa cylindrica* exhibiting dominant monogenic inheritance

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Keywords: Gene A^{dgn}, androgyn-4, 'androgynous monoecious', advancing speciation

Abstract

The normal genotypes of sponge gourd (*Luffa cylindrica*) are 'typical monoecious' in sex form. The vine nodes in flowering stage of these genotypes have genetic potential to possess seven morphological organs viz., a leaf, a bract, a tendril, a true branch, a very occasional adventitious root, an axillary solitary pistillate flower, and an androecious inflorescence. Basically monoecious in nature, a variant land race Androgyn-K, having androgynous inflorescence, was collected from Kushinagar district of Uttar Pradesh in India. Androgyn-K had only 'four organs node potential' viz., a leaf, a tendril, a very occasional adventitious root, and an androecious or an androgynous inflorescence. The rest three organs found in the vine nodes of normal genotypes were remarkably absent. In unusual manner a few of androecious and androgynous racemes of Androgyn-K, got converted into long length branches. Androgyn-K was purified into three distinct lines viz., Androgyn-1, Androgyn-4 and Androgyn-7, having androgynous inflorescence. These lines endowed with androgynous inflorescence were also monoecious and, therefore, called as 'androgynous monoecious'. Homozygous 'androgynous monoecious' line Androgyn-4, with 'four organs node potential' was crossed with another homozygous 'typical monoecious' genotype NDSG-5, having 'seven organs node potential'. All the F_1 plants had 'androgynous inflorescence' with 'four organs node potential', indicating dominance of 'androgynous monoecious' with four organs node potential over 'typical monoecious' trait with 'seven organs node potential'. A gene symbol 'A^{dgn}' is proposed for the mutant gene determining androgynous inflorescence in the 'androgynous monoecious' lines, and the counterpart allele 'a^{dgn}' for expression of 'typical monoecious' genotypes. The F_2 and BC_1 segregation data very vividly revealed monogenic dominance of allele A^{dgn} over that of normal allele a^{dgn}. The four

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organs node potential associated with androgynous inflorescence inherited together with the latter trait i.e., androgynous inflorescence. It is concluded that the gene A^{dgn} has pleiotropic effect on the expression of morphological organs in the node and several other unusual behaviour. Distinct variations in androgynous monoecious lines Androgyn-1, Androgyn-4 and Androgyn-7 suggested that the expression of the gene A^{dgn} is influenced by background minor genes. Occurrence of androgynous inflorescence is a direct evidence of advancing speciation in *L. cylindrica*.

INTRODUCTION

Sponge gourd [*Luffa cylindrica* (L) Roem.] is reported to be strictly monoecious in nature (Singh 1957). A species of diverse use and considerable commercial value, sponge gourd exhibits great variability in its important economic attributes (Porterfield 1955; Whitaker and Davis 1962; Robinson and Decker-Walter 1999; Seshadri and More 2009). In flowering stage, individual vine nodes of common genotypes of *L. cylindrica* have genetic potential to possess five vegetative morphological organs, *viz.*, a leaf, a tendril, a bract, a branch, and a very occasional adventitious/arial root; and two reproductive organs viz., a solitary axillary pistillate flower, and a raceme inflorescence of staminate flowers (Fig. 1a). Thus, the normal 'typical monoecious' genotypes of sponge gourd are characterized by a total of 'seven organs node potential' in the flowering nodes. Three vegetative organs *viz*., leaf, tendril, and bract are the constant feature of every node in both vegetative and reproductive phases (Fig. 1a and 1e) and they may be called as obligatory organs of a vine node. Presence and absence of the remaining four organs i.e., branch, adventitious root, axillary solitary pistillate flower, and androecious raceme, may vary from node to node, and the expression, or development of these organs to their effective growth potential is decided by a combined factor of the growth condition, organ load in the preceding node, and the natural wisdom of the growing vine (Fig. 1f, g, h and i). Therefore, these four organs may be considered as subsidiary organs of the vine nodes.

In January 2008, an unique 'variant' land race of *L. cylindrica* was located in Kushinagar district of Uttar Pradesh (India) endowed with peculiar androgynous inflorescence having both staminate and pistillate flowers in the same raceme (Fig. 2a, 3a and 4b). The variant also had androecious racemes, as found in normal 'typical monoecious' genotypes (Fig. 2a, 3a and 4a). The bracts, 'true branches' and solitary axillary pistillate flowers directly originating from the nodes were conspicuously absent from every flowering vine node (Fig. 1b). Lesser number of branches were present in the variant but they were not true branches, in the sense that they were formed due to unusual conversions of a low frequency of androecious and androgynous racemes, after initial growth as inflorescence originating from the vine nodes (Fig. 2b, 2c, 3b and 3c). The variant was basically monoecious in

nature. It differed from the typical monoecious genotypes only for the position of its pistillate flowers, which were present in the androgynous racemes instead of their natural position as solitary flowers in the axils of leaves. In contrast to the 'seven organs node potential' of typical monoecious genotypes (Fig. 1a), this peculiar genotype in true sense had only 'four organs node potential' viz., a leaf, a tendril, a very occasional adventitious root, and an androecious or androgynous inflorescence (Fig. 1b). To differentiate from the 'typical monoecious' genotypes, the variant was termed as 'androgynous monoecious'. The seeds were collected and the accession was named as Androgyn-K (K for Kushinagar district, the initial source of accession). Androgyn-K was purified through inbreeding and selection in the offing years. A distinctly stable homozygous 'androgynous monoecious' line Androgyn-4 (Fig. 3a), with 'four organs node potential', was crossed with a normal genotype NDSG-5 (Fig. 1a), homozygous for 'typical monoecious' nature and 'seven organs node potential' with an objective to study the inheritance patterns of 'androgynous monoecious' vs 'typical monoecious' traits along with other closely associated node organs.

MATERIALS AND METHODS

The experiments were conducted at Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad (Uttar Pradesh) India, which has humid, sub-tropical climate. In this region sponge gourd is generally planted from mid January to mid August. January to April sown spring-summer crop faces long days, where photo-insensitive genotypes of sponge gourd have no problem of flowering and fruiting, but photo- sensitive genotypes do not bear productive flowers till late in August, when the days become relatively shorter. However, for July sown crop photoperiod is not a problem and both photo-sensitive and insensitive genotypes bear profuse staminate and pistillate flowers within 40-50 days after sowing. Mid March, 2008 sown crop of Androgyn-K seemed to be photo-sensitive and hence, in the meantime, the remnant 25 seeds of the genotype were sown in late July 2008. Out of 25 seeds sown, 22 plants grew well, which bore staminate and pistillate flowers early in the season in about 46 to 55 days. Interestingly, this small population of 22 plants segregated into 16 'androgynous monoecious' with androgynous inflorescence and 6 typical monoecious, which led to the speculation of heterozygous nature of the source plant of Androgyn-K. The collected seeds appeared to be the produce of chance selfing on the heterozygous plant. The segregation pattern indicated dominance of 'androgynous monoecious' trait over 'typical monoecious' trait. All the androgynous monoecious plants were selfed, but at maturity fruits were harvested only from 10 vigorous plants with relatively good fruit shape and yield. In July, 2009, 40 seeds each from the 10 selected androgynous plants were sown in plant to row method for further purification and selection.

Critical observations were made on 'four organs node potential' and 'seven organs node potential' of 'androgynous monoecious' and 'typical monoecious' forms, respectively. Out of 10 lines 3 non-segregating stable lines were selected, exhibiting various proportions of pistillate and staminate flowers combination in individual androgynous inflorescence. These selected lines were named as Androgyn-1, Androgyn-4, and Androgyn-7. The most stable line Androgyn-4, homozygous for 'androgynous monoecious' nature was crossed with another photo-sensitive genotype NDSG-5, homozygous for 'typical monoecious' nature. In July, 2010, 10 F_1 plants of the cross Androgyn-4 x NDSG-5, were raised along with ten plants of each of the parents. All the F_1 plants were 'androgynous monoecious' exhibiting dominance of this trait over 'typical monoecious' trait. Five F_1 plants were selfed and the remaining five were backcrossed by the recessive parent NDSG-5 to procure F_2 and BC_1 seeds, respectively. In July, 2011, 310 F_2 seeds from one F_1 plant and 310 BC_1 seeds also from one plant were sown to grow F_2 and BC_1 generations. Twenty seeds each of the parents viz., Androgyn-4 and NDSG-5 were sown to raise parental lines for a comparison of segregating populations. In F_2 and BC_1 populations critical observations were made on presence and absence of bracts in the vine node, presence and absence of true branches directly origination from vine nodes, presence and absence of solitary axillary pistillate flowers, and presence and absence of androgynous inflorescence. Chi-square test was used to test the goodness of fit of F_2 and BC_1 segregation data.

RESULTS AND DISCUSSION

The salient features of the results recorded in the study are discussed under the following sub-heads:

Glandular bracts- the obligatory node organ of typical monoecious *L. cylindrica*

Bracts are specialized leaves from the axil of which single or group of flowers arise. In normal genotypes of *L. cylindrica*, bracts are obligatory organ in the vine nodes and seem to be well developed, in the axil of which androecious racemes arise (Fig. 1a and 1c). In androgynous monoecious genotypes the bracts were remarkably absent from vine nodes (Fig. 1b). The absence of bracts was so stable that this trait was used as marker for identification of androgynous plants before flowering. However, the bracts were regular feature in the inflorescence of both typical monoecious (Fig. 1d) and androgynous monoecious genotypes (Fig. 3a, 4a, 4b and 4c). The outer surface of the bracts is glandular both in vine nodes (Fig. 1c) and in inflorescence, which is visited by ants (Fig. 1d). From the axils of bracts present in the inflorescence either pistillate or staminate flowers arise. Bracts are to play diverse role in several plant species. If the bracts in the vine nodes of *L. cylindrica* have certain vital role to play then absence of bracts in the androgynous lines is a weakness of the genotypes, and if they are superfluous in the vine nodes

in normal genotypes then androgynous genotypes seem to be at higher ladder of speciation, which requires further investigation.

Evolutionary significance of androgynous inflorescence

The phylogenetic trends suggest that presence of an inflorescence in a plant species is an advance trait, whereas solitary flower is considered primitive in nature (Hutchinson 1969). Incidentally, within the family Cucurbitaceae androgynous racemes are often found in genera *Sicyos* and *Sicyosperma* of the tribe Sicyoideae, which is an advanced tribe of family Cucurbitaceae, compared to the tribe Benincaseae to which *L. cylindrica* belongs (Hutchinson 1967). Occurrence of androgynous inflorescence in the variant genotype Androgyn-K of *L. cylindrica*, otherwise bearing axillary solitary pistillate flowers and androecious racemes, seems to be a result of dominant spontaneous mutation. The occurrence of androgynous inflorescence and absence of solitary pistillate flower is an instant evidence of how complex forms of traits evolve in nature from simpler forms in the process of speciation. It has to be watched as to how this dominant mutant becomes helpful in the advancing speciation of *L. cylindrica*, which is ridden with numerous weaknesses as far as its performance from breeder's point of view is concerned.

Significance of conversion of androecious and androgynous inflorescence into branches

In botanical terms, inflorescence is considered modified branch. Conversion of androecious and androgynous racemes into long branches in Androgyn-1 and Androgyn-4 is a direct evidence of the above fact. It was observed that while the underneath physiological process was on for conversion of an inflorescence into a branch the bracts present in peduncle became larger and finally got converted into true leaves, from the axils of which androecious (Fig. 2c) and androgynous racemes arose (Fig. 3b). As soon as the raceme got converted into a real branch, there was a remarkable absence of the bract in the branch node as found in main vines of these genotypes. This observation of conversion of bracts into leaves has an evolutionary significance from botanical point of view.

Characterization of isogenic lines Androgyn-1, Androgyn-4 and Androgyn-7

In all the three distinct androgynous lines, Androgyn-1, Androgyn-4, and Androgyn-7, bracts and true branches were conspicuously absent from the vine nodes at all stages of plant growth. However, bracts were present in androecious and androgynous racemes of these lines (Fig. 2, 3 and 4). At flowering stage, all the three lines were characterized by consistent absence of solitary axillary pistillate flowers. The three androgynous lines differed in their behaviour of expression of androecious, androgynous and gynoecious racemes as well as conversion of

androecious and androgynous racemes into long branches. Androecious racemes more frequently converted into branches (Fig. 2b, 3b and 3c) than androgynous racemes (Fig. 2c). Fruits were sessile and dissimilar in shape and size. All the three androgynous lines produced quite low yield with poor marketable quality (Fig. 4d). Androgynous lines exhibited several other abnormalities in expression of different plant organs, particularly in floral structures. The gene responsible for androgynous inflorescence with its pleiotropic effect, in turn appears to be highly influenced by modifier genes resulting in distinct androgynous lines. One such abnormality occurred in Androgyn-1 where a pistillate flower was recorded in the axil of bifurcation point of a tendril (Fig. 2d). Tendrils in cucurbits have been studied for their morphological and anatomical details and views differ as far as their origin is concerned (Sensarma 1956; Robinson and Decker-Walters 1999). Abnormalities like the presence of pistillate flowers in the tendrils may throw a little light on the subject, at least in *L. cylindrica*.

Androgyn-1 bore only one pistillate flower in its androgynous raceme, which was constantly at first position of the raceme (Fig. 2a). There was a lower tendency of conversion of a few androecious and androgynous racemes into real long length branches in moderate temperature conditions of September and October (Fig. 2b and 2c). In androgyn-4, androecious racemes were lesser in frequency than androgynous racemes. In androgynous racemes this genotype had 2-7 pistillate flowers, generally placed at initial position of the raceme (Fig. 3a). Androgyn-4 had moderately higher frequency of conversion of androecious and androgynous racemes into real long length branches as compared to Androgyn-1. Androgyn-7 bore only a limited number of androecious racemes that too only in the early stages of flowering (Fig. 4a). With the advancing moderate cool weather of October and November, there were only androgynous racemes (Fig. 4b). This line generally bore 7 to 10 pistillate flowers in androgynous racemes. In lower temperatures of December and January, Androgyn-7 bore only gynoecious racemes (Fig. 4c), which bore 15-20 pistillate flowers. The plants of Androgyn-7 did not bear even a single branch because none of its racemes converted into branch. Hence, it had very limited amount of foliage and its yield was lowest, even though it produced highest frequencies of pistillate flowers.

Inheritance of 'androgynous monoecious' vs 'typical monoecious' sex forms

All the 25 F_1 plants between Androgyn-4 and NDSG-5 were strictly androgynous monoecious having androgynous inflorescence, which revealed complete dominance of 'four organs node potential' of vine nodes associated with 'androgynous monoecious' trait over 'seven organs node potential' of 'typical monoecious' nature. A gene symbol A^{dgn} is proposed for the gene determining 'androgynous inflorescence' trait, which appears to be the result of spontaneous

dominant gene mutation. The counterpart a^{dgn} is proposed for the normal recessive 'typical monoecious' trait. The F_2 population of 289 surviving plants segregated in accordance with expected ratio of 3 androgynous monoecious (A^{dgn} -) : 1 typical monoecious (a^{dgn}, a^{dgn}) forms, whereas, BC_1 generation consisting of 271 plants, segregated in close agreement to the expected ratio of 1 androgynous monoecious (A^{dgn} -) : 1 typical monoecious (a^{dgn}, a^{dgn}) (Tab. 1) revealing, thereby, monogenic dominance of 'androgynous monoecious' form bearing androgynous inflorescence over 'typical monoecious' trait. The segregating F_2 and BC_1 plants with androgynous inflorescence were not exactly similar in behaviour like Androgyn-4. They bore varying proportions of pistillate flowers in the androgynous racemes indicating, therefore, role of modifier genes on the expression of this major gene 'A^{dgn}'.

Close observation on individual segregating F_2 and BC_1 plants revealed that all the associated traits with androgynous inflorescence viz., presence of four nodal organs and absence of the rest three, were found to be tightly linked and all of them inherited together. The above observation, therefore, suggested that either gene A^{dgn} has pleiotropic effect on the expression of associated traits or the genes determining the associated traits are tightly linked with the gene A^{dgn}. Out of the above two speculations pleiotropic effect of gene A^{dgn} seems more likely. The androgynous monoecious lines exhibited several other abnormal minor deviations in its traits, particularly during flowering period, which are of evolutionary significant and require in-depth botanical study. It is concluded that the novel appearance of androgynous inflorescence is the result of spontaneous monogenic dominant mutation with pleiotropic effects, the expression of which in turn is influenced by background minor genes. Occurrence of androgynous inflorescence along with other closely linked remarkable traits is a direct evidence of advancing speciation in *L. cylindrica*.

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Table 1. Segregation patterns of ‘androgynous monoecious’ vs ‘typical monoecious’ genotypes in F2 and BC1 generations

Generation name/total plants	androgynous monoecious plants	typical monoecious plants	Observed ratio	Chi-square	probability
F_2/289	210	79	2.66:1	0.84	35.9%
BC_1/271	130	141	0.92:1	0.44	50.4%

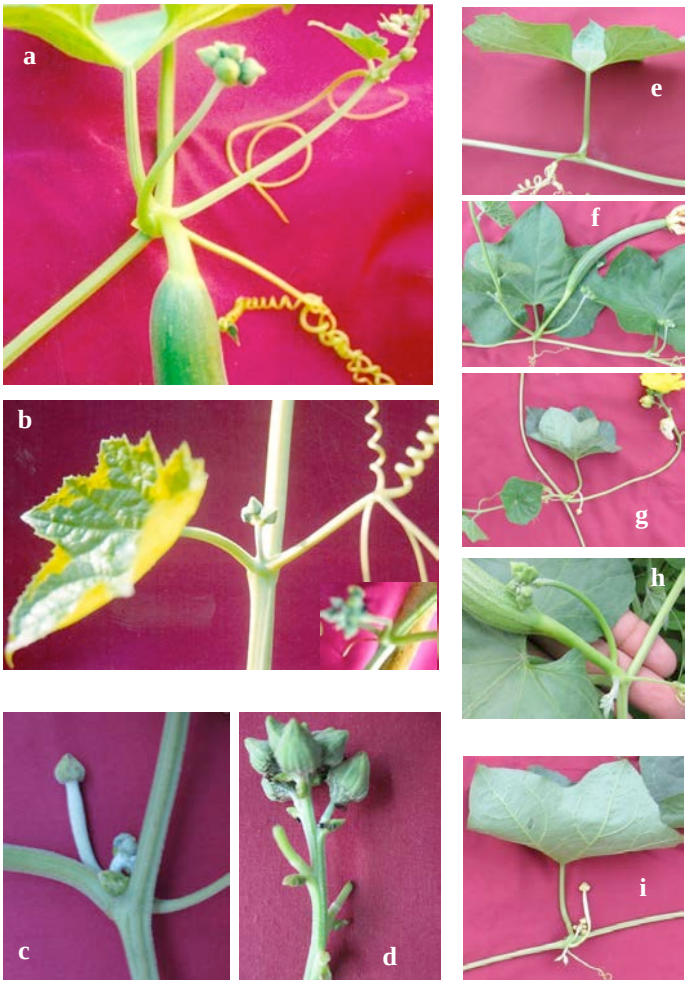

Fig. 1. Features of vine nodes: a, ‘seven organs feature’ in vine node of ‘typical monoecious’ genotype; b, ‘four organs feature’ in ‘androgynous monoecious’ genotype; c, a magnified picture of glandular nodal bract; d, bracts in androecious raceme visited by ants; e, three obligatory organs in vegetative node of a typical monoecious genotype; and f, g, h, i, reproductive nodes of ‘typical monoecious’ genotype in various combination of developed, underdeveloped and withering stages of various subsidiary vine nodal organs.

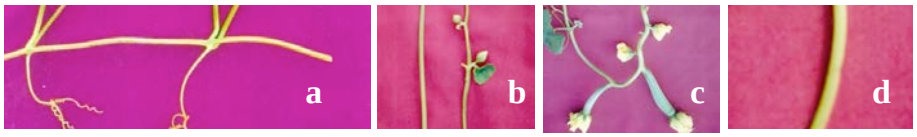

Fig. 2. Types of racemes in Androgyn -1 and an abnormal tendril: a, a branch bearing an androecious and an androgynous raceme; b, androecious racemes converting into branch; c, androgynous raceme converting into branch; and d, an abnormal tendril

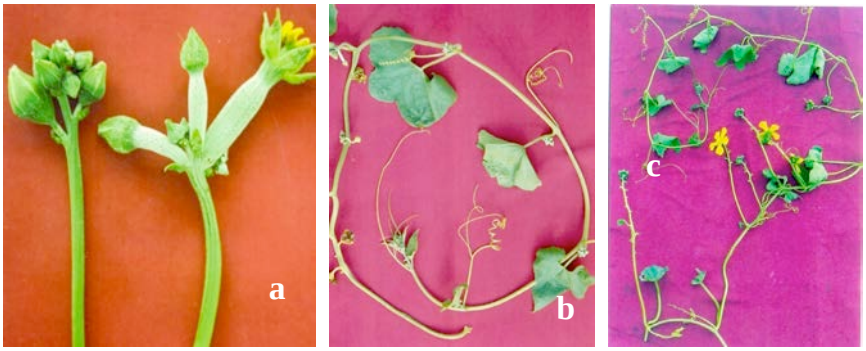

Fig. 3. Types of racemes in Androgyn-4: a, an androecious and an androgynous raceme; b, an androecious raceme converting into branch; c, a node with androecious raceme and another node with androecious raceme converting into branch

Fig. 4. Types of racemes in Androgyn-7 and deshaped fruit: a, an androecious raceme; b, androgynous raceme; c, a gynoecious raceme; and d, sessile fruits in dissimilar shape and size

Determination and analysis of drought resistance index for watermelon during germination stage

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Keyword: *Citrullus lanatus*, seed germination, germination drought resistance index, relative germination energy, injury rate

Abstract

To determine an efficient method and provide proper indexs for evaluating drought resistance of watermelon at the early germination stage, 5 watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) cultivars of different ecotypes were conducted seed germination test under drought conditions simulated by sucrose within different concentrations. Three indices including germination drought resistance index (GDRI), relative germination potential (RGP) and injuring rate (IR) were examined. Genotypic variability was found among the cultivars for all the parameters analyzed in the test. Results showed that the best concentration to the evaluation was observed at $50g \cdot kg^{-1}$ of sucrose. Seed germination was inhibited by sucrose, the higher the concentration of sucrose, the better effect of the inhibition; while the higher germination drought resistance index of the variety, the less suffering of the inhibition. It was also observed that with the decrease of GDRI of the stressed watermelon, the RGE of it decreased as well, indicating a significant relationship between them.

INTRODUCTION

Drought is the major abiotic stress having severe adverse effects on the normal growth of crop plants and the crop yield. Mining and identifying germplasms with potential drought tolerance properties is essential for developing drought-resistance breeding programs, thus to help to alleviate such an destructive effect and develop water-saving agriculture. Bouslama et. al (1984) reported that water stressed germination test simulated by PEG was able to identify drought tolerance genotypes. In his study, he firstly pointed the concept of germination drought resiatance index (GDRI), and considered it as an effective indicars to screen drought tolerance varieties. Many other previous researchs have also demostrated that drought stress

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treatment simulated by hypertonic solution was as reliable as by soil water stress (Jing et al. 1998; Xue et al. 1997; Zhang et al. 2007; Xu et al. 2003).

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a major crop in northwest China. Where with high altitude, sufficient sunshine, and a large temperature difference between day and night, makes high quality watermelon available. For each one year, the plant area of watermelon is as wide as 300 million mu and the output value is as high as 7 billion. However, due to the low rainful in this dryland region, drought is by far the main environment fator limiting local watermelon industry. Thus, identification of cultivars with drought tolerance properties is urgent for us to achieve high-quality and high-yield watermelon production in that drought occuring dry-land place.

The objectives of this study were to evaluate the discriminating ability of drought resitance in five watermelon cultivars and to provide reliable indexs to be used to preliminarily predict the drought tolerance of some other selected germplasms or cultivars at the early germination stage.

MATERIALS AND METHODS

Plant materials

Five watermelon cultivars including Xinong No.8, Hongguanlong, Shannong No.9, Nongkeda No.5 and Nongkeda No.6 were screened germination by osmotic stress. All the cultivars were newly bred cultivated varieties selected from watermelon breeding project at Northwest A&F University, and were the main planted cultivars at present in China.

Seed germination test

Drought stress was simulated using water solutions of sucrose at three levels (30g·kg^{-1}, 50g·kg^{-1} and 70g·kg-1), the control was distilled water. The experiment was conducted by a randomized complete block design with a total of 20 treatments and three replications. For each treatment, 25 seeds of the tested cultivars were placed on a 9-cm-diameter Petri dishe with three layers of filter paper moistened with 5 mL distilled water or the appropriate sucrose solution and incubated in an air-conditioned growth cabinet for 4 days at 30^{o}C. Germination was recorded when the radicle reached 2 mm in length. Counts of germinated seeds were made every day. At the end of 4th day, radicles were cut off and placed in oven at a starting temperature of 105^{o}C for 5min and an end temperature of 70 ^{o}C till they reaching at a constant weight. Germination drought resitance index (GDRI), promptness index (PI), relative germination potential (RGP) and injury rate (IR) were calculated as follows:

GDRI = promptness index in the treatment / promptness index in the control;

PI = $nd_1(1.00) + nd_2\ (0.75) + nd_3\ (0.50) + nd_4(0.25)$;

where: nd_x = number of seeds germinated by the xth day of observation.
RGP% = (germination potential in the treatment / germination potential in the control) ×100%
IR% = [(A－B)/A]×100%
where: A= treatment value, B= control value.

Data analysis

Data were analyzed using DPS for the analysis of variance and Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of hypertonic solution by sucrose on seed germination

Hypertonic solution creates high osmotic pressure to produce high water potentials. Thus, sucrose solutions, which could be easily used to create different moisture stress conditions within different concentrations, were suggested used in the laboratory germination experiment for screening drought tolerance cultivars. Under the moisture stress conditions, GDRI can well reflect seeds germination status (Ma 2002). The results (Table 1) showed that lower germination drought index in the treatment was correlated with lower relative germination potential, and the correlation coefficients (r) ranged from 0.9493, 0.9264 to 0.9934 under the treatment of sucrose at 30g·kg−1, 50g·kg−1, 70g·kg−1 respectively, with a very significant level ($P<0.01$). Sucrose stress inhibited seed germination, the higher the concentration of sucrose, the better effect of the inhibition, while the higher germination drought resistance index of the variety, the less suffering of the inhibition. With 30g·kg−1 Sucrose stress, GDRI values of the five tested cultivar ranged from 0.311 to 0.985, no significant difference observed among them. Similarily, with 70g·kg−1 Sucrose stress, there was also no significant difference of GDRI value in the five clutivars. While with $50g·kg^{-1}$ Sucrose stress, it was found that there were significant differences among the five with a wide range of GDRI value from 0.089 to 0.630. Based on results above, $50g·kg^{-1}$ of sucrose was selected to be the best concentration to the drought resistance evaluation since it gave the largest differential response in germination.

Cultivars Xinong No.8 and Nongkeda No.6 were the most tolerant with respective values of 0.630 and 0.505 for GDRI, 96.00% and 93.34% for RGP at the level of $50g·kg^{-1}$ sucrose. While Cultivars Nongkeda No.5 and Shannong No.9 were among the most succptible with respective values of 0.084 and 0.161 for GDRI, 28.00% and 61.43% for RGP at the level of $50g·kg^{-1}$ sucrose.

Effect of hypertonic solution on the growth of radicle

Radicle growth was affected by osmotic stress. Radicle dry weight injure rate reflected the inhibitory effects of osmotic stress (Table 2). High injure rate has

been associated with high inhibition of radicle growth. Under the concentration of sucrose at 30g·kg−1, IR of Xinong No.8 was as low as 3.7% while Hongguanlong, Nongkeda No.6, Nongkeda No.5 and Shannong No.9 reached 17.74%, 45.33%, 58.97% and 76% respectively. There was no coherence between this finding and the result determined by GDRI. When at $50g \cdot kg^{-1}$ of sucrose, the values of radicle dry weight IR varied from 23.08% for Xinong No.8 to 92.31% for Shannong No.9. This made a high coherence to the result determined by GDRI. While with $100g \cdot kg^{-1}$ of Sucrose stress, IR of Xinong No.8, Nongkeda No.5, Shannong No.9 were all as high as 100%. Thus, $50g \cdot kg^{-1}$ of Sucrose could be as a critical concentration to simulate water stress for measuring IR of tested cultivars as above in this study.

CONCLUSION

Five watermelon cultivars from Northwest A&F were screened for drought tolerance during germination stage, seeds of the tested cultivars were subjected to osmotic stresses at three levels induced by Sucrose. Germination drought resistance index, relative germination energy and injuring rate were examined in this evaluation test. Based on the correlation analysis of these three indicators, we selected 50g·kg−1 of sucrose as the best concentration for the drought tolerance evaluation in watermelon during germintion. The experiment has revealed genotypic differences in the response of the tested cultivars to the conditions of simulated drought. The cultivar Xinong No.8 had the highest GDRI value indicating the best adaptive capacity to drought stress. Followed by Hongguanlong and Nongkeda No.6, the cultivars Shannong No.9 and Nongkeda No.5 having the lowest GDRI were the most susceptible.

Drought resistance in crop is a complicated quantitative trait (Zhang 2007). Because internal aspects of seeds such as seed size and viability may affect the emergence of seedlings under stress conditions as well, germination may not be as reliable as growth in the seedling stage to predict cultivar performance (Kpoghomou et al. 1990), so furhter study under field conditions still needs to be continued.

ACKNOWLEDGEMENTS

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Table 1. Germination potential (GP) and germination drought resistance index (GDRI) of watermelon cultivars in sucrose solutions at 30 g·kg−1, 50 g·kg−1, 100 g·kg−1.

Cultivars	Concentration of sucrose solution ($g \cdot kg^{-1}$)	Relative germination potential (%)	Germination drought-resistant index
Xinong No.8	30	98.67 aA	0.895 aA
	50	96.00 cdABC	0.630 cdBCD
	70	52.00 deDE	0.150 ghHI
Hongguanlong	30	95.95 aA	0.685 bcABC
	50	93.34 aAB	0.505 deCDE
	70	18.67 fF	0.050 hI
Shannong No.9	30	78.57 abcABCD	0.311 fgFGH
	50	61.43 cdCD	0.161 ghHI
	70	20.00 fF	0.039 hI
Nongkeda No.5	30	76.00 abcdABCD	0.411 efEFG
	50	28.00 efEF	0.084 hHI
	70	13.33 fF	0.026 hI
Nongkeda No.6	30	96.00 aA	0.759 abAB
	50	92.13 aAB	0.441 deDEF
	70	65.33 bcdBCD	0.173 ghGHI

a-h Means within columns followed by the same letters do not differ significantly at 0.01 level of probability according to Duncan's multiple range test.

A-I Means within columns followed by the same letters do not differ significantly at 0.01 level of probability according to Duncan's multiple range test.

Table 2. Injury rate of radicle in watermelon cultivars under sucrose solution conditions

	30 g·kg^{-1} sucrose solution			**50 g·kg^{-1}** sucrose solution			**100 g·kg^{-1}** sucrose solution		
	Radicle weight(g)		Injury rate (%)	Radicle weight(g)		Injury rate (%)	Radicle weight(g)		Injury rate (%)
	CK	**T**		**CK**	**T**		**CK**	**T**	
	0.053	0.051	3.70 gF	0.053	0.040	23.08 fgEF	0.053	-	100.00 aA
n	0.062	0.051	17.74 fgEF	0.062	0.043	30.65 efgDEF	0.062	0.006	90.32 abAB
g	0.050	0.012	76.00 abcABC	0.050	0.007	86.00 abcAB	0.050	-	100.00 aA
a	0.039	0.016	58.97 cdeBCD	0.039	0.003	92.31 aAB	0.039	-	100.00 aA
a	0.075	0.041	45.33 defCDE	0.075	0.029	62.67 bcdBCD	0.075	0.008	89.33 abAB

CK is for the control, T is for treatment.

a-g Means within columns followed by the same letters do not differ significantly at 0.01 level of probability according to Duncan's multiple range test.

A-F Means within columns followed by the same letters do not differ significantly at 0.01 level of probability according to Duncan's multiple range test.

Dynamic characteristics of enzymes and transcriptome related to sugar metabolism and accumulation in sweet and non-sweet watermelon fruits

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Keywords: *Citrullus lanatus*, sugar accumulation, photosynthate assignment, enzyme activity, gene expression

Abstract

Sugar accumulation and content are major characteristics of watermelon fruit quality. The sweet watermelon inbred 97103 and the non-sweet watermelon accession PI296341-FR were used to study the enzymology and transcriptome dynamics of sugar metabolism and accumulation during watermelon fruit development. We found that differences of the sugar metabolism enzyme activities lead to the different photosynthate assignment. Acid α-galactosidase and insoluble invertase activities may play an important role in oligosaccharides hydrolyzation and sucrose accumulation in 97103. Soluble acid invertase may contribute to the decomposition from sucrose to glucose and fructose. The high activities of sucrose synthase and sucrose phosphate synthase are necessary for accumulating sugars in sweet watermelon fruits. The digital expression profiling of both flesh and mesocarp at four critical stages of fruit development of 97103 further confirmed these enzyme activities in transcriptome level. These results indicate that the corporate action of photosynthate phloem unloading, partitioning and metabolism determine the sugar content in sweet watermelon and non-sweet watermelon.

INTRODUCTION

Sucrose, glucose and fructose are the main sugars accumulated in the fruit of watermelon (*Citrullus lanatus*). Sucrose content is very low at early stage of fruit development and it starts to increase rapidly during late fruit developmental stage, while fructose and glucose contents are almost constant during watermelon fruit

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development (Brown and Summers 1985).

It was reported that α-galactosidase (AGA) play an important role in cucumber and melon sink tissue during phloem raffinose-family oligosaccharides (RFOs) unloading (Gao and Schaffer 1999). Soluble acid invertase (SAI) activity is negatively correlated with sucrose accumulation in tomato fruit (Miron and Schaffer 1991) and melon fruit (Schaffer et al. 1987; Lester et al. 2001). Insoluble acid invertase (IAI) activity has been found to be almost constant during the sucrose accumulation stage in melon (Schaffer et al. 1987). Neutral invertase (NI) was positively correlated with sucrose accumulation during melon fruit development (Burger and Schaffer 2007). The positive correlations between Sucrose synthase (SuSy) activity and fruit sucrose accumulation were reported in melon (Burger and Schaffer 2007) and watermelon (Yativ et al. 2010). Sucrose phosphate synthase (SPS) activity was positively related to sucrose accumulation in melon (Hubbard et al. 1989; Burger and Schaffer 2007) and in watermelon (Yativ et al. 2010). While there is less knowledge about the comparison of sugar related enzymology and transcriptome dynamics in sweet and non-sweet watermelon fruit tissues during watermelon fruit development.

Large-scale transcriptome sequencing has proved to be efficient and cost-effective for gene discovery and gene expression analysis. Recently, several large expressed sequence tag (EST) datasets have been generated in cucurbit species. These include approximately 589,000, 1.2 million, 514,000 and 508,000 ESTs generated from watermelon, melon, cucumber and *Cucurbita pepo*, respectively, using the Roche/454 sequencing technologies (Guo et al. 2011), and an additional ~127,000 ESTs generated from melon using the traditional Sanger sequencing approach (Clepet et al. 2011).

In this study, the enzyme and transcriptome dynamic characteristics of invertases, AGA, SuSy and SPS were comparatively analyzed using the center flesh and mesocarp of sweet and non-sweet watermelon fruit. These combined analyses of enzyme and transcriptome dynamics provide a new insight of sugar mechanism and accumulation in watermelon fruit.

MATERIALS AND METHODS

Plant material

The sweet watermelon 97103 (*Citrullus lanatus* subsp. *vulgaris* East-Asia Ecotype) and the non-sweet PI296341-FR (*Citrullus lanatus* subsp. *lanatus*) were used in this study. Seeds were germinated and grown in greenhouse with nutrition pots containing a soil mixture (peat:sand:pumice, 1:1:1, v/v/v). Flowers were hand-pollinated and tagged. Center fruit flesh and mesocarp samples of 97103 were collected at stages of 10, 18, 28, and 34 DAP, respectively. Center fruit flesh and mesocarp samples of the late maturity wild germplasm PI296341-FR were collected

at stages of 10, 18, 28, 34 and 50 DAP, respectively. Two separate, biological replications were performed.

Sugar determination

Sugar was assayed as previously described by Bethke et al. (2009) and Yativ et al. (2010). Each time point is the average of 6 fruit samples for dry matter content and soluble sugar contents.

Extraction and assays of AGA, invertase, SuSy and SPS

AGA activity was extracted and assayed according to the method of Gao and Schaffer (1999) Invertase activity was extracted and assayed according to the method of Miron and Schaffer (1991) SuSy and SPS were extracted according to the method of Hubbard et al. (1989) and Lowell et al. (1989).

Fruit transcriptome sequencing and analysis

RNA extraction and strand-specific RNA-Seq library preparations were performed as described (Zhong et al. 2011) and RNA-seq libraries were sequenced on the Illumina HiSeq 2000 system. Two independent biological replica samples were prepared. Differentially expressed genes during flesh or mesocarp development, and between flesh and mesocarp at the same stages were identified using the LIMMA (Smyth et al. 2004) and DESeq packages (Anders and Huber 2010), respectively. Raw p-values of multiple tests were corrected using false discovery rate (Benjamini and Hochberg 1995).

RESULTS AND DISCUSSION

Sugar accumulation during fruit development

Watermelon fruit mainly accumulated glucose and fructose at the early stage of watermelon fruit development, while sucrose increased rapidly 3 or 4 weeks after fruit setting (Brown and Summers 1985). In our study, similar results were observed in sweet watermelon fruits. Total sugar content in central flesh of 97103 increased rapidly from 10DAP (immature white) to 26 DAP (red flesh) and with slightly increase from 26DAP to 34 DAP (full-ripe). Central flesh of 97103 mainly accumulated glucose and fructose at the early stage (before 18DAP) (Fig. 1).

It was reported that in high-sucrose-accumulating watermelon, sucrose accumulated from about 3 weeks after pollination and subsequently with a sharp increase in sucrose content (Yativ et al. 2010). However, in our study, sucrose content increased about 2-3 weeks (18DAP to 26DAP) after pollination in 97103 central flesh (Fig. 1). The most possible reason is that 97103 is an early-mature fruit.

The flesh of PI296341-FR (*Citrullus lanatus* subsp. *lanatus*) was hard, white, low sugar and rich with water. Fructose, glucose, and sucrose content in both central flesh and mesocarp of PI296341-FR were much lower than those of central flesh of 97103 and decreased gradually during fruit development (Fig. 1). Total sugar content in central flesh was higher than that in mesocarp at each of the five stages (Fig. 1). These results showed that sugar accumulation between cultivated and wild type watermelon fruits were completely different, providing an ideal model for comparative genetic analyses and studies of sugar accumulation in watermelon fruit.

Enzyme activity dynamics of AGA

Alkaline AGA was suggested as an important enzyme in photosynthate unloading and partitioning in cucurbit sink tissue (Gao and Schaffer 1999). However, enzyme activity of alkaline AGA in both 97103 and PI296341-FR declined through fruit development. Moreover, alkaline AGA activities had no significant difference among 97103 central flesh and mesocarp, PI296341-FR central flesh and mesocarp (Fig. 2a). It indicated that alkaline AGA may be only involved in photosynthate unloading but not in determining fruit sugar content.

Acid AGA activity in 97103 central flesh was 2-4 times higher than that in 97103 mesocarp. Furthermore, activity of acid AGA in 97103 mesocarp was a little higher than that in flesh and mesocarp of PI296341-FR. Activities of acid AGA in both flesh and mesocarp of PI296341-FR were very close during most developing stages except in mature stage (Fig. 2b). These results suggested that acid AGA may be an important enzyme in determining sugar accumulation in watermelon central flesh.

Enzyme activity dynamics of invertase

One important role for IAI has been suggested as determining sink strength in various species, like tomato (Jin et al. 2009) and carrot (Tang et al. 1999). However, a sharp decline of IAI activity was observed 4 weeks after pollination in high sucrose accumulating watermelon lines and enzyme activity remained high in low sucrose accumulating lines (Yativ et al. 2010). In our study, IAI activity in central flesh of 97103 increased quickly at early stage of fruit development and reached a peak level at 26DAP. In contrast, the IAI in central fresh of PI296341-FR kept in a low level during fruit development. IAI activity in mesocarp for both 97103 and PI296341-FR was constant at all stages in both acidic and basic buffer (Fig. 3). These results indicate that IAI may contribute to total soluble sugar accumulation.

SAI is considered as a key enzyme regulating sucrose accumulation in melon (Lester et al. 2001) and tomato (Miron and Schaffer 1991). The correlation coefficient between SAI activity and sucrose levels was significant in watermelon

fruit (Yativ et al. 2010). However, in our study, SAI activity of central flesh of 97103 decreased dramatically from 10DAP to 18DAP and reached the lowest level at 34DAP. Meanwhile, SAI activity in 97103 mesocarp was higher than in central flesh. SAI activity was also very high in PI296341-FR fruit (Fig. 4a). SAI located in cell vacuole, which was the main organelle for sugar storage. SAI cleaves sugar stored in vacuole to glucose and fructose, which are subquently transferred out of vacuole to take part in other metabolism, for instance, building of cell composition. We assumed that SAI may play an important role in cell vacuole sucrose catabolism, instead of the direct role for sugar accumulation in watermelon fruit.

NI was positively correlated with sucrose accumulation during melon fruit development (Burger and Schaffer 2007). In our study, both for central flesh and mesocarp, the value of NI activity is high and constant in 97103 while low and constant for PI296341-FR during fruit development. As to the mesocarp of 97103, NI activity was also high while the sugar content was very low. The role of NI in watermelon fruit sugar accumulation still remains unclear based on current results (Fig. 4b).

Enzyme activity dynamics of SuSy and SPS

Previous study in sweet watermelon showed that both SuSy and SPS activities were positively related to sucrose concentration and correlation coefficient was higher for SuSy than that for SPS (Yativ et al. 2010). In our study, activity level of SuSy in 97103 central flesh declined slightly before 26DAP and subsequently with a sharp increase (from 26DAP to 34DAP). SuSy activity in mesocarp of 97103, central flesh and mesocarp of PI296341-FR was constant and lower than that in central flesh of 97103 during all stages (Fig. 5a).

SPS activity in central flesh of 97103 increased and reached a peak at 34DAP. Activity level of SPS in mesocarp of 97103, central flesh and mesocarp of PI296341-FR was lower than that of central flesh of 97103 (Fig. 5b). These results indicated that high activity of SPS and SuSy is necessary for sucrose accumulation.

Transcriptome dynamics of watermelon fruit development

We performed strand-specific RNA-seq of both flesh and mesocarp at four critical stages of fruit development in the inbred line 97103 (Table 1). A total of five AGA, two insoluble acid invertase, a soluble acid invertase, a neutral invertase and a sucrose phosphate synthase were differently expressed during fruit development.

It's worth noting that *AGA* gene WF06123 was highly expressed during fruit development. It may be the key genes involved in photosynthate unloading. The digital expression of *IAI* gene WF20872 was consistent with the enzyme activity of IAI at all stages. As to SAI, the gene expression in flesh of 97103 is significantly lower than that in mesocarp of 97103. This is similar to the enzyme activity of SAI

in flesh and mesocarp of 97103 during fruit development. Similar result also was found in the gene expression and enzyme activity of NI. As to SPS, different profiling between gene expression and enzyme activity was found. This result indicates again that regulation is not limited to enzyme specific activity or expression pattern but it is also in mRNA stability, rate of protein synthesis, protein turnover rates and stability, as well as possible different posttranscriptional controls.

CONCLUSION

This study provided the dynamic characteristics of enzymes and transcriptome expression related to sugar metabolism and accumulation during watermelon fruit development. More unloaded photosynthates were assigned to central flesh and storage as soluble sugars in sweet watermelon than in non-sweet fruit. The consistency among sugar accumulation profiles, enzyme activities and transcriptome expressions indicated that the sugar metabolism related enzymes play significant roles in regulating sugar content in watermelon fruits. The corporate action of photosynthate phloem unloading, partitioning and metabolism determined the sugar content in sweet watermelon and non-sweet watermelon.

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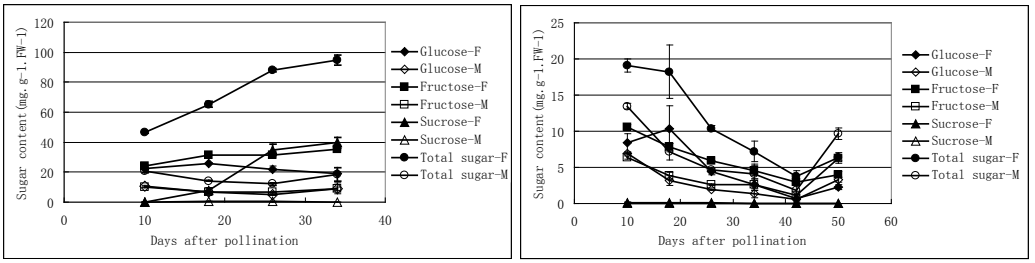
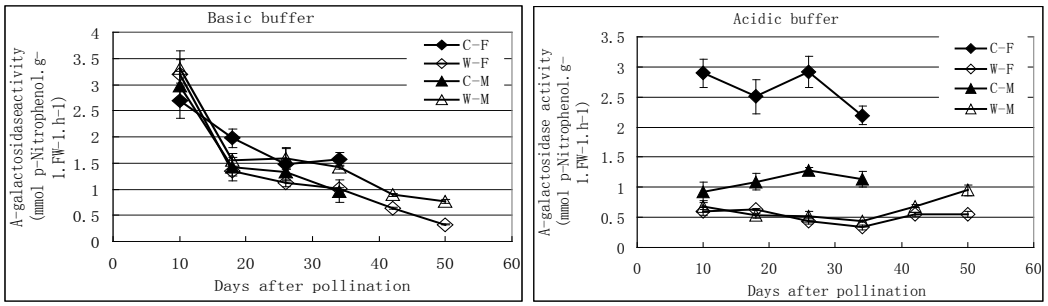


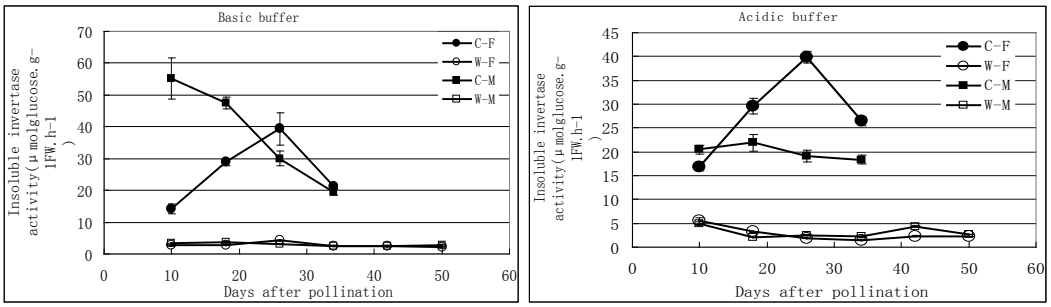
97103 PI296341-FR

Fig. 1. Sugar accumulation in central flesh and mesocarp of 97103 (cultivated type) and PI296341-FR (wild type). The first letter represents genotypes and the second letter represents different tissues, other following figures are the same. C: cultivated type (97103); W: wild type (PI296341-FR); F: central flesh; M: mesocarp. Other following figures are same.



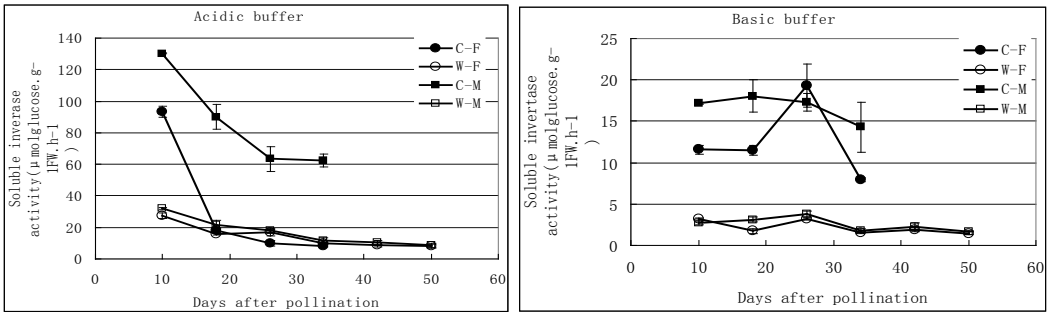
a b

Fig. 2. In-vitro activity of AGA under acidic (pH5.5) and basic (pH7.5) conditions and in watermelon fruits collected at various developmental stages.



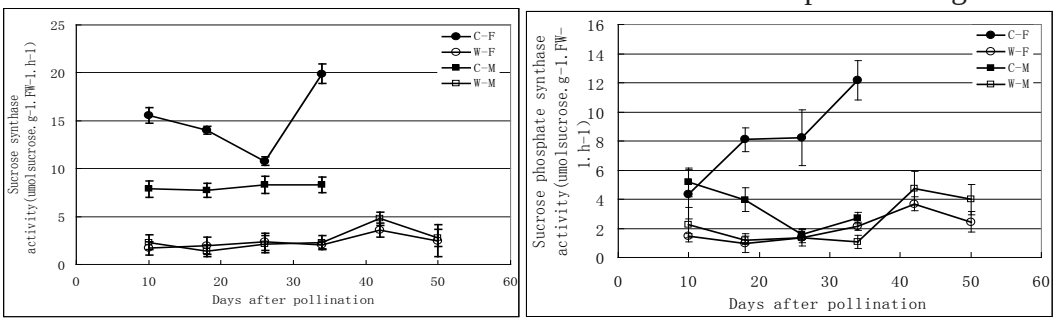
a b

Fig. 3. *In vitro* activity of IAI under acidic (pH5.0) and basic (pH7.5) conditions and in watermelon fruits collected at various developmental stages.



a b

Fig. 4. In-vitro activity of soluble invertase under acidic (pH5.0) and basic(pH7.5) conditions and in watermelon fruits collected at various developmental stages.



a b

Fig. 5. In-vitro activity of SUSy (a) and SPS (b) in watermelon fruits collected at various developmental stages.

Table 1. Expression of sugar metabolic genes during watermelon fruit development.

Enzyme	Gene	Normalized expression (FPKM)							
		Flesh				Mesocarp			
		10 DAP	18 DAP	26 DAP	34 DAP	10 DAP	18 DAP	26 DAP	34 DAP
AGA	WF06123	630.59	1677.93	2140.69	1659.28	233.49	707.17	291.04	403.08
	WF12211	109.48	137.26	18.11	18.32	186.33	386.72	284.79	257.85
	WF22734	21.81	14.58	1.26	1.78	27.58	32.63	29.59	29.12
	WF22735	48.84	31.12	3.06	6.37	78.26	58.87	77.24	80.25
	WF22883	204.33	362.19	117.36	109.11	67.15	81.57	51.61	44
IAI	WF17674	30.67	12.86	1.98	1.04	25.04	26.4	24.53	15.91
	WF20872	15.83	20.68	46.14	36.93	9.59	12.54	10.14	12.43
SAI	WF02328	6.54	1.51	0.39	1.25	21.48	9.04	6.1	13.5
NI	WF21809	23.19	21.46	11.53	5.29	37.33	29.67	31.67	26.33
SPS	WF11923	43.17	20.58	11.3	6.49	42.22	35.84	29.28	12.6

eth3.5 and *eth6.3* control climacteric fruit ripening in the near isogenic line SC3-5-1

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Keywords: fruit ripening, QTL analysis, RNA-seq

Abstract

The coexistence of climacteric and non-climacteric genotypes and the availability of a set of genetic and genomic resources make melon a suitable model for fruit ripening studies. In a previous work we described two interacting QTLs, *eth3.5* and *eth6.3*, for climacteric fruit ripening in the near isogenic line SC3-5-1. In this study, a high-resolution genetic map of the region containing *eth6.3* has been developed identifying some candidate genes. Additional studies about the interaction between the two QTLs were carried out confirming a faster climacteric ripening process and a higher ethylene production when both *eth3.5* and *eth6.3* are present. A large number of different processes occur during fruit ripening, with the presence of ethylene dependent and independent mechanisms, suggesting that a complex and highly regulated network controls this trait. To understand the transition between unripe and ripe fruits and the differences in ripening behavior between climacteric and non climacteric melon genotypes a RNA-Seq experiment was performed comparing the climacteric line SC3-5-1 and its parental PS at two different fruit developmental stages.

INTRODUCTION

Fruit ripening is a complex metabolic and physiologic process, highly regulated and has a direct impact in the organoleptic quality of fruit. An important compound during fruit ripening is ethylene, and based upon the presence or absence of an autocatalytic ethylene burst during ripening, fruit can be divided in climacteric and non-climacteric. In climacteric fruit, ethylene coordinates and accelerates the ripening process through the regulation of several genes (Giovannoni 2004). It is believed that ethylene-dependent and independent regulatory pathways coordinate

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both climacteric and non-climacteric ripening (Lelievre et al. 1997). The existence of genotypes showing both types of ripening and the availability of a set of genomic tools make melon a good alternative model system for the study of fruit ripening.

The Cantalupensis climacteric melon type has been often used as a model to study climacteric fruit ripening (Ayub et al. 1996). In melon several processes that take place during fruit ripening are ethylene-dependent as fruit softening, aroma production and color change of the rind. Ethylene production was drastically reduced in antisense 1-aminocyclopropane-1-carboxylate oxidase (ACO1) cantaloupe plants (Ayub et al. 1996). Both exogenous ethylene treatment and application of 1-methyl-cyclopropene (1-MCP) in ACO1 antisense melons confirmed that fruit softening is an ethylene-dependent process (Nishiyama et al. 2007). Genetic analysis of a population of RILs from the Charentais x PI 161375 cross, segregating for fruit abscission and ethylene production, indicated that both characters are controlled by two independent loci, abscission layer *Al-3* and *Al-4* (Perin et al. 2002). Much less is known about ripening mechanisms in non-climacteric fruits (Lelièvre et al. 1997).

In previous work we have detected two QTLs, *eth3.5* and *eth6.3*, which are involved in climacteric fruit ripening in the near isogenic line SC3-5-1. Both QTLs are localized in linkage groups III and VI in two introgressions from the donor parent "Sonwhan Charmi" (SC) in the background of "Piel de Sapo" T111 (PS) (Moreno et al. 2008; Vegas, unpublished)0, and low-resolution genetic maps in both regions have been developed. The interaction between both QTLs was previously examined and we showed that the presence of both QTLs produced the strongest climacteric fruit phenotype. In this work we have generated a high-resolution genetic map around *eth6.3.* At the same time we have performed a transcriptomic study of the ripening process using 454 next generation sequencing, which will eventually allow identifying genes differentially expressed between PS and the climacteric line SC3-5-1. The integration of both approaches may help to identify candidate genes for *eth6.3*.

MATERIALS AND METHODS

Plant material and DNA extraction

Genotypes used for this study were the parental line PS, the climacteric line SC3-5-1 and 967 individuals of an F2 population fixed for *eth3.5* and segregating for *eth6.3*. All plants were grown in a greenhouse in peat bags and drip irrigated as described previously (Moreno et al. 2008). Fruits were harvested when they showed the characteristic ripening features for the climacteric lines and at 45 days after pollination for the non-climacteric ones. For the RNA-seq experiment, fruit flesh samples were collected in triplicate 25 days after pollination (DAP) and in harvest time (30 days for SC3-5-1 and 40 for PS). Genomic DNA from all the

plants was isolated from young leaves according to Doyle and Doyle (1990) with modifications (Garcia-Mas et al. 2000).

Fine mapping *eth6.3*

DNA extracted from each F2 individual was genotyped with markers PSI_41-H06 and CMCTN41. Fruit from F2 recombinant plants were phenotyped measuring external change color, fruit abscission and aroma production. Recombinant plants were selfed in order to perform progeny test. New SSR and SNP markers in the PSI_41-H06/CMCTN41 interval were developed from the melon genome sequence (Garcia-Mas et al. 2012).

RNA-seq

mRNA was extracted from each fruit sample and libraries were prepared in the CRAG sequencing service. Two 454 GS-FLX (Roche) runs were performed using the Titanium+ chemistry. Low quality reads and MID identifiers were removed using Newbler 2.6 (Roche). Sequences were aligned to the melon reference genome (Garcia-Mas et al. 2012) with TopHat-1.4.1 and TopHat2.0.0. The transcriptome was reconstructed and differential expression was measured using Cufflinks-1.3.0 and Cufflinks 2.0.0 (Trapnell et al. 2012).

RESULTS AND DISCUSSION

Construction of a high-resolution genetic map of *eth6.3*

In order to increase the mapping resolution of *eth6.3*, which was previously obtained with a segregating population of 150 F2 individuals, 967 individuals from a new F2 population fixed for the SC alleles of *eth3.5* and segregating for *eth6.3* were used. The F2 population was genotyped with the flanking markers PSI_41-H06 and CMTCN41. We identified 54 recombinants, and defined the position of the QTL after saturating the genomic interval with additional markers that were developed from the melon genome sequence. We selected 5 informative recombinants and performed a progeny test, which allowed narrowing down the position of *eth6.3* to an interval of 4.5 Mb. Several candidate genes have been identified and a new F2 population has been obtained in order to obtain additional recombinants in the interval, which will allow narrowing down the region around *eth6.3*.

The availability of a NIL population (Eduardo et al 2005), with introgression lines that are genetically and phenotypically well characterized, greatly facilitates the study of complex traits as they permit mendelisation of QTLs (Zamir 2001). We report the fine mapping of the QTL *eth6.3* involved in the climacteric ripening behavior of NIL SC3-5-1.

Transcriptomic analysis of SC3-5-1

Two 454 runs were performed with mRNA obtained from unripe (25 DAP) and ripe (harvest) flesh fruit samples from PS and SC3-5-1. Both runs showed a correlation of 0.87 and data was merged resulting in a total of 1.504.873 reads (451 Mb) with an average read length of 300 pb. The total number of genes expressed in

any of the four conditions is 13,716 (49.97% of the annotated genes in the melon genome), including 18 genes not annotated in the reference genome.

A first analysis reveals that some genes known to be involved in fruit ripening are differentially expressed between SC3-5-1 and PS, as genes encoding ethylene biosynthesis enzymes and ethylene receptors, proteins from the ethylene signaling pathway and transcriptional factors from the APETALA/ERF or NAC families, among other.

The transcriptomic experiment can help to understand the differences between climacteric and non-climacteric ripening in melon, allowing the identification of genes with differential expression that may be located in the intervals of the SC introgressions in SC3-5-1, with the final objective of characterizing candidate genes for *eth3.5* and *eth6.3*.

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Effect of carpel primordia-targeted inhibition of ethylene perception on sex expression, fruit set, and fruit ripening in melon (*Cucumis melo* L.)

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Keywords: CRABSCLAW, *Cucumis sativus*, *etr1-1*, floral development, fruit development, sex determination

Abstract

Ethylene is a key factor regulating sex expression in cucurbits, such as melon (*Cucumis melo* L.). Our previous studies found that transgenic andromonoecious melon plants expressing the dominant negative ethylene perception mutant gene, *etr1-1*, under the control of the carpel and nectary primordia-targeted *CRABSCLAW* (*CRC*) promoter showed increased and earlier carpel bearing buds and flowers in the greenhouse. To further investigate this phenotype and its potential usefulness for earlier fruit production, we observed T_2 transgenic *CRC::etr1-1* plants in the field for sex expression, fruit set, and fruit ripening phenotypes. *CRC::etr1-1* plants in the field showed an increased number of carpel bearing buds and open flowers, earlier onset of first open carpel-bearing flower, and increased early fruit set relative to controls. However, fruit from the transgenic melon plants were smaller, resulting in equivalent total kg/plot. *CRC::etr1-1* lines also showed earlier fruit ripening phenotypes, manifested either as earlier external signs of ripening in the field (line M5), or no obvious external signs of ripening despite extensive internal ripening phenotypes (i.e. mesocarp and septum degradation, and separation of seeds from septum) (line M15). M15 fruit that appeared green externally were found to have elevated levels of internal ethylene, equivalent to those seen in WT orange stage fruit. These observations likely reflect differences in the requirement for ethylene for different ripening processes, and suggest localized effects of perception inhibition. Thus, targeting inhibition of ethylene perception to the carpel and nectary led to increased and earlier carpel-bearing buds and open flowers in the greenhouse and field, and increased early fruit set, however, transgenic fruit exhibited decreased fruit weight, and altered ripening.

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INTRODUCTION

The Cucurbitaceae family contains species that show a variety of heritable sex phenotypes (Roy and Saran 1990; Grumet and Taft 2011). The most common sex type in melon (*Cucumis melo* L.) is andromonoecious. As the plant develops, vegetative nodes are produced first, followed by nodes with male flowers, and then a mixture of male and bisexual nodes. A key factor regulating sex determination in cucurbits is ethylene. Exogenous application of ethylene-related chemicals has led to increased femaleness (Rudich et al. 1969; Karchi 1970). Likewise, an endogenous increase in ethylene driven by over-expression of the ethylene biosynthetic enzyme *ACS* in transgenic melon also showed increased femaleness (Papadopoulou et al. 2005). Inhibition of ethylene biosynthesis with aminoethoxyvinylglycine (AVG), or perception with silver ions leads to increased maleness after application (De Nijs and Visser 1980; Owens et al. 1980). Similarly, transgenic melons that constitutively express an *Arabidopsis thaliana* mutant ethylene receptor protein, etr1-1, that blocks ethylene response, showed complete loss of carpel-bearing nodes (Little et al. 2007). These results demonstrated that ethylene perception is required for carpel primordia development during melon sex determination (Little et al. 2007). Our previous studies using stamen or carpel primordia-targeted expression of *etr1-1* in transgenic melons, indicated inhibition of ethylene perception by stamen primordia prevented production of carpel bearing buds (Little et al. 2007). In contrast, inhibition of ethylene perception in carpel primordia resulted in increased and earlier carpel bearing buds and flowers in the greenhouse (Little et al. 2007).

Ethylene also plays an important role in fruit ripening for a wide range of species including melon. Studies conducted using ACO-antisense melons have characterized ethylene-dependent and independent pathways of ripening (Guis et al. 1997; Pech et al. 2008). Loss of chlorophyll and increase in carotenoids in the rind, flesh softening, peduncular abscission, and aroma production are ethylene-dependent processes, while changes in acidity level, and flesh carotenoid and sucrose accumulation are not regulated by ethylene (Pech et al. 2008). If CRC-driven *etr1-1* expression persists during fruit development, it could influence the ripening process in melon.

In this work we studied transgenic melons targeting expressing of *etr1-1* to the carpel and nectary primordial under control of the *CRABSCLAW* (*CRC*) promoter (*CRC::etr1-1*) to determine if the increased femaleness phenotype previously observed in the greenhouse carries into the field and allows for earlier or increased fruit set. Fruit set and development was also assessed to see if ethylene perception inhibition in the carpels has an effect on fruit growth and ripening.

MATERIALS AND METHODS

Plant material and growth conditions

The genotypes used for these experiments were wild type, andromomoecious Hales's Best Jumbo (Hales) and T_2 progeny of two *CRC::etr1-1* transgenic lines, M5 and M15, that were originally produced from Hales as described by Little et al (2007). M5 and M15 plants were self-pollinated in the greenhouse to obtain the T_2 generation for *CRC:etr1-1* lines. All transgenic plants used for greenhouse or field trials were verified by PCR analysis of seedlings as described by Little et al. (2007). Greenhouse growth conditions were as described by Little et al. (2007). Field experiments, including germination in the greenhouse, transplanting to the field, row and plant spacing, irrigation and pest control were performed as described by Papadopoulou et al. (2005).

Field trials were performed in East Lansing, MI during the summer of 2009 with 6 replicates and 6 plants/genotype/plot, and the summer of 2011 using 4 replicates and 10 plants/genotype/plot, with a randomized complete block design. Main stems of the plants in the 2009 field experiment were scored for number of male and carpel-bearing buds and open flowers, and first node appearance of carpel-bearing buds and open flowers. In 2011, main stems were observed for first node of set fruit and total fruit set throughout the growing season. Estimated fruit maturity was based on diameter readings taken on fruit set on plants at 26, 40, and 46 days after planting, and visual rind characterization at time of harvest (10 weeks after planting). Fruit length, diameter, and weight were recorded for all harvested fruit.

qRT-PCR analysis

Total RNA was prepared from 200mg of liquid nitrogen frozen melon apex tissue (~3-4mm in size) or fruit tissue, and cDNA preparation was performed using a procedure based on Schilmiller et al. (2009) as described in Ando and Grumet (2010). RNA from fruit mesocarp, or exocarp minus rind was extracted from 200-300mg of corresponding tissue from 10mm and 60mm diameter field grown fruit (~3 days post pollination (dpp), and ~14 dpp) as described above. Gene-specific primers were designed using Primer Express 3.0 software (Applied Biosystems); the *etr1-1* transgene-specific primers used for detection were 5' end – 5'GTTGGGGATGAGAAACGGC (RG317) and 3' end 5'-GCCCACTACCACCCGAGC (RG318). qRT-PCR analysis was carried out on the Stratagene Mx4000 system using Power SYBR Green PCR Master Mix (Applied Biosystems) for PCR quantification via standard curve reference. *C. melo Polyubiquitin 7* (ICuGI: MU43954) was used as an endogenous control for normalization.

Ethylene measurements

Ethylene measurements were performed on apices of greenhouse grown Hales, *CRC:etr1-1* M5 and M15 plants. Two lateral apices (~3-4 mm in size) per plant were excised from branches 8-10 nodes long, using a razor blade, and weighed to record fresh weight. Apices were placed in a 10mL plastic syringe with a 5mm diameter Whatman filter paper #2 disc moistened with distilled water, and placed at room temperature for 1 hour to allow for diffusion of wound ethylene. Ethylene measurements were performed by gas chromatography as per Papadopoulou et al. (2005). The experiment was repeated twice with six plants/genotype in a randomized complete block design. Three samples/plant were taken for ethylene analysis with two apices/sample.

To perform fruit ethylene measurements, fruit harvested from the field were classified by rind color as green (green rind, unripe), turning (mixture of green and orange), or orange (fully orange rind, ripe) and weighed. To measure ethylene evolution, three fruit/rind color category (one replicate) were placed in separate 10 gallon buckets and sealed for 1 hour with air tight covers with a rubber septum prior to sampling for ethylene concentration. Internal fruit ethylene was measured from each fruit after whole fruit head space was analyzed. An 18 gauge needle attached to a 1mL syringe was inserted in each fruit to draw air from the cavity. The experiment was repeated on three harvest dates, with three replicates/experiment.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using Proc GLM or Mixed protocol in SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Ethylene and sex expression

Transgene expression was verified in apices of PCR-positive *CRC::etr1-1* transgenic melons by qRT-PCR analysis (Fig. 1a). The PCR positive apices also showed a 2-6 fold increase in apical ethylene levels (Fig. 1b). Elevated ethylene production likely results from lack of feedback inhibition as has been reported frequently in non-ethylene perceiving mutants and transgenics (e.g., Barry and Giovannoni 2007; Little et al. 2007; Pech et al. 2008). We previously observed that carpel primordia-targeted inhibition of ethylene perception in *CRC::etr1-1* melons led to earlier onset and an increase in the number of carpel-bearing buds in transgenic plants (Little et al. 2007). A possible explanation is that the stamen primordia, which promote carpel development in response to ethylene, can perceive the elevated ethylene, thereby resulting in increased carpel development. Studies of ethylene evolution in gynoecious cucumber apices have shown two-fold greater ethylene compared to their monoecious counterparts, providing evidence for the

biological significance of two-fold differences in modulating sex determination (Rudich et al. 1972; Yamasaki et al. 2003).

Earlier or increased production of carpel-bearing flowers could have a possible application for increased and earlier fruit set. Experiments were conducted to see if this phenotype is also expressed under field conditions. The first field experiment focused on sex expression. As had been observed in the greenhouse (Little et al. 2007; Taft et al. 2010) both *CRC:etr1-1* lines in the field showed an increase in the number of carpel-bearing buds and open flowers, and earlier onset of the first open carpel-bearing flower on the main stem by approximately 7-10 nodes, or 8-10 days (Fig. 2a-c).

Fruit set and yield

The second field experiment assessed fruit set and development. Transgenic fruit showed increased average fruit set, especially early in the season, consistent with the earlier appearance of carpel-bearing flowers (Fig. 2d, Table 1). The difference declined over the season. Total yield, as measured by kg/plot, did not significantly differ from wild type plants (Table 1). Average fruit weight of the *CRC::etr1-1* melons was lower. Collectively these observations indicate that the increased fruit set was compensated by abscission of young fruit and smaller final fruit size. This suggests that higher competition for photosynthetic resources of the plant might have occurred in the *CRC::etr1-1* plants, either due to increased fruit number or earlier fruit set on younger, smaller plants. Competition for resources has been frequently observed to regulate balance between grain or fruit size and number (Sadras and Denison 2009). Cucumber thinning has been demonstrated to increase fruit size and decreased assimilates within the plant leads to fruit abortion of young fruits (Hikosaka and Sugiyama 2005).

Fruit ripening

To monitor fruit development and ripening, fruit of equivalent size (90-120mm diameter) from each of the different lines were tagged on the same day, and ripening stage was determined by exocarp color at final harvest as: green, turning (mix of green and orange), or orange. Fruit categorized as full slip were orange, but could be easily removed from the vine. At the final harvest date, 100% of the tagged fruit of the *CRC::etr1-1* M5 line showed external ripening (classified as turning, orange, or full slip), compared to ~50% of wild type, and ~30% of the M15 line (Fig. 3a). This result indicates early ripening occurring in the M5 line, likely due to earlier fruit set. In contrast, the majority of M15 fruit did not show external ripening. However, when full size green fruit were cut open, the mesocarp was orange and frequently accompanied by over-ripe characteristics (i.e. mesocarp degradation, septum separation from seeds, and/or septum degradation).

Measurement of ethylene evolution from whole fruit showed that orange fruit of all lines had much higher ethylene evolution than green fruit, as would be expected during climacteric ripening (Fig. 3b). Similarly, samples of internal ethylene taken from the seed cavity of green fruit showed that Hales, azygous, and M5 lines all had low ethylene levels. However, M15 green fruit showed high ethylene levels comparable to those in orange fruit, consistent with the accelerated mesocarp ripening that was not externally visible (Fig. 3c).

A possible explanation for the green exterior, but overly ripe interior is that expression of the *etr1-1* transgene was inhibiting perception of ethylene in the exocarp but not the mesocarp. A study by Fernandez et al. (2009) looking at tomato plants with the CRC promoter fused to GUS observed that expression was specifically localized to the fruit epidermis, but not mesocarp, throughout the ripening process. Earlier work with transgenic *35S::ACO*-antisense melons inhibiting the ethylene ripening processes showed that rind yellowing, abscission, and a majority of flesh softening processes are ethylene-dependent (Ayub et al.1996; Pech et al. 2008). qRT-PCR analysis of young fruit (~3 and 14 dpp) did not show increased transgene expression within the exocarp compared to mesocarp of transgenic fruit (data not shown). Full size fruit are currently being analyzed to determine if the expression of the *etr1-1* transgene expression in different tissues of mature fruit is the cause of this phenotype.

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Table 1. Field observations of fruit yield parameters.

Parameter	Hales (WT)	Azygous	*CRC::etr1-1*		Non-transgenic	*CRC::etr1-1*
			M5	M15		
Total fruit/plot	42 ± 4.2a[z]	40 ± 3.8a	52.5 ± 4.6a	51.5 ± 4.1a	41 ± 2.6**a**[y]	52 ± 2.9**b**
Total fruit yield (kg/plot)	60.3 ± 4.6a	56.0 ± 4.7a	63.2 ± 6.8a	60.5 ± 6.1a	58.2 ± 3.1a	61.9 ± 4.3a
Average fruit weight (kg)	1.5 ± 0.0**a**	1.40 ± 0.1**ab**	1.20 ± 0.0**bc**	1.17 ± 0.0**c**	1.45 ± 0.1**a**	1.19 ± 0.0**b**

[z]Values are presented as average of 4 replicates with 10 plants/rep ± SE. Different letters represent significant differences by LSD at $P<0.05$.
[y]Comparison of non-transgenic (Hales and azygous) vs. CRC::etr1-1 transgenic plants by orthogonal contrast. Different letters represent significant differences at $P<0.05$.

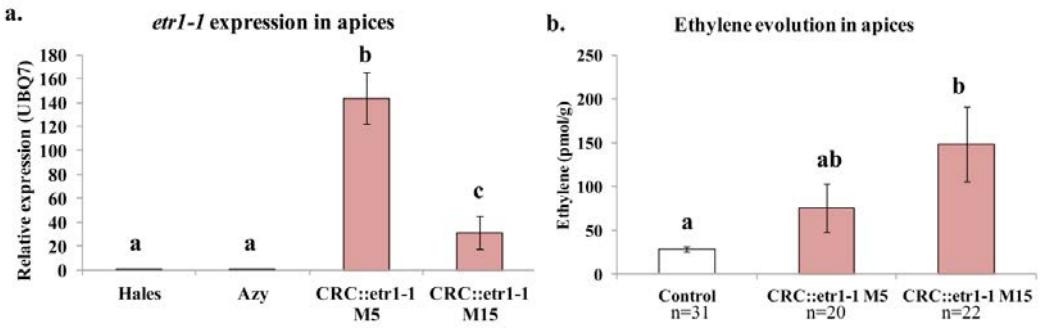

Fig. 1. qRT-PCR analysis of expression of the etr1-1 transgene (a), and ethylene evolution (b) in apices of Hales (WT) Azygous (Azy; control is a combination of Hales and Azygous), and *CRC::etr1-1* M5 and M15. Values in qRT-PCR are the average of three biological replicates/technical replicate and three technical replicates ± SE. Values presented in ethylene evolution are the average of 6 replicates with 2 plants/rep ± SE. Different letters represent significant differences by LSD at $P<0.05$.

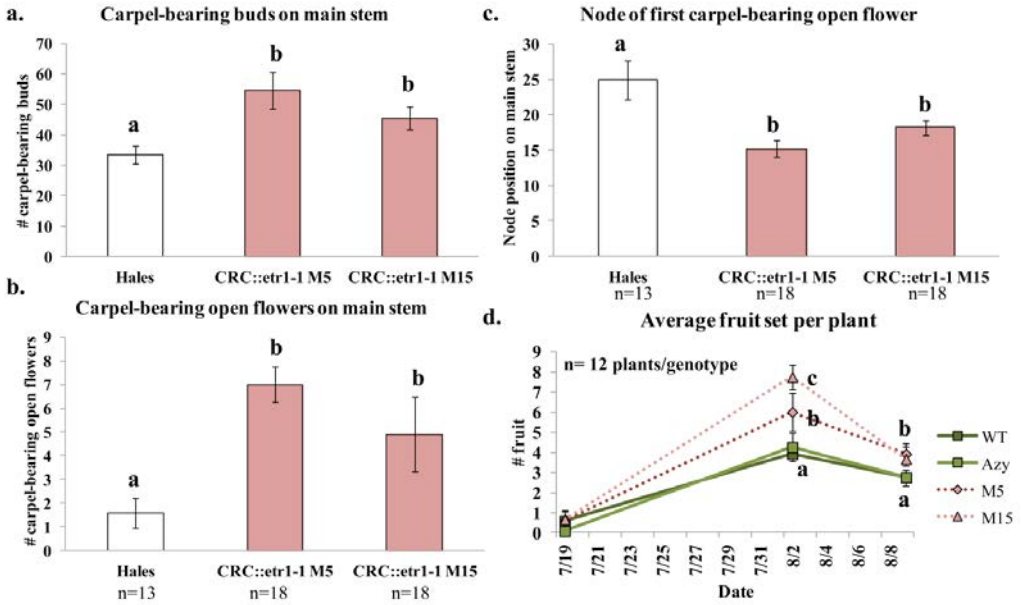

Fig. 2. Field observations of number of carpel-bearing buds (a), and open flowers (b), and node of first carpel-bearing open flower on main stem (c), and average fruit set per plant (d) of Hales (WT), and *CRC::etr1-1* M5 and M15. Values for sex expression data are presented as the average of 6 replicates with 4 plants/rep ± SE. Average fruit set per plant values are the average of 4 replicates with 3 plants/rep ± SE. Different letters represent significant differences by LSD at $P<0.05$.

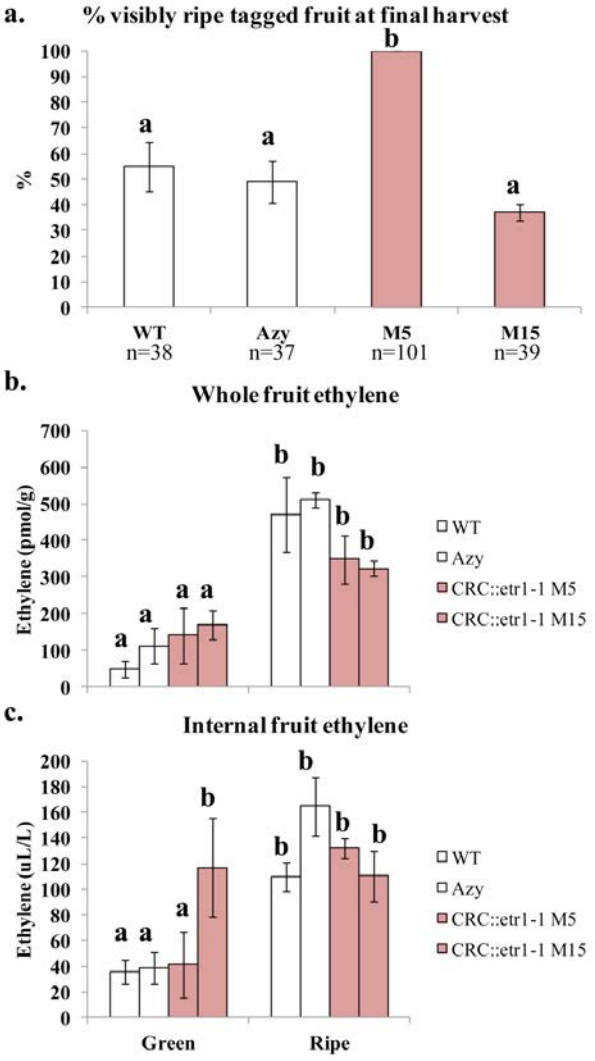

Fig. 3. Percentage of tagged fruit that are visibly ripe at final harvest (a; visibly ripe fruit were classified as turning, orange and full slip), whole fruit ethylene evolution (b) and internal fruit ethylene (c) of wild type, azygous, and *CRC::etr1-1* M5 and M15. Values for visibly ripe tagged fruit are presented as the average of data combined from three sample dates with four replicates of 10 plants/rep. Values for whole and internal fruit ethylene are the average of data combined from three sample dates with three replicates of 3 fruit/rep ± SE. Different letters represent significant differences by LSD at $P<0.05$.

Enzyme activity and Cucurbitacin content in relation to fusarium wilt resistance in melon

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Keywords: *Cucumis melo*, defense responses, fusarium, peroxidase, polyphenol oxidase, triterpenoids

Abstract
Fusarium wilt (*Fusarium oxysporum* f.sp. *melonis*) is one of the most destructive and widely distributed wilt diseases of melons. Forty five melon cultigens collected from different parts of Iran, were screened for Fusarium race 1 (*Fom*1) resistance. Analysis of variance revealed highly significant differences among cultigens. Based on the results of the screening, five susceptible and five resistant genotypes were selected for further study. The activity of peroxidase (POX, EC 1.11.1.7) and polyphenoloxidase (PPO, EC 1.10.3.2) and also Cucurbitacin content of the root samples were analyzed during eight days after inoculating with the causal agent. The PPO and POX activity in both groups (susceptible and resistant genotypes) increased in response to infection by the pathogen, but the raising was more prominent in resistant genotypes. The enzymes pick of activity occured four days after infection. Analysis of variance showed there was a correlation between Cucurbitacins B and D content and resistance to the disease, while there was no clear correlation between the content of Cucurbitacins E and I with disease resistance. Resistant genotypes showed highest amount of Cucurbitacin B content at day six after infection. Cucurbitacin D was absent in roots of susceptible genotypes at day 0 (no infection) while the roots of resistant genotypes contained significant amount of this compound. The results suggest that the increased activities of POX and PPO in melon genotypes play an important role in the mechanism of resistance to this disease. Finally it should be investigated whether Cucurbitacins B and D are contributed in antibiosis mechanism of resistance to Fom1.

INTRODUCTION
Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *melonis*, is one of the most devastating diseases in melon production worldwide. Epidemics of Fusarium

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wilt can result in approximately 100 percent yield losses. The fungal soil-borne pathogen remains viable on non-host crop residues and roots grown in rotation or as chlamydospores for decades. Thus, the control of this vascular wilt disease carries out its own difficulties (Gordon and Martyn 1997). The long lasting presence of the pathogen in soil, has led scientists to employ host resistance as the most effective controlling way to avoid economic yield losses caused by the disease (Zuniga et al. 1997). Burger et al. (2003) found resistant genotypes pickling melon (breeding line), PI 161375, F65 (Galia), and I4-6-2-B (Galia) by inoculating melon genotypes with race 1 of *F. oxysporum* f.sp. *melonis*. In 1976, Risser et al. designated physiological races of *F. oxysporum* f.sp. *melonis* including 0, 1, 2, and race 1, 2 (1,2w and 1,2y).

Plants respond to pathogen attack by employing defense mechanisms such as rapid production of reactive oxygen species (ROS) (De Gara et al. 2003); alterations in the cell wall constitution; and accumulation of antimicrobial secondary metabolites (Heath 2000). Various antioxidant enzymes such as peroxidase (POX), superoxide dismutase (SOD) catalase (CAT) and polyphenol oxidase (PPO) are reported to be involved in ROS metabolism during the pathogen attack (Morkunas and Gemerek 2007). Among the proteins induced during the host plant defense, class III plant POXs (EC 1.11.1.7; hydrogen donor: H_2O_2 oxidoreductase, Prxs) participate in the wall-building processes such as oxidation of phenols, suberization and lignifications of host plant cells (Khatun et al. 2009). PPO (E.C. 1.10.3.2; diphenol oxidase or catecholase activity) is also involved in the oxidation toxic PCs into quinones which are not harmful to plants and lignification of plant cells during microbial invasion (Mohammadi and Karr 2002). Cucurbitacins are highly oxygenated tetracyclic triterpenes which are predominantly found in the family *Cucurbitaceae* and are also found in other plants. They are responsible for the characteristic bitter taste of most wild species of *Cucurbitaceae* (Chen et al. 2005). These chemicals have a broad range of potential biological activities such as cytotoxicity, antitumor properties, antimicrobial and anthelmintic activities which are also involved in plants' resistance against insects (Aharoni et al. 2005). In 1981, Nes and Patterson reported that Cucurbitacins have an inhibiting effect on the growth of *Phytophthora cactorum*, causal agent of root rot. It has been shown that Cucurbitacin I has antifungal properties against *Botrytis cinerea* (Bar-Nun and Mayer 1990). In the present investigation attempts were made to find differences in the degree of resistance against Fom race 1 (Fom1) among 45 genotypes of melon and associate these differences with variation in the activity of the enzymes PPO, POX and four types of Cucurbitacin.

MATERIALS AND METHODS

Plants cultivation and pathogen preparation

Forty-five melon genotypes mostly collected from different parts of Iran and

a few imported genotypes provided from market were used in this study (Table 1). The seeds were kindly provided by the Iranian National Gene Bank Department, Seed and Plant Improvement Institute, Karaj, Iran. Seeds were surface-disinfected with sodium hypochlorite (1%) and then sown in growing trays; filled with a pasteurized potting mix of peat, perlite and sand (1:1:1) and were grown under greenhouse conditions (24-28 ºC, 16 h day/8 h night, via natural lighting plus high pressure Sodium Lamps to supply an average lighting level of 10,000 Lux).

Two isolates of Fom1 (dominant race in Iran; Banihashemi 1989) M263 and Msh from Garmsar and Mashhad area, respectively, supplied from the collection of Plant Protection Department, Shiraz University, were used in this study. It was single spore cultured on potato dextrose agar (PDA), incubated at 22±1ºC and a photoperiod of 14h (L: D) for 7 days.

Plant inoculation and disease assessment

At first true leaf stage, seedlings were removed from soil and roots were washed under running tap water, pruned to approximately half length, and dipped in the conidial suspension to obtain a concentration of 1×10^6 spores/ml, for 1-2 minutes and transplanted into new pots. Ten seedlings of each genotype were inoculated, and roots of three plants were dipped in sterile water as control treatment. Disease severity was assessed on leaves using a rating scale from 0 to 4, and four days after inoculation, the disease symptoms were daily monitored and recorded till 4 weeks (Chikh-Rouhou et al. 2008).

Extraction and activity assay of POX and PPO

Enzymatic activities were investigated in resistant and susceptible genotypes at 0 (control), 2, 4, 6 and 8 days after inoculation. The extraction and purification were done according to Janda et al. (2003) method. Then, the supernatant was stored at −80 ◦C for analysis of the POX (EC 1.11.1.7) and PPO enzyme activities and determining protein concentration. The bovine serum Albumin, guaiacol and prolin were obtained from Sigma–Aldrich Co. Protein concentration was determined according to Bradford (1976) with bovine serum Albumin as a standard. Employing method done by Janda et al. (2003), POX activity was measured. Followed by the absorbance variation measured at 475 nm for 1 min at 25°C in a UV–vis spectrophotometer (Perkin Elmer-lambda 25). The activity of PPO was measured according Chen et al. (2000) method. Finally, the variation of absorbance was monitored at 515 nm for 1 min at 25 ◦C.

Extraction and assay of Cucurbitacins content

In the last experiment, the content of four Cucurbitacins present in the melon genotypes was determined by high performance liquid chromatography (HPLC)

analysis. The target contents were measured in resistant and susceptible genotypes at 0 (control), 2, 4, 6 and 8 days post-inoculation. Since highest levels of Cucurbitacin concentrations are normally found in roots (Rymal et al. 1984), the root samples were used for this purpose. The extraction and purification were done according to Balkema-Boomestra et al. (2003). The quantitative analysis of Cucurbitacins was determined by HPLC (Analytical, Knauer, Germany), with UV detector at 235 nm (after much trial and error) and column C18 (125nm×4nm, Nucleosil-100, 5µm).

Statistical analysis

Analysis of variances and mean comparisons were performed using SAS software (version 9). A Duncan test with a probability of 0.05 was used to show significant differences among treatments.

RESULTS AND DISCUSSION

Response of particular genotypes to Fom

Symptoms were observed on susceptible plants as early as five to seven days after inoculation, and seedlings of susceptible genotypes were usually killed within 10 to 15 days. The resistant plants showed only some light yellowing and decreased growth. Analysis of variance revealed significant differences for symptom severity among the genotypes. Genotypes exhibited a wide range of disease incidence (0-4), and inoculation with both isolates (M263 and Msh) (Table 1). While control and resistant plants remained healthy, susceptible ones showed wilt symptoms.

According to the mean *Fom*1 (M263) disease incidence, the most resistant genotypes are Ogen, Chorok-Zard, Khatoni, Mahali, Zard-Shotori and the most susceptible ones are Shahabadi, Yellow Canary, Spanish, Dastanbu 1, and Dastanbu . Reaction to race Msh demonstrated Chorok-Zard, Ogen, Khatoni, Zard-Shotori, and Mahali as the most resistant genotypes and, Yellow Canary, Spanish, Shahabadi, Dastanbu 1, and Dastanbu were classified as the most susceptible resources (Table 1). Race M263 and its corresponding resistant and susceptible genotypes were chosen for following assays to identify the possible defense mechanisms against the pathogen.

Determination of enzymatic activities in root samples

Comparison of means indicated that the basal POX activity is higher in resistant genotypes than in susceptible ones. This difference became even sharper soon after inoculation, as the inoculation induced the enzyme activity. POX activity decreased sharply in resistant genotypes four to six days after inoculation, in such a way that in 6th-8th day after inoculation its activity was even less than those of susceptible genotypes (Fig. 1). Highest amount of POX activity was observed in Ogen, Mahali, and Zard-Shotori and lowest in Shahabadi and Dastanbu 1 (Table

2). Polyphenol oxidase (PPO) activity was higher in resistant genotypes when compared to susceptible genotypes and this difference was significant on all days of experiment. The level of PPO activity after inoculation with isolate M263 increased between the second and fourth day, and highest activity was observed on the fourth day, while the lowest activity was detected in control plants (Fig. 1). Highest amounts of PPO activity were observed in Ogen and Mahali and lowest in Dastanbu and Dastanbu 1 (Table 2).

Determination of Cucurbitacin E and I contents in root samples

Analysis of variance showed that Cucurbitacin I and E contents was not significantly different between the susceptible and resistant genotypes. The highest amount of Cucurbitacin E was observed in Mahalli (2.29 ppm) and Yellow Canary (1.61 ppm) and lowest in Chorok-Zard (0.27 ppm) and Dastanbu (0.75 ppm) (Table 2). The content of Cucurbitacin I in the susceptible and resistant genotypes was almost equal except for Spanish genotype. The highest amount of Cucurbitacin I was observed in Spanish (2.08 ppm) and Ogen (1.94 ppm) and lowest in Yellow Canary (1.61 ppm) and Khatooni (1.65 ppm) (Table 2).

Variations of Cucurbitacin D and B contents between resistant and susceptible genotypes

Comparison of means indicated that Cucurbitacin D content was higher in roots of resistant genotypes than susceptible ones. There was no detectable content of Cucurbitacin D in susceptible genotypes Yellow Canary, Dastanbu and Spanish (Table 2). The production of Cucurbitacin D decreased in resistant genotypes until six post–inoculation day and then demonstrated increase until day eight but in susceptible ones it was continuously increased during the same period (Fig. 2). The highest content of Cucurbitacin D belonged to Chorok-Zard (0.54 ppm) and Zard-Shotori (0.5 ppm) (Table 2). Analysis of variance showed that Cucurbitacin B content was significantly different between the susceptible (lower) and resistant (higher) genotypes. In the susceptible group, its level reached to the peak at day four, and then decreased until day eight post-inoculation. Mean-while, resistant genotypes showed highest amount of Cucurbitacin B content at day six after inoculation. The presence of Cucurbitacin B was not detected in susceptible melons at day 0 (no infection) (Fig. 2). Zard-Shotori (2.07 ppm) and Ogen (1.94 ppm), demonstrated the highest amounts of Cucurbitacin B (Table 2).

On the basis of the greenhouse experiments, significant genetic variation among the Iranian melon genotypes, and resistant and susceptible ones were recognized. This variation in melon and related resistance sources to *Fom* race 1 has been reported, previously (Alvarez et al. 2005). Among the resistant and susceptible genotypes determined in this study, the five most resistant and the five

most susceptible ones were selected to investigate the possible involvement of oxidative enzymes and Cucurbitacin in resistance.

Our results revealed that resistance to *Fom* race 1 in melon is significantly correlated with increased peroxidase and polyphenoloxidase activities. Peroxidase is involved in the production of reactive oxygen species, which are directly toxic to the pathogen. As an indirect effect, they can also reduce the spread of the pathogen by increasing the cross linking and lignifications of the plant cell walls (Hammond-Kosack and Jones 1996). The induced activity of PPO through pathogens has been reported from a variety of plant, including monocots and dicots (Chen et al. 2000). The current obtained results showed a significant correlation between the resistance and POX activity. Although the POX activity in resistant genotypes declined significantly at 6th day after inoculation, the earlier induced activity might have a role on limiting the number of successful infections. However, this significantly different induction of peroxidase might be a reaction of host to pathogen attack in resistant and susceptible genotypes. But, it could be an effort to replace damaged tissues as the enzyme is responsible for lignification and suberization in plant tissues. As an agreement, Tang and Smith (2001) have demonstrated the involvement of an NADPH oxidase and a peroxidase during studies with inhibitors on an isolate of *Verticilium albo-atrum*, nonpathogenic to *Medicago sativa*.

Results obtained from the evaluation of PPO activity clearly showed that resistance to *Fom* 1 in melon is correlated with the increase of this enzyme after infection. The defensive roles of PPO were first directly tested with PPO-overexpressing tomato plants which showed fewer lesions and increased resistance to the bacterial pathogen *Pseudomonas syringae* pv *tomato* (Li and Steffens 2002). Differences in peroxidase and polyphenol oxidase activity between resistant and susceptible groups, after inoculation, implied that these responses are closely associated with defense responses in host after pathogen penetration. Elevated POX and PPO activity was associated with induced resistance to anthracnose in cucumber (Tian et al. 2008). POX is reported to inhibit the spore germination and mycelial growth of certain fungi (Chen et al. 2000). Our results revealed that resistant genotypes of melon demonstrate increased Cucurbitacin B and D contents and accumulation of them in response to *Fom* 1 isolate. Also it seems to be no consistent correlation between disease incidence and Cucurbitacin E and I contents. There is a large variation in the amount of Cucurbitacin E in roots of different genotypes whereas Cucurbitacin I content in the susceptible and resistant genotypes followed the same pattern.

Cucurbitacins B and D have been identified as antagonists to insect steroid hormones acting at the ecdysteroid (regulation of the pupation) receptor in *Drosophila melanogaster* cells. Ecdysteroids are known to be involved in the molting process (Dinan et al. 1997). There was no Cucurbitacin D in susceptible

genotypes at day 0 (no infection) in comparison with resistant genotypes (Fig. 2). Interestingly, Cucurbitacin D was not detected in Yelloww Canary, Dastanbu and Spanish roots which were susceptible genotypes (Table 2).

Results obtained from monitoring of Cucurbitacin B content clearly proved the correlation between resistance to *Fom* 1 in melon and Cucurbitacin B increase post inoculations. There are significant differences between Cucurbitacin B content in resistant and susceptible genotypes. Differences in Cucurbitacin B and D contents (after inoculation) occurred between resistant and susceptible groups implied that these responses are closely associated with defense responses in host, after penetration of the pathogen. Therefore, it is not surprising that Cucurbitacin B level was simultaneously induced after infection by *Fom* 1. The results of Cucurbitacin B and D are strong evidence to suggest their participation in a direct defense mechanism of melon against *Fom* 1. The Cucurbitacin A, B, C, D. and E strongly inhibited growth of *Phytophthora cactorum.* Growth inhibition of *Phytoptora cactorum* caused by Pentacyclic and various tetracyclic triterpenoids indicates that these compounds may be naturally occurring fungistatic agents (Nes and Patterson, 1981). Bar-Nun and Mayer (1990) suggested that the ability of Cucurbitacins I and D to inhibit induction of laccase formation by *Botrytis cinerea* is responsible for its protective effect. Additional genetic analyses, such as co-segregation in a segregating population, are necessary to validate our results.

In conclusion, melon genotypes demonstrated significantly different reactions against race 1 of *Fusarium oxysporum* f.sp. *melonis*. After inoculation, activity levels of PPO, POX and Cucurbitacins B and D content were increased in both susceptible and resistant melon plants, however both induction and basal levels were significantly higher in resistant genotypes. The resistance to Fom1 observed in some melon genotypes, though it could be assumed a biochemical indicator for resistance in melon genotypes which has attributed in antibiotic effects mechanism.

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Table 1. Reaction of melon genotypes against infection by two isolates of Fom (M263 and MSH).

Genotype	Mean disease incidence				Genotype	Mean disease incidence			
	M263		MSH			M263		MSH	
Shahabadi	4†	a	3.66	a-e	Honeydew	3.2	e-j	3.45	a-g
Yellow Canary	3.91	ab	4	a	Garmak	3.2	e-j	2.13	lm
Spanish	3.9	a-c	4	a	Kalegorgi	3.14	f-k	2.19	lm
Dastanbu 1	3.87	a-c	3.65	a-e	Mashhady Kochak	3.11	g-l	2.05	m
Dastanbu	3.85	a-c	3.22	b-i	Sabz	3.1	g-l	2.54	i-m
Bargney	3.85	a-c	3.59	a-f	Zolfaroos	3.02	h-l	1.99	m
Goosht-Narengi	3.76	a-d	2.15	lm	Samsori	2.86	i-m	2.54	i-m
Ghalehatam	3.75	a-d	2.81	g-l	Zard-Jalali	2.85	i-m	3.77	a-d
Rishbaba	3.75	a-d	3.86	ab	Mashhady	2.77	j-m	2.23	lm
Khiarchanbar	3,65	a-e	3.66	a-e	Semnani	2.74	k-m	3.23	b-h
Dastanbu 2	3,64	a-e	3.86	ab	Ahmadi	2.69	l-n	2.4	k-m
Shadegani	3.57	a-e	2.48	j-m	Gharamaleki	2.68	l-n	2.11	m
Ananasi	3.5	b-g	3.03	e-k	Kashefi	2.46	nm	3.25	m
Talebi	3.48	b-h	2.52	j-m	Khaghani	2.46	nm	2.08	m
Amochi	3.45	c-h	2.05	m	Saboni	2.3	n	3.37	a-g
Sefidak	3.38	d-h	3.1	c-j	Jimabadi	2.27	n	2.93	g-k
Sooski-Zard	3.36	d-h	3.8	a-c	Talebi-Chorook	1.86	o	1.11	n
Tusorkh	3.33	d-h	2.65	h-m	Zard-Shotori	1.85	o	1.38	n
Alamdary	3.27	e-i	3.09	d-k	Mahali	1.63	op	1.26	n
Samsoori	3.25	e-i	2,52	j-m	Khatoni	1.37	op	1.17	n
Kharboze	3.25	e-i	3.8	a-c	Chorok-Zard	0.88	p	0.43	o
Amirpangi	3.21	e-j	2.96	e-k	Ogen	0.51	q	0.79	no
Sweet hert	3.2	e-j	3.4	a-g					

Rating scale (0–4); (0): no symptoms, (1): beginning of Yellowwing or wilting, (2): leaves heavily affected, (3): stem standing and leaves completely wilted, and (4): death of plants.

† Data are means of three replications per screening test. Means in each column with the same letters indicate no significant difference according to Duncan's multiple range test (*P = 0.05).

Table 2. PPO and POX activities and Cucurbitacin contents in the resistant (R) and susceptible (S) melon genotypes inoculated by Fom 1 isolate M263.

Genotypes	Cucurbitacin content mean (ppm)				Enzyme activities	
	B	D	E	I	PPO mean	POX mean
Mahalli (R)	1.87 C†	0.27 C	2.29 A	1.71DE	0.052 B	0.31 B
Khatooni (R)	1.75 D	0.26 C	0.94 E	1.65 E	0.044 CD	0.2 E
Zard-Shotori (R)	2.07 A	0.5 AB	0.98 E	1.91 B	0.045 C	0.29 C
Ogen (R)	1.94 B	0.48 B	0.83 F	1.94 B	0.06 A	0.46 A
Chorok-Zard (R)	1.85 C	0.54 A	0.27 G	1.78 CD	0.048 BC	0.27 C
YellowwCanary (S)	0.44 G	0 F	1.61 B	1.61 E	0.035 EF	0.24 D
Shahabadi (S)	0.72 E	0.18 D	1.07 D	1.93B	0.038 DE	0.17 F
Dastanbu (S)	0.45 G	0 F	0.75 F	1.83 BC	0.024 G	0.25 D
Dastanbu 1(S)	0.47 G	0.13 E	0.83 F	1.87 BC	0.027 G	0.19 EF
Spanish (S)	0.67 F	0 F	1.36 C	2.08 A	0.03 FG	0.23 D

† Different letters indicate significant differences among means according to Duncan's multiple range test (*P ≤ 0.05).

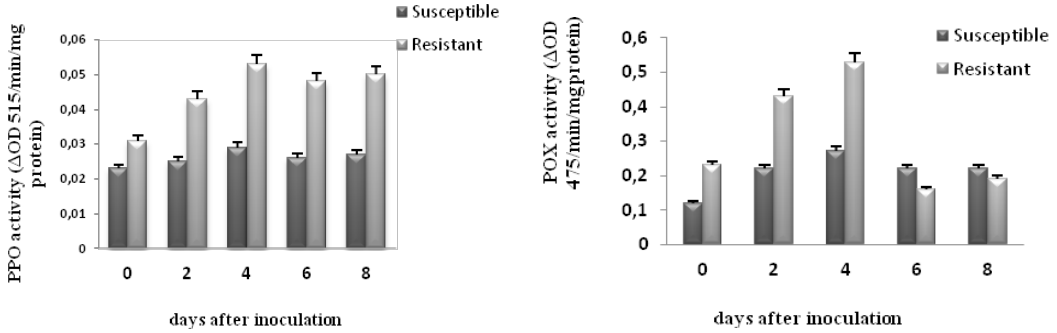

Fig. 1. PPO (left) and POX (right) activities at 0 (control), 2, 4, 6 and 8 days after inoculation with *Fusarium oxysporum* f.sp. melonis race 1 isolates M263 in resistant and susceptible genotype means. Data are average of three replicates. Error bars indicate ±SE (P ≤ 0.01).

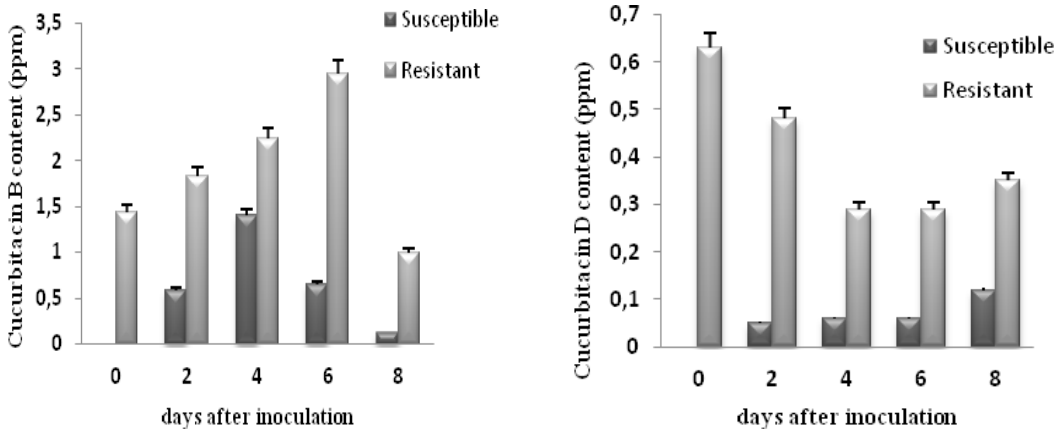

Fig. 2. Cucurbitacin B (left) and D (right) contents in roots after infection with *Fom* 1 isolate M263 in resistant and susceptible genotypes of melon. Data are average of resistant and susceptible genotypes in three replicates. Error bars indicate ±SE (**P ≤ 0.01).

Fusarium wilt of watermelon: A historical review

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Keywords: *Fusarium oxysporum* f. sp. *niveum, Citrullus lanatus*, plant breeding, resistance, pathogen races, cucurbits

Abstract

In the early 1890s a mysterious wilt disease of watermelon was causing heavy losses in the southern United States. E.F. Smith began the seminal research on what became only the second plant wilt disease described, Fusarium wilt of watermelon, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *niveum*. Within a few years, Smith characterized and named the casual agent, established its pathogenicity and host specificity and performed detailed histological studies on the transport of microconidia within the xylem and the formation of 'emboli sms' (tyloses), a characteristic later shown to be common to all Fusarium vascular wilts. Almost immediately, W.A. Orton began hybridization studies to develop a wilt-resistant watermelon cultivar; a concept that at the time was not fully embraced by the scientific community. Over the ensuing decades, numerous breeders improved upon wilt-resistant cultivars and, by the 1950s, the disease was well controlled throughout much of the US and other countries. Resistance, however, was not universal and, in some areas, cultivars succumbed to wilt. Physiologic specialization (races) of *F. o*. f. sp. *niveum* was investigated as early as 1934, as well as host specificity and the concept of formae speciales (f. sp.). The appearance of new races has been a subject of great interest for years and continues to spark debate. Methods to identify and distinguish among the various formae speciales and races, including vegetative compatibility and numerous molecular techniques, continue to generate interest, although inoculation of differential cultivars is still the primary means of identifying pathogenic isolates and races. *F. o*. f. sp. *niveum* was one of the first Fusarium wilt pathogens to be associated with seed, a problem that still exists today. Numerous other control strategies have been investigated over the last 60 years and include nutritional, cultural, and biological methods, but none have replaced genetic resistance as the preferred method of control. Recent research has focused on several main areas including a better understanding of the inheritance of wilt resistance, sequencing and mapping the watermelon genome and

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the development of race 2-resistant varieties derived from the highly resistant PI 296341-FR germplasm. Additional investigations into the histological, molecular, genetic and biochemical mechanisms of resistance and susceptibility are improving our understanding of this tenacious disease. While it has been almost 120 years since Smith first described Fusarium wilt of watermelon, it continues to be a significant cause of economic losses in watermelon production around the world.

INTRODUCTION

In the early 1890s a mysterious wilt disease of watermelon [*Citrullus lanatus* (Thumb.) Matsum. & Nakai)] in the southern U.S. was causing significant damage. At the same time similar wilt diseases of cotton and okra were occurring. In 1892, Atkinson (1892) first reported that the cotton wilt disease was associated with a fungus he named *Fusarium vasinfectum*, although he left numerous questions unanswered and even speculated that bacteria might be the cause. This led Erwin F. Smith, a plant pathologist with the USDA to investigate the other "wilt diseases of the South" (Smith 1899). Smith's early work on watermelon wilt included detailed descriptions of the fungus, its host specific and inoculation and histological studies detailing the transport of microconidia within the xylem and the formation of "embolisms in the vascular system" (tyloses). Fusarium wilt of watermelon was only the second Fusarium wilt disease described; the first being on cotton. By 1899 similar wilt diseases of muskmelon and cucumbers were reported from Ohio and Connecticut.

Smith named the watermelon wilt fungus *Fusarium niveum* E.F.S. and suggested it was the asexual stage of the perithecial ascomycete fungus, *Neocosmospora vasinfectum* Atk., which Atkinson had named as the cotton wilt pathogen. As detailed as Smith's studies were, he actually misidentified these fusaria as having the ascomycete perfect (sexual, teliomorph) stage of *Neocosmospor*a. It was W.A. Orton, who, several years later, established the pathogenicity of *F. vasinfectum* on cotton and that it did not have a *Neocosmospora* teliomorph. Snyder & Hansen's (1940) revision of the Fusarium section Elegans reduced 10 species to one, *F. oxysporum,* and included numerous pathogenic formae speciales. Thus, the watermelon wilt pathogen became *F. oxysporum* Schlechtend.: Fr. f. sp. *niveum* (E.F. Sm.) W. C. Synder & H. N. Hans.

WATERMELON BREEDING PROGRAMS FOR FUSARIUM WILT RESISTANCE

If the early history on the biology and taxonomy of *F. o.* f. sp. *niveum* began with Smith, the seminal research on its control began with William A. Orton. Orton joined the USDA laboratory of E. F. Smith in 1899 to continue investigations into the cotton wilt disease. Orton's first publication (Orton 1900) proposed the use of resistant varieties to mange cotton wilt and within 2 years he had developed wilt resistant selections of cotton. Following his work with cotton he

developed wilt resistant cowpeas his pioneering work on hybridization between an inedible resistant wild citron and the susceptible edible watermelon variety 'Eden,' led to the first Fusarium wilt resistant watermelon, 'Conqueror' (1907).

One of the earliest wilt resistant watermelon breeding programs was established in 1924 at the Iowa Agricultural Experiment Station. Fusarium wilt was so severe there at the time that it was credited with reducing Iowa's watermelon acreage by 90% (Parris 1949). Over the next 10 years Porter and Melhus released several wilt resistant cultivars including 'Improved Kleckley Sweet No.6'. One of most storied watermelon breeding programs began at the Leesburg Unit of the University of Florida's Agricultural Experiment Station in 1930 and continues today. Marion Walker was the first watermelon breeder and the first wilt resistant watermelon released was 'Leesburg', a melon derived from a selection and selfing program using 'Kleckley Sweet' (Walker 1936). Walker later released 'Blacklee', a wilt resistant variety developed from a fortuitous cross of 'Hawkesbury WR' x 'Leesburg' made in 1936. One selection from this cross was designated 'Florida seedling 124' that ultimately was used to develop 'Blacklee'. 'Florida seedling 124' has been recognized as the source of a high-level of resistance to wilt in later cultivars such as 'Calhoun Gray, 'Summit' and 'Calhoun Sweet' (Crall 1981, Netzer and Weintall 1980).

In the mid-1940s, C. Fred Andrus began a watermelon breeding program at the U.S. Vegetable Laboratory in Charleston, SC and, in 1954, released 'Charleston Gray', a cultivar that dominated the world market for more than two decades and became the most widely-grown watermelon in the U.S. and throughout much of the world. 'Charleston Gray' displayed many desirable horticultural traits along with resistance to both Fusarium wilt and anthracnose and it has been estimated that over 5 million pounds of 'Charleston Gray' seed have been sold since its release in the mid 1950s (Adams 1994). Another watershed watermelon breeding program was instituted by Charles Hall at Kansas State University in 1953. Numerous greenhouse crosses, inbreeding and field selections were made over a period of years ultimately resulting in the release of 'Crimson Sweet' in 1963. 'Crimson Sweet' had a remarkably high level of wilt resistance, combined with a deep red, sweet flesh. Other varieties followed, including 'Petite Sweet' and 'Allsweet', but none captured the market like 'Crimson Sweet'. Today, much of the US watermelon market is dominated by seedless varieties, almost none of which have any wilt resistance.

PHYSIOLOGIC SPECILAIZATION AND GENETIC RELATEDNESS

Formae speciales

The Cucurbitaceae plant family is affected by several vascular wilts caused by formae speciales of *Fusarium oxysporum* that are generally considered host-specific. There are reports of cross pathogenicity (Owen 1955; Davis 1966; Martyn & McLaughlin 1983a; McMillan 1986; Gerlagh & Blok 1988) although these are

mostly laboratory and greenhouse phenomena. In the field, the cucurbit formae speciales are generally host-specific. Seven different formae speciales are recognized as causing wilts in cucurbits, but economically, the most important ones are *F. oxysporum* f. sp. *melonis* (FOM; melon), f. sp. *cucumerinum* (FOC; cucumber), and f. sp. *niveum* (FON; watermelon). In addition, several physiological races have been identified within each forma specialis. There is considerable genetic diversity within *F. oxysporum* (Kistler 1997; Baayen et al. 2000) and numerous techniques have been employed to examine relatedness between isolates within and among different formae speciales. Thus far, no single technique or combination of techniques will completely answer the relatedness question among formae speciales and races. Molecular techniques such as mtDNA and rDNA RFLPs and DNA fingerprinting with nuclear repetitive DNA have indicated both monophyletic and polyphyletic origins for different formae speciales. Within the cucurbit wilt fusaria, FON appears to be monophyletic (Kim et al. 1993a) while FOM may be polyphyletic (Jacobson & Gordon 1990b; Namiki et al. 1994). Additionally, the mtDNA RFLP maps of FON and FOM are very similar, if not identical (Jacobson & Gordon 1990b; Kim et al. 1992a).

Vegetative compatibility groups (VCGs) may be the most practical means to distinguish among formae speciales, as pathogenic subgroups often are limited to one or a few VCGs and isolates within a VCG tend to be more similar than isolates in different VCGs. Additionally, they tend to represent clonal lineages (Leslie 1993; Gordon and Martyn 1997). In many cases there is a relatively simple and direct relationship between forma specialis, race and VCG (Correll 1991), including *F. o.* f. sp. *niveum* (Larkin et al. 1990). There are exceptions to this generality, however, and other formae speciales (e.g., f. sp. *melonis*) have a more complex relationship between formae speciales, race and VCG (Jacobson & Gordon 1988, 1990a). Thus, other phenotypic, genetic and nuclear markers should be used in conjunction with vegetative compatibility and pathogenicity. The cucurbit wilt fusaria, *F. o.* f. sp. *melonis* (FOM), *F. o.* f. sp. *niveum* (FON) and *F. o.* f. sp. *cucumerinum* (FOC) have the VCG codes 013-, 008- and 018-, respectively (Kistler et al. 1988).

Host specificity vs. cross pathogenicity

The formae speciales concept was based on strict host specificity of the strains and it was intended to describe the physiological capabilities of the fungi and was not part of the formal taxonomic hierarchy. There are exceptions to strict host specificity and many formae speciales are pathogenic to related plants, often in same genus or family. Bouhot (1981) showed that within the cucurbit wilt fusaria, one forma specialis could be converted into another through laboratory mutagenesis and that the pathogenicity factors in these forms could coexist in one isolate. Owen (1955) indicated an isolate from cucumber was highly pathogenic to muskmelon and slightly pathogenic to watermelon while Martyn & McLaughlin

(1983a) reported an isolate of *F. o.* f. sp. *niveum* caused seedling wilt in some cultivars of *Cucurbita pepo.* MacMillan (1986) reported isolates of *F. oxysporum* obtained from wilted cucumber plants in the Bahamas were pathogenic to cucumber, melon and watermelon, while isolates from Florida cucumbers were host specific. Similarly, isolates from wilted cucumbers in the Netherlands also were pathogenic to melon and watermelon (Gerlagh & Blok 1988). Collectively, these reports indicate that within the cucurbit-infecting formae speciales, strict host specificity is not always the case and that multiple formae speciales share characteristics necessary for pathogenicity. This led Gerlagh & Blok (1988) to suggest that we do away with individual forma specialis within the Cucurbitaceae family and erect a new forma specialis – *F. o.* f. sp. *cucurbitacearum* – one that would encompass all the cucurbit forms. While this was an interesting idea, it has not come about.

Detection and identification of *Fusarium oxysporum* f. sp. *niveum*

Identification of *F. o.* f. sp. *niveum*, or for that matter, any forma specialis, has been based almost exclusively on the isolation of the pathogen from symptomatic plants and inoculation of healthy plants of the same and / or related species. The fact that most formae speciales are host specific gives a generally reliable identification using this method. It is, however, laborious and time consuming, requires growth chamber or greenhouse space, may take up to several weeks to conclude, and is fraught with variables that often go unrecognized or uncontrolled by the researcher. In addition, multiple isolates of the fungus should be tested to guard against using nonpathogenic strains of *F. oxysporum* in the inoculation tests.

Because formae speciales of *F. oxysporum* are morphologically identical, distinguishing between them in culture is not possible. Over the years multiple techniques have been evaluated to accurately identify and distinguish among different formae speciales. Molecular techniques, particularly the polymerase chain reaction (PCR), have become popular for the detection and identification of plant pathogens (Henson et al. 1993; Saikia & Kadoo 2010). In fungi, the genomic ribosomal DNA (rDNA), and particularly the internal transcribed spacer (ITS) regions, have proven to be very useful in distinguishing among a wide range of fungi. The ITS region is typically conserved at the species level but varies in higher taxa, making species identification practical, although it typically is not effective at lower taxa levels. Zhang et al. (2005) developed a PCR protocol based on species-specific primers from ITS sequences that could accurately detect and distinguish *F. o.* f. sp. *niveum* from *Mycosphaerella melonis*, causal agent of gummy stem blight of melons. A single 320 bp fragment was amplified from all 24 Chinese isolates of *F. o.* f. sp. *niveum* and a single 420 bp fragment from 22 Chinese isolates of *M. melonis*. Lin et al. (2010) also developed a PCR assay for the detection and differentiation of *F. o.* f. sp. *niveum* from other formae speciales,. In

this case the primer set was not designed from rDNA ITS sequences, but from a novel RAPD fragment identified in an isolate of *F. o.* f. sp. *niveum* from Taiwan, designated OP-M12$_{411}$. Primer set Fon-1/Fon-2 amplified a single 174 bp fragment specific to all isolates of *F. o.* f. sp. *niveum* from both Taiwan and the U.S., but did not amplify DNA from 13 other formae speciales, including those infecting other cucurbits (ff. spp. *melonis, cucumerinum, luffae and momordicae*).

Physiologic races

Ideally, physiologic specialization in plant pathogens, or races, is identified based on specific disease reactions following inoculation of a differential host set, with each differential cultivar in the set differing by a specific and different resistance gene. This works reasonably well when a single, strong, dominant gene confers resistance, but it becomes more ambiguous when resistance is recessive or conferred by multiple genes or when there are epistatic affects. Although there is no standard nomenclature for designating races, convention states that pathological races are defined by the corresponding resistance genes in the host they defeat. In the cucurbits, race designation of formae speciales of *F. oxysporum* generally follows that proposed by Risser et al. (1976) for *F. o.* f. sp. *melonis*. This protocol has been used for designating races in the other cucurbit formae speciales (Martyn & Vakalounakis 2012), however, in some cases the specific resistance gene(s) have not been identified.

To date, four races of *F. o.* f. sp. *niveum* are described in the literature (race 0, 1, 2, & 3), but there is some disagreement on whether all four are valid descriptions (Martyn & Vakalounakis 2012). Following the genetic model proposed by Henderson et al. (1970), Cirulli (1972) classified isolates from Italy into two pathogenic groups: race 0 and race 1. FON race 1 occurs most commonly around the world and is the pathological phenotype originally described by Smith from South Carolina. FON race 0 was first reported from Florida (Crall 1963) and was distinguished from race 1 by causing wilt only in varieties that carried no resistance genes. As a result, race 0 is of minor economic importance since most commercial cultivars have the *Fo-1* gene. There is growing speculation, however, that the distinction between race 0 and race 1 may be more quantitative than qualitative and, consequently, race 0 and race 1 may all be strains of race 1 varying in aggressiveness (Martyn 1987; Larkin et al. 1990). FON race 2 was described from Israel in 1976 (Netzer 1976) and from Texas in 1981 (Martyn 1985, 1987) and was characterized by overcoming wilt resistance in all commercial cultivars evaluated. In an initial survey of the US, Martyn & Bruton (1989) reported FON race 2 to be in three states: Texas, Oklahoma and Florida. Since then, race 2 has been reported from eight states in the US and a dozen or more countries (Egel & Martyn 2007). Zhou & Everts (2003) found all three races in commercial fields in Maryland and Delaware, with race 2 occurring with one or both of the other races in the some

of the same fields. Over one half of the isolates (57%) recovered were identified as race 1 with race 0 and race 2 accounting for 21% and 24%, respectively. Race 3 was recently described from Maryland and is reported to be pathogenic on all cultivars, including the differential PI296341-FR (Zhou & Everts 2010).

Races and vegetative compatibility

In an extensive study of *F. o*. f. sp. *niveum*, Larkin et al. (1990) showed a direct correlation between vegetative compatibility (VCG) and virulence (race). Race 1 and race 2 isolates were compatible with isolates of the same race but incompatible with the opposite race. All isolates of race 1 were in a single VCG (0081) while all race 2 isolates were in VGC 0082. All pathogenic isolates of *F. o*. f. sp. *niveum* were incompatible with non-pathogenic isolates of *F. oxysporum*. In contrast, other cucurbit formae speciales e.g., f. sp. *melonis*, f. sp. *cucumerinum* and f. sp. *radicus-cucumerinum* have a much more complex relationship, with isolates of a single race belonging to multiple VGCs, multiple races belonging to a single VCG and isolates belonging to two different VCGs (bridging isolates) (Jacobson & Gordon 1988, 1990a; Valalounakis & Fragkiadakis 1999). Zhou & Everts (2007) identified three VCG groups from a regional population of *F. o*. f. sp. *niveum* from Maryland and Delaware in the U.S. Two were the same VCGs previously described by Larkin et al. (0080 and 0082) along with a new VCG – 0083. Unlike that of Larkin et al. there was not a good correlation between VCG and race in the Maryland and Delaware populations. All three races (0, 1 and 2) were associated with both VCG 0080 and 0082 and none of the isolates were in VCG 0081, which consisted of race 1 isolates from Florida. Six isolates were in a new VGC 0083 and not compatible with other race 2 isolates, but were classified as race 2. They were later were reclassified as race 3.

Kim et al. (1995) transformed a FON race 2 isolate using a genomic library from a race 0 isolate. Most of the transformants showed no change in virulence, however, two lost virulence to the cultivars 'Calhoun Gray' and 'Charleston Gray', while retaining pathogenicity to 'Black Diamond', thus expressing a race 0 phenotype. While pathogenicity phenotype changed in these transformants, vegetative compatibility was not altered, as they retained compatibility with the race 2-tester strain and were not compatible with race 0 (Kim et al. 1992b). Little is known about the genetic makeup of *F. o*. f. sp. *niveum*; however, Kim et al. (1993b) reported 5 to 10 putative chromosomes ranging in size from approximately 900 to 4,400 kb with a minimum total genome size of 15.8 to 26.0 Mb. Similar numbers and sizes of putative chromosomes have been identified by others (Migheli et al. 1993; Min 1995).

THE GENETICS OF RESISTANCE

Resistance genes and pathogenic races

Resistance to Fusarium wilt was initially thought to be a recessive trait (Walk-

er 1941), and it wasn't until many years later that the inheritance of resistance to FON race 1 was shown to be conferred by a single, dominant gene, *Fo-1* (Henderson 1970; Netzer and Weintall 1980). Resistance to races 1 and 2 of Fusarium wilt of cucumber (*F. o*. f. sp. *cucumerinum*) also is controlled by a dominant gene, *Foc* (syn. *Fcu-1*). Similarly, resistance to races 0, 1 and 2 of Fusarium wilt of melon (*F. o.* f. sp. *melonis*) is conferred by two different dominant genes, *Fom-1* and *Fom-2* (Martyn & Vakalounakis 2012). Little is known about the mechanism of Fusarium wilt resistance genes, however one study sheds some light on this. Diener and Ausubel (2005) reported that in *Arabidopsis thaliana* ecotype Col-0, resistance to *F. o*. f. sp. *matthioli* is conferred by six dominant loci (*RFO*) but one locus had the strongest effect (*RFO1*). *RFO1* also conferred resistance to *F. o*. f. sp. *raphani* in ecotype Ty-0, suggesting that *RFO1*-mediated resistance is not race-specific. Map-based cloning of $RFO1^{Col-0}$ showed that *RFO1* is identical to a previously named *Arabidopsis* gene *WAKL22* (*WALL-ASSOCIATED KINASE-LIKE KINASE 22*), which encodes a receptor-like kinase that does not contain an extracellular leucine-rich repeat domain.

Over the last two decades, FON race 2 has become more prominent around the world. A screening program was begun early to identify resistance to race 2 and, in 1991, an inbred PI line (PI 296341-FR) highly resistant to race 2 was released (Martyn & Netzer 1991). While this line has exceptional resistance to races 0, 1 and 2, the resistance was not fixed, and it continues to segregate for resistance: susceptibility at a ratio of approximately 95:5. PI 296341-FR was added to the host differentials for race-typing isolates of *F. o*. f. sp. *niveum* While the inheritance of FON race 1 resistance is relatively simple, inheritance of race 2 resistance in PI 296341-FR is more complicated and likely is conferred by one or more major dominant and recessive genes interacting with some minor genes (Zhang and Rhodes 1993; Hawkins et al. 2001; Zou et al. 2011). Zhang and Rhodes (1993) suggested that resistance to FON race 0 in PI 296341-FR is dominant but is affected by nonallelic modifier genes that can overcome the resistance. Inheritance of FON race 1 resistance appears to a dominant gene, similar that described by Netzer and Weintall (1980) but also affected by modifier gene(s). Resistance to FON race 2 is governed by at least one recessive pair of genes, but a dominant gene from a susceptible parent is epistatic over the recessive gene for resistance in PI 296341-FR. As a consequence, transferring the high level of resistance in PI 296341-FR into commercial cultivars has proven difficult. Xu et al. (2000) reported cloning and sequencing a RAPD marker linked to FON race 1 resistance in PI 296341-FR that appeared to be only one copy and was useful in selecting resistant plants in the F_3 population of introgressed plants, while Hawkins et al. (2001) reported numerous RAPD markers linked to Fusarium resistance but the marker loci were too large for effective use in marker-assisted selection. Recently, a diploid watermelon hybrid 'Super Pollinizer-5®' (SP-5) with the PI 296341-FR resistance has been developed by Syngenta Seeds for use as a

pollinating parent in seedless hybrid watermelon production (X. Zhang, Syngenta Seeds, personal communication). Other sources of resistance to FON race 2 have been reported (Dane et al. 1998; Wechter et al. 2012). Two plant introductions from Zimbabwe (PI 482246 and PI 482252) and one from South Africa (PI 271769) displayed partial resistance to FON race 2 in initial tests (40-56%) but resistance was increased significantly (89-100%) after a self-pollinated generation (S_1).

Effect of inoculum concentration on resistance

Resistance to plant diseases is known to be affected by, among other things, the amount of inoculum. There are only a few studies that have been conducted with watermelons. Numerous authors have evaluated watermelon cultivars for wilt resistance. In spite of reports indicating a gradation in wilt resistance from none to very high, most commercial cultivars are described simply as resistant or susceptible to Fusarium wilt and the degree of resistance is not usually indicated. Elmstrom & Hopkins (1981) reported almost a five-fold reduction in yield of 'Charleston 76' when grown in a field that had been continually cropped to watermelon for 6 years. While they did not determine the population levels of *F. o.* f. sp. *niveum* in the soil, it can be assumed that it increased over time and, thus, may have affected the apparent wilt resistance. In another study, wilt severity increased each year in both susceptible and resistant cultivars over a 4-year monoculture with the exception of 'Crimson Sweet', which remained low (Hopkins & Elmstrom 1984). Sumner (1972) examined several different concentrations of *F. o.* f. sp. *niveum* on wilt development in cultivars of known wilt resistance. Slightly resistant or susceptible cultivars wilted severely at 10^2 and 10^3 conidia/ml concentrations while progressively more inoculum was needed to cause wilt in resistant cultivars. Martyn & McLaughlin (1983b) evaluated 17 watermelon cultivars varying in resistance from highly susceptible to highly resistant against inoculum levels from 1×10^3 to 1×10^6 conidia / ml. In general, the apparent wilt resistance ranking of each cultivar (susceptible, slightly resistant, moderately resistant and highly resistant) dropped one level with each 10-fold increase in inoculum. Thus, cultivars that were moderately or highly resistant at the lowest inoculum levels were highly susceptible at the highest inoculum levels. An exception was noted for two cultivars, 'Dixielee' and 'Smokylee', which remained highly resistant at each of the inoculum levels. Zhou & Everts (2003) surveyed numerous watermelon fields in Maryland and Delaware to determine the level of *F. o.* f. sp. *niveum* present in each field and the subsequent amount of wilt observed in a susceptible cultivar at harvest. They reported values ranging from 5 to 5,100 CFU/g soil with wilt severity ranging from 15 – 100% across the fields. They concluded that the minimum amount of inoculum necessary to cause wilt was 166 CFU/g soil and that 367 CFU/g was enough to cause wilt in 50% of the plants (ID_{50}). In most of the fields examined (73%) the CFU/g soil values ranged between 100 and 1,200.

INFECTION, COLONIZATION AND SURVIVAL

The early studies by Smith (1899) and others suggested that *Fusarium* enters the plants via the roots and proceeds to grow into the xylem vessels, where it causes a blockage of water transport, resulting in wilt. As simple as this seems, the controversy as to the exact mechanism(s) of wilt still rages on.

Histopathology

Fusarium oxysporum is a very good colonizer of plant roots and it is one of the most common fungi isolated from asymptomatic roots of crop plants. That saprophytic isolates of *F. oxysporum* do not cause a wilt disease is presumably due either to their inability to enter the vascular tissue or to a rapid response of the host that localizes the infection (Gao et al. 1995). Strains that enter into the parasitic phase, however, make their way through the root tissue and into the xylem elements (space-0) (Beckman & Roberts, 1995) where they become full fledged vascular wilt pathogens. Microconidia are produced and carried in the transpiration stream until they are blocked by the vessel end-walls where they must then germinate, penetrate through the end-wall and produce a conidium on the other side that then can be carried to the next vessel (space-1). This process is repeated every 2 -3 days until the fungus has colonized much of the host's xylem vessels. Extracellular cell wall degrading enzymes (endopolygalacturonases, cellulases, hemicellulases, pectinases and others) of both host and pathogen origin are produced resulting in the formation of gums and gels that occlude the vessels, limiting water transport. Additionally, tyloses may be induced adding to the blockage resulting in permanent wilt and ultimately plant death. Di Pietro et al. (2003) and Recorbet et al. (2003) provide excellent reviews of the current understanding of the underlying mechanisms and molecular determinants of pathogenicity of Fusarium wilt diseases.

Resistance to Fusarium wilts has been correlated to the rate of colonization of the xylem tissue by the fungus, or conversely, the speed at which the host can block the pathogen's spread (Beckman 1995). Much of the information stems from work done with Fusarium wilt of tomato and only limited work has been done with watermelon. Di Pietro et al. (2001) identified a MAP kinase gene in f. sp. *lycopersici*, *fmk1* that was essential for root penetration and pathogenesis. Similarly, Jonkers et al. (2009) concluded that the F-box protein Frp1 is required for f. sp. *lycopersici* to colonize and invade tomato roots. They concluded that the inability of the *Δfrp1* strain to colonize and invade roots was the result of reduced expression of several cell wall degrading enzymes. Using a GFP-tagged isolate of f. sp. *niveum* race 1, Lü et al. (2011) showed that by 12 hours post inoculation (hpi), the pathogen attached and grew on the root surface of the resistant watermelon line, PI 296341-FR. By 24 hpi more than 50% of conidia on the root surface germinated and grew along the axis of the root. By 3 dpi, the hyphae penetrated into the epider-

mal cells and formed appressoria at the penetration sites. At 5 dpi, most of the root surface was covered with mycelium and by 8 dpi the fungus began to sporulate on the root surface. However, the fungus did not grow into the xylem vessels and no wilt symptoms occurred in the FON race 1 / PI 296341-FR incompatible reaction. In contrast, hyphae colonized the central cylinder of the roots, grew into the xylem vessels and fully colonized and destroyed the roots by 7 dpi in the compatible reaction between FON race 1 and the susceptible watermelon 'Black Diamond'. Zhou & Everts (2004) showed there was significantly less colonization of the roots and stems of resistant watermelon cultivars by f. sp. *niveum* than susceptible cultivars. Percent wilt was positively correlated with colonization in the roots and lower stems and there was a link between cultivar resistance and a reduced rate of spread of the fungus in the stems. Similar results were observed by Chang et al. (2008).

Seed-borne nature of *F. o.* f. sp. *niveum*

Numerous plant pathogens are capable of surviving in or on propagative plant parts, including seeds. Each of the major cucurbit wilt Fusarium formae speciales (watermelon, melon and cucumber) can be seed borne (Martyn & Vakalounakis 2012). Fulton and Winston (1915) first reported that the watermelon Fusarium could be seed-borne. In this case, the pathogen was recovered from the external seed coats after washing. Porter (1928) showed conclusive evidence that the watermelon wilt pathogen could be seed-borne and isolated '*F. niveum'* from seeds obtained from melons attached to wilting vines, as well as from seeds obtained from commercial seed suppliers in Georgia and Texas. Martyn (1987) isolated the highly aggressive FON race 2 from the seeds used to produce a seedless watermelon hybrid in Texas. Most recently, using nit$^-$ mutants Kleczewski & Egel (D Egel, person commun) showed that *F. o.* f. sp. *niveum* could enter into the flesh and seeds of watermelon when inoculated directly into the peduncle, although they were not able to recover the pathogen from seeds when inoculated directly onto the flowers.

Survival in the soil

F. oxysporum survives season to season in the soil primarily in the form of chlamydospores that are produced both in the hyphae and by the conversion of microconidia formed in sporodochia. Saprophytic survival of *F. oxysporum* in the soil occurs, but, in most cases, it is probably short-lived, owing to the limited capabilities of the hyphae to survive periods of stress. Soils rich in organic matter and crop residues may provide a better environment for saprophytic survival, but still not to the extent that chlamydospores provide. Numerous studies indicate that *F. o.* f. sp. *niveum* can remain in soil for extended periods of time. As early as 1895, Smith knew it could survive long periods in the absence of a host and recommended that "fields already infested with this fungus must not be planted to melons for along

series of years". Porter (1928) stated that in Iowa that a watermelon field that experienced a slight amount of wilt in 1910 was not planted with a cultivated crop of any kind for 16 years, yet when watermelons were again planted in 1917, about 50% of the plants were partially or wholly infected. Laboratory studies of the long-term survival of formae speciales of *F. oxysporum* were conducted by McKeen & Wensley (1961). Using ff. spp. *lycopersici, melonis*, and *niveum*, they demonstrated that all three could survive unchanged morphologically and pathogenically for 11 years or more in soil tube cultures stored at 3 - 4 °C and that *F. o*. f. sp. *melonis* remained viable after 17 years of storage. As a consequence, management recommendations for Fusarium wilt of watermelon include crop rotation schemes of 5-7 years.

MANAGEMENT OF FUSARIUM WILT

Watermelon genomics and resistance to Fusarium wilt

Much of the effort in watermelon genome sequence and mapping projects has been directed primarily at identifying horticultural markers and genes involved in fruit and plant development, including fruit shape, rind, flesh and seed color, sweetness, etc. and resistance genes to foliar pathogens. Only recently have they focused on identifying markers for Fusarium wilt resistance (Hawkins et al. 2001; Levi et al. 2001; Guo et al. 2010). Several linkage maps based on different markers have been constructed (Levi et al. 2002, 2006; Zhang et al. 2004; Guo et al. 2010; Ren et al. 2012) and the watermelon genome has recently been sequenced (http://www.icugi.org/cgi-bin/ICuGI/genome/home.cgi) by collaborators in the International Watermelon Genomics Initiative, led by the National Engineering Research Center for Vegetables, Beijing, China (Ren et al. 2012). Using a high-resolution genetic map, 93.5% of the assembled sequence was anchored onto eleven chromosomes. Based on the complete sequence and current assembly, there is an estimated 23,440 predicted genes in *C. lanatus*. Marker Assisted Selection (MAS) has helped speed up the identification of important genetic traits, including disease resistance genes. Lin et al. (2009) developed a PCR protocol for the differentiation of watermelon lines resistant to *F. o*. f. sp. *niveum*. Using sequence characterized amplified region (SCAR) markers they developed a PCR protocol that amplified a single 898 bp fragment in FON-resistant watermelon lines but not in susceptible lines. An interesting approach to developing transgenic resistant watermelons was used by Chen et al. (1998). Using a genomic fragment of squash DNA fused with the GUS marker, they transformed watermelon via the pollen-tube pathway by injecting the ovaries 48 hr after hand pollination with the foreign DNA. Approximately 5% of the transgenic plants obtained from seeds from the parents were resistant to Fusarium wilt after inoculation and transplanting to the field. Resistance was apparent in both the T_1 and T_2 progeny. The nature of the squash DNA fragment responsible for the resistance is unknown.

Lü et al. (2011) performed a transcriptome profile analysis of an incompatible and compatible reaction of watermelon inoculated with *F. o.* f. sp. *niveum*. Using PI 296341-FR and FON race 1 as the incompatible reaction and an Agilent custom microarray containing approximately 8,200 watermelon genes they showed significant differential expression in 24 to 592 genes in roots at different times post inoculation of the incompatible reaction. Genes that were differentially induced during the incompatible reaction but not in the compatible reaction included pathogenesis-related (PR) genes, transcription factors, signaling/regulatory genes and cell wall modification genes. Most genes involved in jasmonic acid (JA) biosynthesis also were expressed stronger and for longer periods of time in the incompatible reaction compared to the compatible reaction, as were those genes associated with the shikimate-phenylpropanoid-ligin biosynthesis. In contrast, genes for transporter proteins such as aquaporins were down-regulated in the incompatible reaction, indicating that transporter proteins might contribute to the development of wilt symptoms after infection. Inoue et al. (2002) identified a mutant strain of *F. o.* f. sp. *melonis* (B60) that displayed greatly reduced virulence to melon. Molecular analysis revealed that the affected gene, *FOW1*, encodes a protein with strong similarity to mitochondrial carrier proteins (MCP) of yeast. *FOW1* is conserved in other formae speciales and *fow1* mutants of f. sp. *lycopersici* also are avirulent on tomato.

Cross protection and induced resistance

Cross protection (induced resistance) in plants is the ability of one pathogen or strain to protect a plant from a similar or related pathogen or strain and the phenomenon was first described by with tobacco mosaic virus and tobacco. Several authors have examined induced resistance in cucurbits (Davis 1967; Shimotsuma et al. 1972; Mas et al 1981; Gessler & Kuć 1982; Biles & Martyn 1989). In almost all cases, each demonstrated protection of susceptible plants against Fusarium wilt or a foliar pathogen such as *Colletotrichum lagenarium*, when the plants were preinoculated (induced) with either a nonhost forma specialis or an avirulent race. In most cases too, the more closely related the inducing organism was to the challenge organism (e.g., formae speciales in the same family or avirulent races) the greater the protection. In addition, protection was generally higher when the inducing inoculum was applied 24 h before the challenge inoculation. Most research on the mechanisms of induced resistance has focused on the shikimate and phenylpropanoid metabolic pathways and key enzymes such as peroxidases, polyphenoloxidase, phenylalanine ammonia lyase, and chalcone synthase. Numerous correlations between increased enzyme activity and resistance have been reported but the exact mechanisms are still largely unresolved.

Grafting watermelon onto *Cucurbita* rootstock

Control of diseases, especially Fusarium wilt, is the most important and common reason for grafting watermelons (King et al. 2008). The phase out of methyl bromide used in soil fumigation has been a major driver in the increase in vegetable grafting. Japan was an early adopter of vegetable grafting and it is now widespread throughout much of East Asia. The first record of a grafted vegetable crop was in 1927 from Japan in which watermelon was grafted onto *Cucurbita moschata* rootstock to control Fusarium wilt of watermelon. Because most formae speciales of *F. oxysporum* are host specific, roots of other cucurbit species would not become infected by *F. o.* f. sp. *niveum*. In the 1930s, bottle gourd (*L. siceraria*) rootstock gradually replaced *C. moschata* as a rootstock for watermelon. In 1957, Matuo & Yamamoto (1957) described a new forma specialis in Japan that caused Fusarium wilt in bottle gourd, *F. o.* f. sp. *lagenariae,* thus rendering bottle gourd rootstock no longer effective in controlling wilt. Bitter gourd (*Momordica charantia*) also was being used as a rootstock on watermelon in East Asia, but it too, succumbed to a new Fusarium wilt, *F. o.* f. sp. *momordicae,* first described from Taiwan (Sun & Huang 1983). Thus, the two major cucurbit rootstocks used to control Fusarium wilt of watermelon were now susceptible to their own Fusarium wilt pathogens. Vegetable grafting is becoming increasingly more popular around the world, especially in areas were labor costs are low. Grafted watermelons now account for about 95% of all watermelons grown in Japan and Korea. Robotic grafting machines are being used in parts of Asia and can generate up to 1200 grafted seedlings per hour; more than 10 times that of an experienced technician. In spite of mechanized operations, vegetable grafting has not become mainstream in the United States, possibly because of the perceived cost. And this is likely to remain the case, at least until the use of methyl bromide is mostly eliminated (King et al. 2008). The cost of a grafted watermelon seedling now averages about $1.00 per seedling, down from over $2.00 per seedling only a few years ago, but is still higher than soil fumigation using methyl bromide.

Disease suppressive soil and biological control

A disease suppressive soil, opposed to a disease conducive soil, is one in which disease severity remains limited in spite of a high pathogen inoculum density. Disease suppressive soils have been described for many diseases and in many areas of the world, but probably none have been studied as much as the Chateaurenard soils in southern France (Alabouvette et al. 2009) for their suppressiveness against Fusarium wilt of melon. In general, disease suppressiveness is essentially microbiological in nature and, as such, subject to alteration and soil properties greatly influence the degree of suppressiveness. Disease suppressiveness may be eliminated or reduced in soil, it may be induced in new soils, and it often can be transferred to different soils. The only detailed studies on Fusarium wilt of watermelon were

done in Florida. Fusarium wilt suppressive soil was induced after 7 years of watermelon monoculture (Hopkins et al. 1987). Overtime, wilt severity increased among all cultivars, regardless of the resistance level, except in two cultivars, 'Smokylee' and 'Crimson Sweet'. When soil from the 'Crimson Sweet' plots was seeded with wilt-susceptible cultivars they did not succumb to wilt. The suppressive nature of the soil was sensitive to both moist heating to 70˚C or methyl bromide. Successive cropping of 'Crimson Sweet' resulted in an increase in indigenous populations of *F. oxysporum* in the suppressive soils, along with increases in actinomycetes, fluorescent pseudomonads and overall bacteria (Larkin et al. 1993). They later concluded that a specific indigenous isolate of *F. oxysporum* was primarily responsible for the disease suppressiveness and that the mechanism was not directly related to its ability to colonize the roots, but more likely induced resistance (Larkin et al. 1996).

Harveson & Kimbrough (2002) isolated a novel mycoparasitic fungus from roots of watermelons with Fusarium wilt. The fungus was identified as *Sphaerodes retispora* var. *retispora* and when encapsulated into alginate pellets and put into FON-infested soil, significantly reduced plant mortality and increased dry weights compared to inoculated plants without the mycoparasite.

Cultural control

There are numerous reports in the literature of the effect of various crop residues and amendments on soilborne diseases, including various Fusarium wilts, but there are relatively few specific to Fusarium wilt of watermelon. Zhou & Everts (2004a) evaluated numerous plant and animal residues for their ability to suppress wilt in watermelons. A pulverized, dry cover crop of hairy vetch (*Vicia villosa*) was the best amendment and reduced the severity of wilt by up to 87% and increased plant biomass, fruit weight and sugar content of the fruit, comparable to those attained with soil fumigation. Reduced disease was not correlated, however, with a decrease in the pathogen population in the soil. In contrast, Njoroge et al. (2008) did not obtain control of Fusarium wilt of watermelon when *Brassica napus* or *B. juncea* residue was incorporated into the soil, in spite of higher levels of glucosinolates. In the same study, however, soil fumigation with methyl bromide also did not reduce the severity of Fusarium, leading the authors to conclude that neither biofumigation nor methyl bromide was an effective disease management tool in their location.

CONCLUSION

It has been almost 120 years since Fusarium wilt of watermelon was described and while we have learned an extraordinary amount of information about its biology, pathology, epidemiology and genetics it still represents the single most important disease of watermelons worldwide. With a little more than a few years of observations and greenhouse studies behind him, Smith (1899) provided the first recommendations for managing Fusarium wilt of watermelon. He stated that 1) "*Fields*

already infested with this fungus must not be planted to melons for a long series of years; 2) Fields free from this disease may become infected by the wash from lands already infested, and probably also, by means of the dirt adhering to agricultural implements and to the feet of horses and cattle; 3) Plants should be removed as soon as they show distinct symptoms of the wilt. They should be pulled while green, stacked with brush, and burned; ... 5) Farmers whose lands have become generally infected are advised to grow other crops on their own fields, and to rent uninfected land from their neighbors for the purpose of growing melons" [emphasis added]. If these recommendations sound familiar it is because they are still applicable today.

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Resistance of melon to *Cucumber Vein Yellowing Virus* (CVYV)

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Keywords. Germplasm evaluation, *Cucumis melo*, inheritance, genetic control, necrosis, mosaic

Abstract

Cucumber vein yellowing virus (CVYV) belongs to the *Ipomovirus* genus, family *Potyviridae*. It is transmitted by the whitefly *Bemisia tabaci*, but can be transmitted mechanically in artificial inoculation. A collection of 1188 melon accessions has been inoculated with a CVYV strain isolated from melon in Spain. Five phenotypes have been observed: 46 % of the accessions are susceptible with mosaic and vein-banding symptoms like the control Védrantais; 50 % are highly susceptible with a severe mosaic, yellowing and stunting. Ouzbeque 2 is one of these accessions; few accessions exhibited necrotic symptoms with a rapid death of the plants. HSD 93-20-A from Sudan is a representative of this phenotype; only one accession (HSD 2458 from Sudan) was tolerant with very mild mottle but the virus can be detected by DAS-ELISA or RT-PCR in the plant apex; only an inbred line derived from the accession PI 164323 from India was resistant, exhibiting no symptom and the virus cannot be detected in non-inoculated leaves either by DAS-ELISA or RT-PCR. Inheritance of these behaviours was studied in F_1, F_2 and BC progenies between the above mentioned accessions. Three loci seem to be involved. At a first locus tentatively named *Cucumber vein yellowing* resistance (symbol *Cvy-1*), three alleles have been identified: *Cvy-1*$^+$ for susceptibility (present in Védrantais), *Cvy-1*^{1} controlling resistance in PI 164323 and *Cvy-1*^{2} controlling necrosis in HSD 93-20-A. At an independent second locus, the recessive allele *cvy-2* present in HSD 2458 controls the tolerance. And at a third locus the allele *Cvy-3*, present in Ouzbèque 2, controls the highly susceptible type of symptoms.

INTRODUCTION

*Cucumber vein yellowing virus (*CVYV) has been first described in Israel (Cohen and Nitzany 1960), then in other countries of the Middle-East and more recently in the western part of the Mediterranean area (Cuadrado et al. 2001;

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Lecoq et al. 2007; Lecoq and Desbiez 2012). It belongs to the *Ipomovirus* genus, family *Potyviridae* and is transmitted in the semi-persistent manner by the whitefly *Bemisia tabaci*, but can be transmitted mechanically in artificial inoculation. It induces mosaic and yellowing symptoms on the leaves and can severely reduce the yield and the fruit quality.

Resistance has been described in cucumber as controlled by a dominant gene (Picó et al. 2008). Wild *Cucumis* species (*C. prophetarum, C. africanus, C. dipsaceus*) have been described as resistant (Marco et al. 2003) but up to date no accession of *C. melo* has been observed to be resistant (Montoro et al. 2004).

In this paper, we describe the screening of melon germplasm and the inheritance of observed different phenotypes.

MATERIALS AND METHODS

Plant material

A total number of 1188 accessions of a melon collection maintained in France by a network associating INRA and private seed companies have been sown in trays with 10 seeds per accession. Some accessions with a typical phenotype were then used to study the inheritance of these traits, namely: 'Védrantais', 'Ouzbèque 2', PI 164323, HSD 2458 and HSD 93-20-A. The F_1, F_2 and back-cross progenies were produced. A recombinant inbred lines population (126 RILs) was developed between Védrantais and PI 164323.

Virus material and inoculation method

A CVYV strain isolated from melon in Spain in 2003 was used for mechanical inoculation on the cotyledons at the pointing first leaf-stage. Symptoms were visually rated. DAS-ELISA and RT-PCR tests were performed on plants with no symptoms or very weak symptoms to assess the presence or absence of the virus. DAS-ELISA was done using an antiserum produced against the CVYV coat protein expressed in bacteria and RT-PCR using primers previously described (Lecoq et al. 2007).

RESULTS

Germplasm evaluation

Among the 1188 accessions, a first group (46.0% of the accessions) exhibited typical mosaic symptoms like the control 'Védrantais' and a second group (50.3%) exhibited very severe mosaic symptoms with yellowing and stunting. The accession 'Ouzbèque 2' was selected as a representative of this second group for inheritance studies. A majority of accessions belonging to the botanical varieties *cantalupensis, reticulatus* and *flexuosus* were in the first group with mosaic symptoms. A majority of accessions of the botanical varieties *agrestis, acidulus, ameri, chate, chinensis,*

dudaim, *makuwa*, *momordica*, and *tibish* were in the second group with severe symptoms. About 50% of the accessions of the botanical varieties *chito*, *conomon* and *inodorus* had mosaic symptoms and 50% severe symptoms. Twenty-three accessions exhibited a systemic necrosis a few days after inoculation; most of them originated from Sudan. HSD 93-20-A was used in the inheritance studies as a representative of this group. One accession from Sudan, HSD 2458, exhibited very mild symptoms of mottling but the virus can be detected by DAS-ELISA or by RT-PCR. Finally, an inbred line in an accession from India, PI 164323, was the only resistant accession with no symptoms and no detection of the virus in non-inoculated leaves, although very rarely few plants presented the necrotic phenotype. Interestingly, all plants from other inbred lines of PI 164323 developed a systemic necrosis few days after inoculation.

Genetic control

Inheritance of resistance

In F_1, F_2 and BC progenies from the cross 'Védrantais' × PI 164323, four phenotypes were observed: no symptom (like PI 164323), necrosis (like HSD 93-20-A), mosaic (like 'Védrantais') and severe mosaic, yellowing and stunting (like Ouzbèque 2) (Table 1a). The necrotic symptom, of the F_1 and a majority of plants in the F_2 and BC progenies, correspond to the heterozygous status. In the segregating progenies, the pooled numbers of plants with no symptom or necrosis *versus* mosaic or severe mosaic can be explained by one dominant gene. In the F_2, 267 *vs* 100 (χ^2 for 3:1 = 0.989, Prob = 33%); in the BC_s 85 *vs* 87 (χ^2 for 1:1 = 0.002, Prob = 88%) and no susceptible plant in the BC_r but one; in the RILs population, 44 RILs *vs* 38 RILs (χ^2 for 1:1 = 0.439, Prob = 51%). In the BCr, no susceptible plant (but one with severe mosaic) was observed. These segregations fitted a "one dominant gene for resistance" hypothesis with necrosis at the heterozygous status. We propose to name this gene *Cucumber vein yellowing virus* resistance (symbol *Cvy-1*).

This genetic control was confirmed in crosses between PI 164323 and HSD 2458 (Table 1c), when pooling the numbers of plants with no symptom or necrosis *versus* mild mottle, mosaic or severe mosaic. In the F_2, 393 *vs* 134 (χ^2 for 3:1 = 0.051, Prob = 82%); in the BC_s 45 *vs* 50 (χ^2 for 1:1 = 0.263, Prob = 61%) and no susceptible plant in the BC_r but one.

Inheritance of necrosis

The necrotic symptom observed in the accession HSD 93-20-A (Table 1b and 1e) was also observed occasionally in PI 164323 and frequently in the F_1, F_2 and BC progenies with PI 164323 as a parent (Table 1a and 1c). The segregation observed in the F_2 between Ouzbèque 2 and HSD 93-20-A (Table 1b) fitted a 3 (resistant or necrotic) *vs* 1 (mosaic) segregation corresponding to a monogenic dominant control

(χ^2 = 2.881, Prob = 9%).

In crosses between PI 164323 and HSD 93-20-A, the F_1 was resistant and in the F_2 no susceptible plant was observed (157 resistant and 38 necrotic). It can be concluded that the same locus *Cvy-1* is involved in the genetic control of necrosis in HSD 93-20-A but that another allele is present in HSD 90-20-A. We propose the symbol *Cvy-1*^{1} for the allele in PI 164323 and *Cvy-1*^{2} for the allele in HSD 93-20-A with the following symptoms: resistance for the homozygous *Cvy-1*^{1} / *Cvy-1*^{1} or the heterozygous *Cvy-1*^{1} / *Cvy-1*^{2} and necrosis for the homozygous *Cvy-1*^{2} / *Cvy-1*^{2} or the heterozygous (*Cvy-1*^{1} / *Cvy-1*$^+$ or *Cvy-1*^{2} / *Cvy-1*$^+$).

Inheritance of tolerance

The accession HSD 2458 exhibited very weak symptoms (mild mottle) but the virus can be detected in DAS-ELISA or RT-PCR. In the F_2 between HSD 2458 and 'Védrantais' (Table 1d), a 1 tolerant: 3 mosaic segregation was observed (χ^2 = 0.114, Prob = 74%) and in the F_2 with Ouzbèque 2, a 1 tolerant: 3 mosaic or severe mosaic segregation was observed (χ^2 = 0.346, Prob = 56%). We propose the symbol *cvy-2* for this recessive gene controlling tolerance in HSD 2458. This genetic control was confirmed in the F_2 between HSD 2458 and PI 164323 (Table 1c) with a 1 tolerant: 15 resistant or necrotic or mosaic or severe mosaic segregation (χ^2 = 0.790, Prob = 37%). The same 1:15 segregation was also observed in the F_2 between HSD 2458 and HSD 93-20-A (Table 1e): 11 tolerant plant *vs* 166 plants with necrosis, mosaic or severe mosaic (χ^2 = 0.0004, Prob = 98%).

Inheritance of severe mosaic, yellowing and stunting

In the F_2, BC_s and RILs between 'Védrantais' and PI 164323 (Table 1a), and similarly in the F_2 between 'Védrantais' and HSD 93-20-A (data not shown), plants with mosaic or severe mosaic were observed. The "severe mosaic" symptom was dominant over the mosaic symptom. The segregation observed (Table 1a) in the F_2 (χ^2 = 0.754, Prob = 39%), in the BC (χ^2 = 5.069, Prob = 2%) and the RILs (χ^2 = 1.684, Prob = 19%) fitted one dominant gene hypothesis. We propose the symbol *Cvy-3* for this gene with the allele *Cvy-3*$^+$ for mosaic symptom.

The allele *Cvy-3* is present in PI 164323, HSD 93-20-A and Ouzbèque 2 and the allele *Cvy-3*$^+$ is present in Védrantais and HSD 2458 as shown (Table 1d) by the absence of plants with severe mosaic in the F_2 between 'Védrantais' and HSD 2458 and the presence of plants with severe mosaic or mosaic in the F_2 between Ouzbèque 2 and HSD 2458.

Linkage and epistasis between the three loci

The locus *Cvy-1* is independent from *Cvy-3* as shown for instance by the 1:1 segregation for mosaic *vs* severe mosaic among the susceptible RILs (Table 1a).

The alleles *Cvy-1*^{1} and *Cvy-1*^{2} are epistatic and dominant on *Cvy-3* according to the higher number of resistant or necrotic plants over the susceptible (mosaic or severe mosaic) or tolerant plants. Moreover the two lines PI 164323 and HSD 93-20-A have respectively the genotypes [*Cvy-1*^{1} *Cvy-3*] and [*Cvy-1*^{2} *Cvy-3*] confirming the epistatic effect over *Cvy-3*. However the allele *Cvy-3* is not necessary for the resistance as it can be concluded from the observation of F_2 progenies between 'Védrantais' and RILs which were resistant: some of these F_2 were segregating with plants with no symptom and plants with mosaic but no plants with severe mosaic indicating that these RILs had the genotype [*Cvy-1*^{1} *Cvy-3*$^+$]

The F_1 between HSD 2458 and Ouzbèque 2, heterozygous for both *cvy-2* and *Cvy*-3, exhibited mosaic symptoms (Table 1d). In the F_2 progeny, we observed about 25% of tolerant plants corresponding to the homozygous [*cvy-2 cvy-2*] (see above inheritance of tolerance). Plants heterozygous for *cvy-2* or without the *cvy-2* allele [*cvy-2 cvy-2*$^+$] or [*cvy-2*$^+$ *cvy-2*$^+$] exhibited mosaic or severe mosaic symptoms. The observed 7:5 segregation (mosaic *vs* severe mosaic) ($\chi^2 = 2.489$, Prob = 11%), could be explained by the following interactions: plants heterozygous for both genes [*cvy-2 cvy-2*$^+$ *Cvy-3 Cvy-3*$^+$] like the F_1 or homozygous for *Cvy-3*$^+$ exhibited mosaic symptoms and plants homozygous *cvy-2*$^+$ and homo- or heterozygous *Cvy-3* [*cvy-2*$^+$ *cvy-2*$^+$ *Cvy-3* -] exhibited severe mosaic symptoms.

From the segregation observed in the F_2 and BC between PI 164323 and HSD 2458 (Table 1c), we can conclude that *Cvy-1* is epistatic dominant over *cvy-2*: the plants homozygous *Cvy-1*^{1} *Cvy-1*^{1} were resistant whatever the allele at the *cvy-2* locus and the plants heterozygous at the locus *Cvy-1* were necrotic whatever the allele at the *cvy-2* locus.

DISCUSSION AND CONCLUSIONS

After the evaluation of the melon genetic resources with one isolate of CVYV, five phenotypes have been observed. Resistance has been found in only one accession as was the case for other viruses, *Zucchini yellow mosaic virus* (ZYMV) or *Papaya ringspot virus-watermelon strain* (PRSV-W), where a low number of resistant accessions have been identified. This resistance is controlled by one dominant allele but the heterozygous plants exhibited a necrotic reaction. In commercial F_1 cultivars resulting from breeding programs, both parents must be resistant. This necrotic reaction was also observed in some accessions which were homozygous for the allele *Cvy-1*^{2}. This type of symptom has been observed in melon in interaction with other viruses. Two pathotypes of ZYMV have been described: the pathotype NF induces mosaic symptoms on 'Védrantais' and 'Doublon' while the pathotype F induces mosaic symptoms on 'Védrantais' and wilting and necrosis on 'Doublon' (Lecoq and Pitrat 1984). One dominant gene (*Flaccida necrosis*, symbol *Fn*) present in 'Doublon' controls this phenotype (Risser et al. 1981). A similar

situation has been described with *Moroccan watermelon mosaic virus* (MWMV) and the gene *Nm* (Quiot-Douine et al. 1988). The interaction with PRSV-W is more similar to what is observed with CVYV. At the locus *Prv*, the allele Prv^1 controls resistance to all strains of PRSV and the allele Prv^2 controls resistance to some strains and systemic necrosis with other strains (Pitrat and Lecoq 1983).

The phenotype of partial resistance with very light symptoms has also been identified in only one accession and is controlled by one recessive allele *cvy-2*. Up to now only a few recessive genes have been described in melon for virus resistance: *cab-1* and *cab-2* for *Cucurbit aphid borne yellows virus* (CABYV), *nsv* for *Melon necrotic spot virus* (MNSV) and QTLs for *Cucumber mosaic virus* (CMV). The practical interest of the gene *cvy-2* in association with $Cvy\text{-}1^1$ or alone for the level and/or the durability of resistance has to be evaluated.

About half of the accessions presented severe yellowing and stunting symptoms. This type of symptom cannot be related to geographical origin or botanical types. It is controlled by one dominant allele *Cvy-3*. Surprisingly, this allele is present in the resistant accession PI 164323 with $Cvy\text{-}1^1$ or in the necrotic accession HSD 93-20-A with $Cvy\text{-}1^2$. But the allele is not necessary for the phenotype of resistance as plant with the [$Cvy\text{-}1^1$ $Cvy\text{-}3^+$] genotype are resistant. Nevertheless its interaction with the locus *Cvy-1* for the durability of resistance should be investigated. Indeed, in preliminary tests, CVYV isolates from other geographical origins could induce necrotic or stunting symptoms on the PI 164323 inbred line described in the paper.

The genotypes of the five lines studied in this paper are the following: 'Védrantais' [$Cvy\text{-}1^+$ $cvy\text{-}2^+$ $Cvy\text{-}3^+$], 'Ouzbèque 2' [$Cvy\text{-}1^+$ $cvy\text{-}2^+$ *Cvy-3*], PI 164323 [$Cvy\text{-}1^1$ $cvy\text{-}2^+$ *Cvy-3*], HSD 93-20-A [$Cvy\text{-}1^2$ $cvy\text{-}2^+$ *Cvy-3*] and HSD 2458 [$Cvy\text{-}1^+$ *cvy-2* $Cvy\text{-}3^+$].

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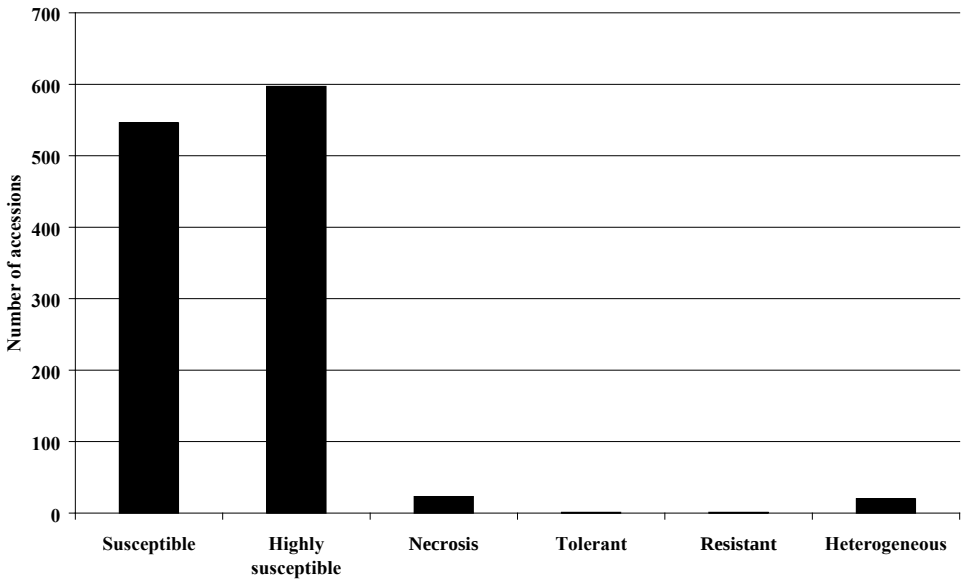

Fig. 1. Number of melon accessions according to the symptoms observed after inoculation with a strain of CVYV.

Table 1. Number of plants observed with different types of symptoms in progenies between different lines of melon (HSD 2458, HSD 93-20-A, Ouzbèque 2, PI 164323 and Védrantais) following inoculation with CVYV.

	Progeny	Symptoms				
		No symptom	Necrosis	Mild mottle	Mosaic	Severe mosaic
a	Védrantais				50	
	PI 164323	43	2			
	F_1 Védrantais × PI 164323		34			
	F_2 (Védrantais × PI 164323) ⊕	30	237		23	77
	BC_s (Védrantais × PI 164323) × Védrantais	1	85		33	54
	BC_r (Védrantais × PI 164323) × PI 164323	53	116			1
	RILs Védrantais × PI 164323	44			15	23
b	Ouzbèque 2					7
	HSD 93-20-A	4	12			
	F_1 Ouzbèque 2 × HSD 93-20-A		12			
	F_2 (Ouzbèque 2 × HSD 93-20-A) ⊕	10	147		4	63
c	HSD 2458	1		50		
	PI 164323	65	2			
	F_1 HSD 2458 × PI 164323	4	51			
	F_2 (HSD 2458 × PI 164323) ⊕	106	287	28	44	62
	BC_s (HSD 2458 × PI 164323) × HSD 2458	1	44	37	11	2
	BC_r (HSD 2458 × PI 164323) × PI 164323	29	70		1	
d	HSD 2458			20		
	Védrantais	1			19	
	Ouzbèque 2					25
	F_1 Védrantais × HSD 2458			13	7	
	F_1 Ouzbèque 2 × HSD 2458			1	34	
	F_2 (Védrantais × HSD 2458) ⊕	2		57	180	
	F_2 (Ouzbèque 2 × HSD 2458) ⊕	3		101	175	149
e	HSD 2458			10		
	HSD 93-20-A		20			
	F_1 HSD 2458 × HSD 93-20-A		5			
	F_2 (HSD 2458 × HSD 93-20-A) ⊕		110	11	22	34

Genetic studies on resistance to downy mildew in cucumber

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Keywords: Downy mildew, *Pseudoperonospora cubensis*, cucumber, *Cucumis sativus*, disease, resistance, quantitative inheritance

Abstract

The objectives of this study were to determine the type of gene action controlling resistance in Ames 2354 (P_1) under controlled conditions in the growth chamber. PI 175695 (P_2) was used as the susceptible parent in crosses with Ames 2354 to make seven generations for study: P_1, P_2, F_1, $F_{1\ reciprocal}$, F_2, BC_{1P1} and BC_{1P2}. The F_2 population showed a continuous segregation and did not segregate into distinct categories. For genetic analysis, the additive-dominance model of Mather (scale A, B, C) and the six-parameter inheritance model of Jinks and Jones were used. The additive-dominance model was rejected, because two of three tests of Mather scale A, B, C were significantly different from 0. Therefore non-allelic gene interactions were important in the inheritance of resistance. The results of a six-parameter model of Jinks and Jones showed that additive and dominance genetic effects were significant for resistance to downy mildew in cucumber. This can also be seen from the F_1 mean which was close to the midparent value. Resistance to downy mildew caused by *Pseudoperonospora cubensis* is quantitatively inherited, and may be controlled by several factors (loci).

INTRODUCTION

Downy mildew (DM) of cucumber, caused by the oomycete *Pseudoperonospora cubensis*, is a devastating, worldwide disease of cucurbit crops, both in field and protected culture. *P. cubensis* outbreaks over the past several decades have been responsible for annual yield losses of up to 95% in some US fields (Colucci et al. 2006). In Central Europe, it has been a serious problem since 1984 (Lebeda 1991), and in Poland, the pathogen was first reported in 1985. Since that time, it continues to occur every year in cucumber growing areas, and has the potential to cause severe damage to the foliage with associated yield losses. Through intensive

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breeding, several resistant cucumber hybrids have been developed in Poland since 1990 (Klosinska et al. 2010), but none with high enough resistance to eliminate the need for fungicides to control the disease.

Previously, the available USDA Plant Introduction (PI) collection of cucumber germplasm was studied to identify new accessions having a higher level of resistance under natural field epidemics in Poland and North Carolina (Klosinska et al. 2010). Among about 1300 cucumber cultigens tested in a four-year study in Poland, six had high resistance: PI 330628, PI 197088, PI 197086, PI 197085, Ames 2353, and Ames 2354. These six cultigens were more resistant than the currently available resistant Polish F_1 hybrids Rodos and Aladyn, and American cultivars Poinsett 76 and Slice. Interestingly, data for all cultigens from Poland showed a greater range of mean DM ratings compared with data from North Carolina (0.3 to 9.0 compared with 1.0 to 7.3, respectively) probably indicating that European populations of *P. cubensis* are highly variable and may have many pathotypes (Lebeda and Urban 2004).

The objective of this study was to determine the type of gene action controlling resistance *P. cubensis* in the resistant cultigen Ames 2354.

MATERIALS AND METHODS

Plant material

The parental genotypes used in this study were resistant Ames 2354 (P_1) and susceptible PI 175695 (P_2), chosen on the basis of their reaction to DM in previous studies (Klosińska et al. 2010). Ames 2354 is a tolerant selection from PI 234517 (SC 50, Carroll Barnes breeding line) made in 1982 by Gregory Tolla from a field infested with Verticillium wilt. PI 175695 is a plant introduction accession identified as susceptible in a screening by Klosinska et al. (2010). The plant material studied consisted of reciprocal F_1, F_2, and backcross populations from crosses of the F_1 with Ames 2354 and PI 175695. All crosses were made by hand pollination in the greenhouses of The Research Institute of Horticulture, Skierniewice, Poland.

Experimental conditions and disease evaluation

Resistance screening tests were conducted under controlled environment conditions in growth chambers and the greenhouse. Seeds were sown in plastic pots (10 cm diameter) filled with a peat substrate (Kronen-Klasmann). One seed was planted in each pot. Seedlings were grown at 26/22°C (day/night) temperatures with day consisting of 12 hours of light.

Cucumber leaves heavily infected with *P. cubensis* were collected from experimental fields in Skierniewice, that had not been sprayed with fungicides. In the laboratory, infected leaves were soaked in distilled water and rubbed gently with a glass rod to dislodge sporangia. The concentration of spore suspension was

determined with the use of hemocytometer and adjusted to a final concentration of 5 x 10^4 sporangia·mL^{-1}. Plants were inoculated at the 1 to 3 true leaf stage by misting the adaxial side of leaves with the sporangial solution until runoff using a hand sprayer (1L size). Pots were placed in a moist dark growth chamber at 20°C and 100% humidity for 48 h. The inoculated seedlings were then removed from the moist chamber and placed on the greenhouse bench, where temperatures ranged from 25 to 30 °C.

Disease ratings and data analyses

Disease ratings were made 14 to 16 days after inoculation using a scale of 0 to 9 (0=no disease, 1-2=trace, 3-4, slight, 5-6=moderate, 7-8=severe, 9=dead) (Jenkins and Wehner 1983). Ratings are based on percentage of infected leaf area, from which a disease severity index (DSI) was calculated. Data were analyzed using STATISTICA 8.0, SAS-STAT statistical package (SAS Institute, Cary, NC) and the SASGene 1.2 program (Liu et al. 1997). Significant differences among means of P_1, P_2, F_1, $F_{1reciprocal}$, F_2, BC_{1P1}, and BC_{1P2} populations were tested using Tukey's HSD (5% probability).

The additive-dominance model (Mather and Jinks 1982) and the six-parameter model of Jinks and Jones (1958) were used for genetic analysis. The 6-parameter model of inheritance was also used to estimate genetic parameters connected with (1) an additive component, describing the difference between homozygotes at any single locus, (2) a dominance component arising from interactions of alleles (intra-allelic interaction, (3) an epistatic component associated with non-alleles (interallelic interaction or epistasis). The standard errors of the Mather scale (A, B, C) and six-parameter model (m, d, h, i, j, l) were mean square roots calculated from the variance of each individual parameter for each model. The significance of these parameters for each model was tested (verified) separately using Student's t test.

RESULTS AND DISCUSSION

Seedlings of the inbred Ames 2354 (P_1) were highly resistant, with a disease severity index of infection (DSI) of 1.6 (Table 1). In contrast, seedlings of PI 175695 (P_2) exhibited high susceptibility (DSI= 7.5). The F_1 and $F_{1\ reciprocal}$ showed no observable difference and the same DSI value (4.2), indicating no maternal effects (Table 1). There was a significant difference between the F_1 mean (4.2) and the parental midpoint (4.6), indicating slight dominance in the direction of resistance (Table 1). This was confirmed by the segregation ratios in the backcross populations. The BC_{1P1} progeny was moderately resistant (DSI=2.9) while the BC_{1P2} generation was susceptible (DSI=6.6). The F_2 population showed a continuous distribution with no distinct categories, indicating that the inheritance of DM resistance was a quantitative trait. Therefore, the effects of genes contributing to downy mildew

resistance in cucumber were estimated using generation means analysis.

Based on Mather's analysis, the additive-dominance model was rejected, because in two of three tests of Mather's scale A, B, C was significantly different from 0 (Table 2). This indicates that non-allelic gene interactions were important in the inheritance of resistance.

Estimates of the six genetic parameters of Jinks and Jones (1958) provided evidence that total genetic variation was due to additive gene effects [d] that had negative values (Table 3). The negative values for parameter [d] contributed to a decrease in downy mildew severity, thus increasing resistance. The results of a six-parameter model showed that the dominance effect [h] was significant. A perfect fit of the genetic parameters indicated epistatic effects associated with interactions of non-allelic loci (interallelic interaction or epistasis) (Table 3). Resistance was increased by the interaction of heterozygous with homozygous *loci* and it was expressed by negative values of the parameter [j]. There were no significant interactions of heterozygous with heterozygous *loci* [l]. The parameter [l] was opposite in sign to the dominance effect [h] indicating the presence of epistasis with duplicate gene action. Therefore, some or all of the genes contributing to downy mildew resistance interact with each other in producing their effects.

Previous results regarding the inheritance of resistance to downy mildew indicate that there are different genes involved: three recessive genes (Doruchowski and Lakowska-Ryk 1992; Shimizu et al. 1963), three partially dominant genes (Pershin et al. 1988), an interaction between dominant susceptible and recessive resistance genes (Badr and Mohamed 1998; El-Hafaz et al. 1990), one or two incompletely dominant genes (Petrov et al. 2000), and a single recessive gene (Angelov 1994; Fanourakis and Simon 1987; Van Vliet and Meysing 1974; 1977). Van Vliet and Meysing (1977) reported that the resistance found in 'Poinsett' and PI 234517 was controlled by the same single recessive gene, designated as *dm*, and likely originating from PI 197087. Ames 2354 is a selection from self-pollination of PI 234517, also known as SC-50. In our study, resistance in Ames 2354 was quantitatively inherited, and not due to a single gene. Therefore, it is probable that Ames 2354 contains at least the *dm* gene, and likely one or more additional resistance genes. Different results in these studies were likely due to multiple factors including different pathogen populations, different resistant lines used, the resistance trait evaluated according to a different scale, and different environmental conditions.

CONCLUSIONS

Our study indicates that resistance to downy mildew in Ames 2354 is quantitatively inherited. Resistance is controlled by several factors (gene loci). There was no maternal effect on resistance. Both additive and dominance genetic

effects were significant. Additionally, estimates of non-allelic interaction effects (homozygous with homozygous loci, and homozygous with heterozygous loci) were significant contributing to the total genetic variation. The presence of epistasis with duplicate gene action was also observed.

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Table 1. Frequency distribution and disease severity index (DSI) of downy mildew symptoms on leaves of Ames 2354 (P_1), PI 175695 (P_2) and their progenies.

Generation	No. of plants	No. of plants in classes										DSI
		0	1	2	3	4	5	6	7	8	9	
P_1	155	24	41	69	18	3						1,6 a
P_2	213						1	15	74	79	44	7,5 f
F_1	204		3	22	62	33	38	21	23	2		4,2 c
$F_{1reciprocal}$	160		4	12	44	28	22	29	11			4,2 c
F_2	853		24	44	99	137	132	184	155	68	10	5,2 d
BC_{1P1}	250	2	51	68	42	42	25	16	4			2,9 b
BC_{1P2}	227			2	9	16	19	32	83	58	8	6,6 e

Values followed by the same letter within a column do not differ significantly according to Tukey's test at 5% probability.

Table 2. Genetic analysis of resistance to downy mildew in cucumber leaves of Ames 2354 (P_1), PI 175695 (P_2) and their progenies (tests of Mather scale A, B, C).

Mather tests		
A	B	C
0,05 ±0,12 ^{ns}	0,28±0,09*	0,81±0,15*

Significance of parameters was by the t-test (5%)

Table 3. Genetic analysis of resistance to downy mildew in cucumber leaves of Ames 2354 (P_1). PI 175695 (P_2) and their progenies (effects of six parameters model of genes action according to Jinks and Jones 1958).

Model					
[m]	[d]	[h]	[i]	[j]	[l]
2,74±0,17*	-0,68±0,03*	-0,63±0,44*	-0,48±0,16*	-0,24±0,14*	0,15±0,29 ^{ns}

[m] = total effect, [d] = additive, [h] = dominance, [i] = epistatic effect of homozygous and homozygous *loci* interaction, [j] = epistatic effect of homozygous and heterozygous *loc*i interaction, [l] = epistatic effect of heterozygous and heterozygous *loci* interaction.
*Significance of parameters was calculated by the t-test (5%)

Application of a new approach for characterization and denomination of races of cucurbit powdery mildews–a case study of Czech pathogen populations

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Abstract

Seventeen pathogenic races were identified in axenic culture among 18 Czech isolates of *Golovinomyces cichoracearum* (*Gc*, six isolates) and *Podosphaera xanthii* (*Px*, 12 isolates) on a set of 21 melon cucurbit powdery mildew (CPM) race differentials and using a triplet septet code. The 18 isolates were collected in 2010 from four cucurbit species: *Cucurbita pepo* (10 isolates), *Cucurbita maxima* (four isolates), *Cucurbita moschata* (two isolates) and *Cucumis melo* (one isolate). The results of this research are interesting from several views. One, the diversity for pathogenicity among the isolates of the two CPM pathogens on the melon CPM race differentials. The pathogenic diversity of among these isolates confirmed that Czech CPM populations are unique, and highly variable in race structure. There was no obvious relationship between source species and race identity for either CPM species. Two, the concomitant reactions of the 21 melon differentials reveal a rich reservoir of potentially useful CPM resistance genes for pyramiding. Three, the distinct differences in reactions by two pairs of closely related melon lines thought to be equivalent in their genes for resistance to *Px* emphasize the complexity of host plant resistance to CPM. Differentials PI 234607, 'PMR 6', PI 124112, MR-1, and PI 124111 exhibited broad resistance to *Gc* and *Px*.

INTRODUCTION

Golovinomyces cichoracearum (*Gc*) and *Podosphaera xanthii* (*Px*) (Ascomycetes, Erysiphaceae) are the most important fungal species causing cucurbit powdery mildew (CPM), a serious disease of field and greenhouse

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cucurbits. Pathogenicity variation of CPM species has been described at the levels of pathotypes and races (Bardin et al. 1997, 1999). Pathotypes express pathogenicity variation on the level of their cucurbit host range; in contrast races characterize pathogenicity on a set of selected genotypes of one cucurbit host species (e.g., *Cucumis melo*) with different resistance factors (Lebeda et al. 2008). Both CPM species are highly variable, as indicated by the existence of large number of different pathotypes and races (Lebeda et al. 2011; McCreight 2006). Races of *Gc* and *Px* have, to date, been reported only on melon; two races of *Gc* and about 25 races of *Px* have been worldwide identified on melons (Lebeda et al. 2011; Coffey et al. 2006; McCreight 2006; McCreight et al. 2005). Recent results suggest that even more races exist (Lebeda and Sedláková 2004; Lebeda et al. 2004; McCreight 2006). According our previous studies from the Czech Republic (CR) (Křístková et al. 2004; Lebeda and Sedláková 2004), Czech CPM populations are highly variable in their pathogenicity, and are unique and markedly different compared to those of some western and southern European countries and other parts of the world. Forty-eight *Px* and 86 *Gc* races were identified in CR from 2000 to 2007; 44 of the *Px* and all of the *Gc* races have been detected only in CR (Lebeda et al. 2011).

Various independent systems of CPM pathotype and race determinations and denominations have been used worldwide. The most frequently cited set of melon differentials includes 11 genotypes (Bertrand 1991; Bertrand et al. 1992; Lebeda and Sedláková 2006, 2007; McCreight 2006) that differentiate CPM races originating from melon (McCreight 2006) and other cucurbits, e.g., cucumber, *Cucurbita* spp. and watermelon (Lebeda and Sedláková 2006; Lebeda et al. 2007). Lebeda et al. (2008) critically reviewed the current state, gaps, and perspectives in our understanding of pathogenicity variation in these two CPM pathogens at the pathotype and race levels. They proposed two sets of differential cucurbit genotypes for the identification of CPM pathotypes and races, and an objective, efficient, uniform and comprehensive coded system for meaningful, concise designation of CPM pathotypes (sextet code) and races (septet code).

A new set for differentiation of *Gc* and *Px* races is composed of 21 differential melon genotypes (Table 1). This enlarged set is based on the set of 11 melon CPM differentials developed by French investigators (Bertrand 1991; Bertrand et al. 1992), and is supplemented with 10 additional melon genotypes that revealed new *Px* and *Gc* races (Bertrand 2002; Hosoya et al. 2000; Lebeda and Sedláková 2006; Lebeda et al. 2007; McCreight 2006). Lebeda et al. (2008) also proposed a numerical system for designation of pathotypes and races that is derived from a previously developed sextet based system for plant pathogens (Limpert and Müller 1994; Limpert et al. 1994). The CPM race differentials are arbitrarily divided into three groups due to their large number. The race differentials are assigned an arbitrary, permanent order with a group prefix (1, 2 or 3) and values for compatible (susceptible) reactions

within each group (1, 2, 4, 8, 16, 32, or 64). The binary results of any CPM assay are thus translated into a triple-part, septet code; one part for each group of seven differentials. The three sums are then presented as an unique triplet-septet code in the format: sum of group 1.sum of group 2.sum of group 3, which serves as a unique identifier for each race. This unified denomination of triplet septet codes standardized the communication on international level.

Here we present a case study using Czech CPM populations for application of the above new approach for characterization and denomination of CPM races. The data are interesting from the viewpoints of pathogenicity and host plant resistance variability.

MATERIALS AND METHODS

The tests were done in spring of 2011 in a greenhouse and a growth chamber at the facilities of the senior author.

Plant materials

The 21 *C. melo* race differentials are a diverse set of genotypes and origins (Table 1). They include universal, or nearly so, susceptible lines, e.g., Iran H, genotypes used to define previously defined major and widespread races, e.g., 'PMR 45', and genotypes that have revealed one or more variants of well known races, e.g., 'Noy Yizre´el'.

Seeds were sown in Perlite and grown in a growth chamber. Seedlings were transplanted at the cotyledon stage and grown in a CPM-free greenhouse (for details see Lebeda and Sedláková 2010).

Pathogen isolation, multiplication and maintenance

CPM samples were microscopically examined before isolation; those determined to be a mixture of *Px* and *Gc* were excluded. Conidia of pure cultures were transferred by tapping onto primary leaves of highly susceptible cucumber (*Cucumis sativus*) 'Stela F_1'. Eighteen Czech CPM (6 *Gc*, 12 *Px*) isolates collected in 2010 from four cucurbit species were used for the study: *Cucurbita pepo* (10 isolates), *Cucurbita maxima* (four isolates), *Cucurbita moschata* (two isolates) and *C. melo* (one isolate). Isolates were cultured on leaf discs in plastic boxes (one isolate per box; 24°C/18°C day/night) for 12 h. Multiplication and maintenance of isolates were done as previously described (Lebeda and Sedláková 2010).

Determination and denomination of pathogenic variability

The 18 isolates (6 *Gc*, 12 *Px*) described above were screened for pathogenic variability (races) by a leaf-disc method (Bertrand et al. 1992; Lebeda 1986) using the set of 21 *C. melo*–CPM race differentials proposed by Lebeda et al. (2008)

(Table 1). Each genotype was represented by three leaf discs (15 mm diam.) from true leaves (2 to 3-leaf stage) in three replicates (one replicate per plant). Discs were inoculated by tapping a primary leaf of cucumber 'Stela F_1' covered with 3 to 4-day-old, sporulating mycelia and incubated under the conditions described above. CPM infection of each disc was evaluated 6 to 14 days post-inoculation using a 0 to 4 scale (Lebeda 1984). Data were used to calculate a degree of infection (DI) value for each genotype that was classified as resistant ($0 \leq DI \leq 1$), or susceptible ($1 < DI \leq 4$). Race identifications of the 18 isolates were determined using the triplet-septet system proposed by Lebeda et al. (2008) and described above.

RESULTS AND DISCUSSION

The results of this research are interesting from several views. One, the diversity for pathogenicity among the isolates of the two CPM pathogens on the melon CPM race differentials. Two, the concomitant reactions of the 21 melon differentials reveal a rich reservoir of potentially useful CPM resistance genes for pyramiding. Three, the distinct differences in reactions by two pairs of closely related melon lines thought to be equivalent in their genes for resistance to *Px* emphasize the complexity of host plant resistance to CPM.

Seventeen races (5 *Gc* and 12 *Px*) were identified among the 18 CPM isolates (6 *Gc*, 12 *Px*) using the triplet septet code. *Gc* race 55.63.119 was isolated from two locations: Olomouc-Holice and Kojetín, ca. 34 km apart the other 16 races (4 *Gc* and 12 *Px*) occurred once. There was no obvious relationship between source species and race identity for either CPM species. Cucumber and squashes, which are commonly grown in Czech Republic have not been reported to exhibit CPM–race specific interactions. Melon is a minor crop in the Czech Republic limited to home gardens, yet the Czech CPM populations exhibited many virulence factors on the 21 melon differentials.

The race diversity of among these 18 CPM isolates verified previous observations of great racial diversity of both CPM pathogens in Czech Republic (Lebeda et al. 2007 2011; Lebeda and Sedláková 2006), and confirmed that Czech CPM populations are unique, and highly variable in race structure. The new set of 21 differentials and septet code revealed and characterized more completely the race variation among CPM populations.

None of the races infected all 21 differentials. Isolate *Px* 45/10 infected all 20 differentials but not PI 124111 (2.7). Isolate *Px* 51/10 infected 16 of the 21 race differentials.

Golovinomyces cichoracearum–race differential observations

PI 234607 (1.4), 'PMR 6' (1.7), PI 124111 (2.7) and 'Negro' (3.4) were notable in that they were resistant to all five races (Table 2). Ten differentials were,

in contrast, susceptible to all five races: 'Védrantais' (1.2), ARHBJ (1.5), 'PMR 45' (1.6), WMR 29 (2.1), 'Edisto 47' (2.2), PI 414723 (2.3), 'PMR 5' (2.4), PI 313970 (3.1), 'Nantais Oblong' (3.6), 'Solartur' (3.7). 'Nantais Oblong' (3.6) was susceptible to all five races, but was reported to have a single, dominant gene for resistance to *Gc* to another unique race (Epinat et al. 1993).

Three differentials were resistant to race *Gc* 54.15.113 (isolate 50/10): Iran H (1.1), 'Noy Yizre'el' (3.2), PI 236355 (3.3.). Iran H at one time was considered susceptible to all races of *Gc* (Pitrat et al. 1998). Two differentials were resistant to race *Gc* 51.15.103 (isolate 1/10 6) and 51.31.103 (isolate 24/10): PI 179901 (1.3) and 'Amarillo' (3.5).

MR-1 (2.6) and PI 124111 (2.7) are closely related. MR-1 was selected from PI 124111 for uniform reaction to downy mildew incited by *Pseudoperonospora cubensis* and *Px* (Thomas 1986), but were distinctly different from each other in response to the five *Gc* races: MR-1 was resistant to four races and PI 124111 was resistant to all five races.

Podosphaera xanthii-race differential observations

Iran H (1.1) has been included in the *Px* differentials as the universal susceptible genotype, and it was so in response to these 12 *Px* races (Table 2). It was, however, resistant in China to race pxCh1 (Liu et al. 2010). Six other differentials were also susceptible to all 12 races in this study: 'Védrantais' (1.2), ARHBJ (1.5), PI 236355 (3.3), 'Amarillo' (3.5), 'Nantais Oblong' (3.6), 'Solartur' (3.7).

None of the differentials was resistant to all 12 *Px* races, but three differentials were resistant to 11 of the 12 races. PI 234607 (1.4) and PI 124112 (2.5) were susceptible to race *Px* 127.63.127 (isolate 45/10). PI 124111 (2.7) was susceptible to one race, *Px* 55.78.142 (isolate 10/10). PI 124111 and MR-1 differed in response to *Px*. MR-1 (2.6) was susceptible to two races: *Px* 127.63.127 (isolate 45/10) and *Px* 55.47.125 (isolate 59/10).

'Edisto 47' (2.2) was resistant to seven races. It was the differential used to first identify race 3 (Thomas 1978). WMR 29 (2.1) was resistant to six races. It is resistant to race 3 (Pitrat M, pers. commun.).

PI 313970 (3.1) was resistant to four races. It is the differential used to first identify races S and SD (McCreight and Coffey 2011).

General race differential observations

'PMR 45' (1.6) was generally susceptible to both Czech CPM pathogens, but did express resistance to two *Px* races. Resistance in 'PMR 45' is still effective in the desert southwest U.S. (see McCreight et al. in this proceedings).

'PMR 6' (1.7) was generally resistant to these races of *Gc* and *Px*, while, in contrast, 'PMR 5' (2.4) was generally susceptible. These two lines resulted from

the same breeding program and have been generally regarded as equivalent in their resistance to *Px*, but these data revealed differences between them for CPM-resistance genes (Pryor et al. 1946).

MR-1 (2.6) and PI 124111 (2.7) have been generally regarded as equivalent in their resistance to *Gc* and *Px* (Pitrat et al. 1998), but were distinctly different from each other in response to these races of *Gc* and *Px*.

These data showed PI 234607 (1.4), 'PMR 6' (1.7), PI 124112 (2.5), MR-1 (2.6), and PI 124111 (2.7) to have broad resistance to *Gc* and *Px.*

The set of melon CPM race differentials used in this study revealed great variation for pathogenicity within *Gc* and *Px*. The set could be further refined as the International Seed Federation develops guidelines for melon CPM race coding. A change in the order of the differentials across the triplets to follow the order that developed from 1998 through 2006 (McCreight 2006; Pitrat et al. 1998; see McCreight et al in this proceedings) would permit those familiar with the changes in race descriptions in recent years to more easily relate the current, open system of race designations with the proposed triplet-septet system applied in the present study. Also, the recent designation of race pxCh1 (Liu et al. 2010) may warrant inclusion of Ames 31282, which superseded the designation PI 134198 (Reitsma KR, pers. commun.) as a differential in lieu of 'Solartur', which was included in this study to fill-out the differential set (Lebeda et al. 2008). Other race differential substitutions may also enhance the set.

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Table 1. Proposed CPM race differentials by triplet group and number. All are *Cucumis melo* (according to Lebeda et al. 2008).

Differential		Other	Original	Country
Group.No.	Designation	designation(s)	source	of origin
1.1	Iran H	–	–	Iran
1.2	Védrantais	–	–	France
1.3	PI 179901	Teti	–	India
1.4	PI 234607	Sweet Melon	–	South Africa
1.5	ARHBJ	AR Hale's Best Jumbo	USDA	USA
1.6	PMR 45	–	USDA	USA
1.7	PMR 6	–	USDA	USA
2.1	WMR 29	–	USDA	USA
2.2	Edisto 47	Ames 8578	Koelz 2563	USA
2.3	PI 414723	LJ 90234	PI 371795	India
2.4	PMR 5	–	USDA	USA
2.5	PI 124112	Koelz 2564	–	India
2.6	MR-1	–	PI 124111	USA
2.7	PI 124111	–	–	India
3.1	PI 313970	VIR 5682	–	India
3.2	Noy Yizre´el	–	–	Israel
3.3	PI 236355	–	–	England
3.4	Negro	–	–	Spain
3.5	Amarillo	–	–	Spain
3.6	Nantais Oblong	–	–	France
3.7	Solartur[z]	–	–	Czech Republic

[z] Not included in the proposed set (Lebeda et al. 2008) and could be replaced by another genotype after discussion.

Table 2. Triplet-septet codes for isolates/races of the CPM species *Golovinomyces cichoracearum* (six isolates/five races) and *Podosphaera xanthii* (12 isolates/12 races) on leaf discs of 21 CPM race differentials in axenic culture. CPM isolates were collected from different cucurbit host species at 12 locations in the Czech Republic in 2010. See Table 1 for identification of the race differentials.

Isolate	Host[z]	Location	Triplet / Race differential / Septet value																					Triplet-septet code
			1							2							3							
			1.1	1.2	1.3	1.4	1.5	1.6	1.7	2.1	2.2	2.3	2.4	2.5	2.6	2.7	3.1	3.2	3.3	3.4	3.5	3.6	3.7	
			1	2	4	8	16	32	64	1	2	4	8	16	32	64	1	2	4	8	16	32	64	
Golovinomyces cichoracearum																								
1/10 6	CP	Ol-Holice	1	2	0	0	16	32	0	1	2	4	8	0	0	0	1	2	4	0	0	32	64	51.15.103
3/10 2	CM	Ol-Holice	1	2	4	0	16	32	0	1	2	4	8	16	32	0	1	2	4	0	16	32	64	55.63.119
24/10	CM	Seletice	1	2	0	0	16	32	0	1	2	4	8	16	0	0	1	2	4	0	0	32	64	51.31.103
41/10	CM	Ostrožská[y]	1	2	4	0	16	32	0	1	2	4	8	0	0	0	1	2	4	0	16	32	64	55.15.119
48/10	CP	Kojetín	1	2	4	0	16	32	0	1	2	4	8	16	32	0	1	2	4	0	16	32	64	55.63.119
50/10	CP	Polkovice	0	2	4	0	16	32	0	1	2	4	8	0	0	0	1	0	0	0	16	32	64	54.15.113
Podosphaera xanthii																								
10/10	CP	Ořechov	1	2	4	0	16	32	0	0	2	4	8	0	0	64	0	0	4	8	16	32	64	55.78.124
33/10	CP	Strážnice	1	2	4	0	16	0	0	0	0	0	0	0	0	0	0	0	4	8	16	32	64	23.0.124
36/10	CP	Vnorovy	1	2	4	0	16	32	0	1	0	4	8	0	0	0	1	0	4	8	16	32	64	55.13.125
44/10	CP	Kvasice	1	2	0	0	16	32	0	0	0	4	8	0	0	0	0	0	4	0	16	32	64	51.12.116
45/10	CP	Kvasice	1	2	4	8	16	32	64	1	2	4	8	16	32	0	1	2	4	8	16	32	64	127.63.127
51/10	CM	Polkovice	1	2	4	0	16	32	64	1	0	4	8	0	0	0	1	2	4	8	16	32	64	119.13.127
52/10	CP	Tovačov[x]	1	2	4	0	16	32	0	0	2	4	8	0	0	0	1	0	4	8	16	32	64	55.14.125
53/10	Cme	NJ-Kojetín	1	2	4	0	16	0	0	0	0	4	0	0	0	0	1	0	4	8	16	32	64	23.4.125
54/10 2	Cmo	OL-Holice	1	2	4	0	16	32	0	1	0	4	0	0	0	0	1	0	4	8	16	32	64	55.5.125
55/10 2	CP	NJ-Kojetín	1	2	4	0	16	32	0	1	2	4	8	0	0	0	1	0	4	8	16	32	64	55.15.125
56/10 2	CM	NJ-Kojetín	1	2	4	0	16	32	0	0	0	0	0	0	0	0	0	2	4	8	16	32	64	55.0.126
59/10	Cmo	NJ-Kojetín	1	2	4	0	16	32	0	1	2	4	8	0	32	0	1	0	4	8	16	32	64	55.47.125

[z]Cme–*Cucumis melo,* CP–*Cucurbita pepo,* CM–*Cucurbita maxima,* Cmo–*Cucurbita moschata*
[y]Ostrožská Nová Ves-Chylice, [x]Tovačov-Annín

Cucurbit powdery mildew of melon incited by *Podosphaera xanthii*: Global and western U.S. perspectives

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Keywords: *Cucumis melo*, pathological race, physiological race, *Sphaerotheca fuliginea*

Abstract

Cucurbit powdery mildew (CPM) is a major problem of melon (*Cucumis melo* L.) production worldwide, that is mostly caused by two fungi: *Podosphaera xanthii* (*Px*; formerly *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (DC) V.P. Heluta (*Gc*; formerly *Erysiphe cichoracearum*). The two species may co-infect in some areas of northern Europe, but *Px* dominates in warmer climates around the world. Forty-six races of *Px* have been reported on melon based on sets of race differentials that range in number from as few as two to as many as 28. The CPM research community and seed industry are undertaking steps to define uniform sets of CPM race differentials and objective criteria for race nomenclature and designations. Breeders and pathologists must also consider another aspect of CPM that stems from its obligate parasitic nature: race stability as defined by a given set of CPM race differentials. This review summarizes the reported races of *Px* largely from the published literature. It also presents annual profiles from 2002 through 2011 of *Px* populations in the Central and Imperial valleys of California, and Yuma, Arizona. It is suggested that a large fraction of the races are not relevant to most *Px* resistance breeding, which will be done on a regional basis for subsets of races.

INTRODUCTION

Two fungi are commonly credited for inciting cucurbit powdery mildew (CPM) worldwide: *Podosphaera xanthii* (*Px*; formerly *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (DC) V.P. Heluta (*Ec*; formerly *Erysiphe cichoracearum*) (Shishkoff 2000). *Px* predominates in warmer areas while *Ec* does

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so in cooler areas, and mixed infections (*Px* and *Gc*) have been found in Europe (Bertrand 1991; Křístková et al. 2009). The two CPM pathogens were confounded for many years as *Gc* (McCreight 2004), but *Px* appears to be the predominant pathogen in major melon production areas. The increased numbers of reported *Px* races has stimulated concern over the future of breeding melons resistant to *Px*, in particular the need for a concise and objective system for *Px* race denomination and designation (see Lebeda et al. 2012).We review here the gradual increase in the complexity of *Px*- and *Gc*-melon interactions over 87 years, expressed as pathogenic races, which has recently escalated (Fig. 1).

Reported pathological races

Imperial Valley, California melon growers suffered losses to *Px* starting in 1925. Scientific breeding for resistance to *Px* was initiated in 1928 upon discovery of variation for reaction to *Px* in germplasm from India and resulted in the release of 'PMR 45' in 1934 (Jagger and Scott 1937). Pathogenic race variation within *Px* was first observed in 1937 when 'PMR 45' was widely infected in Imperial Valley (Jagger et al. 1938; Fig. 1). Resistance to this new form of *Px*, designated race 2, was quickly found and resulted in the release of 'PMR 5' in 1942 (Pryor et al. 1946).

Thomas (1978) first observed a third *Px* race on melons the lower Rio Grande Valley of Texas in 1976. Race 3, as this new strain became known, did not become widespread in Texas or the U.S, but was later reported in the Punjab of India (Kaur and Jhooty 1986) and widespread in Israel (Cohen et al. 1996).

PI 414723 and WMR 29 revealed variation within *Px* race 2: 2US and 2F (France) in 1986 (McCreight et al. 1987). Four *Px* races were known on melon in 1986 (Fig. 1).

By 1998, seven *Px* races, including 2US and 2F, were reported (Pitrat et al. 1998). Race 0 revealed *Px*-resistance factors in 'Védrantais' and 'Top Mark' that had been regarded as universal *Px*-susceptible genotypes (Bardin et al. 1997). Races 4 and 5 were observed at about the same time (1997-1998) in France (M. Pitrat, pers. commun.) and Czech Republic (Křístková and Lebeda 1999a,b) and later in Israel (Cohen et al. 2004). The number of reported *Px* races increased dramatically from this time (Fig. 1).

Four new races (N1, N2, N3, N4) were reported from Japan in 2000 (Hosoya et al. 2000). Two variants each of *Px* races 2 and 3 were reported in 2002 (Cohen et al. 2002). Bertrand (2002) reported race 6 using AR Hale's Best Jumbo.

Race S was first observed in Imperial Valley in 2003 (McCreight et al. 2005). Races F, G, H were found in Czech (Lebeda and Sedláková 2006). Then, there were 20 *Px* races (Fig. 1). *Px* race SD was isolated from Imperial Valley in 2004 or 2005 (Coffey et al. 2006), and shortly afterwards was present in a greenhouse at Salinas (J.D. McCreight, unpub. data). Races 3.5 and P6 were reported in 2005 (M. Pitrat,

pers. commun.). Pitrat and Besombes (2008) reported *Px* race 4.5 in 2008.

Comparative studies of reported *Px* resistance sources identified in California, Japan, and Spain differentiated isolates/populations of *Px* race 1 (eight variants; seven new) and race 2 (six variants; two new) populations in these countries (McCreight 2006).

A unique race, pxCh 1 was reported in China in 2010 (Liu et al. 2010). Twelve new *Px* races were isolated in 2010 in Czech Republic (Lebeda et al. 2012).

Forty-five *Px* and 13 *Gc* races have thus been reported to date on melon (Table 1). This total for *Px* ignores the variation in *Px* at the pathotype level, e.g, isolates of races S and SD vary in their ability to infect watermelon C*itrullus lanatus* (Coffey et al. 2006).

Race stability in California and Arizona

Px Race 2 was the presumed race in California after many years of the deployment of *Px* race 2-resistant cultivars. *Px* race 1 was, however, detected at the Univ. Calif. (UC), Desert Crops Res. and Ext. Ctr. (DREC), Holtville in Imperial Valley six of 10 yrs from 2002 through 2011; *Px* race S was present three years, and *Px* race 2.1 was present one year (Table 2). In Yuma, Arizona, *Px* race 1 was present in Spring 2003, but *Px* race S was isolated from that field via single spore transfer and was consistently present in four subsequent Spring tests (Table 2). *Px* race 1 was detected in four, Fall field tests in Yuma. *Px* race 1 was present at the UC West Side Res. and Ext. Ctr., Five Points in San Joaquin Valley in 2003 (ca. 668 km north of DREC); *Px* race S was found there in 2007. *Px* race S was detected at two sites in 2011 in the Davis–Woodland area, which is ca. 340 km north of Five Points. *Px* race S is becoming more widespread throughout the Central Valley of California. It remains to be seen whether the *Px* populations will be consistently race S, or vary annually, like in Imperial Valley.

DISCUSSION

The apparent challenge of 46 races of *Px* on melon is formidable for pathologists and breeders alike. It is very likely that additional races will be reported as intensive monoculture of melon increases and new germplasm is used in melon improvement. The recent increase in number of *Gc* races is also of interest in this regard. The demonstrated genetic variation in pathogens and host is of practical significance in terms of cultivar development and, perhaps, deployment of cultivars to "manage" *Px* and *Gc* populations so that resistance genes are not rendered ineffective. The large number of races poses, too, an interesting and complex biological puzzle at the genetic and molecular level.

Genetic diversity in the Czech populations of *Px* and *Gc* is of particular interest (Lebeda and Sedláková 2006; Lebeda et al. 2012) in that melon is not

widely cultivated in that area of Europe, and the cultivars grown there likely, in the absence of a catalog of their types and origins, represent a narrow genetic base. The 15 *Px* and 11 *Gc* races reported in Czech Republic are unexpected in the seeming absence of selection pressure for "new" pathogenic races by a limited number of *Px* or *Gc* resistance genes in melon. The diversity in Czech Republic may, therefore, reflect natural, random genetic variation in the respective pathogen populations that is maintained on diverse cucurbit hosts (Křístková et al. 2009) that are generally susceptible to *Px*, and a diverse array of alternate hosts.

Races N1, N2, N3, N4 may reflect selection of *Px* virulence factors by recently introduced melon germplasm in Japan. The unique *Px* race reported from China (px Ch 1) represents another unique and isolated population. There are no data on race variation of *Px* on melons across China. Notably absent are reports of *Px* variation in Mexico, Central America and Brazil, where melons are important export crops from late Fall through early Spring for U.S.A., Canada and Europe. There are not any recent CPM race data from Australia or India, which is a rich source of CPM-resistant melon germplasm (Dhillon et al. 2012; Dogimont 2010-2012).

It is suggested that breeding cultivars resistant to the many *Px* and *Gc* races be done on a regional basis, whether at one or more locations. The 15 *Px* and 11 *Gc* races identified in Czech are thus less relevant as melon production there is insignificant. The general instability of races in California indicates the need to pyramid multiple *Px* resistance genes to control the predominant races in each respective region, but each region will have different sets of genes as it does horticultural and market-specific traits.

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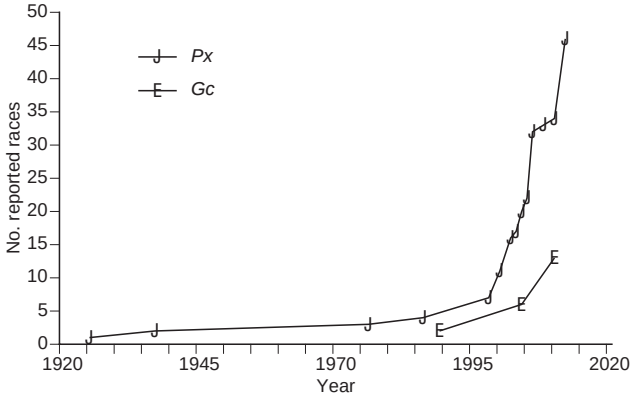

Fig. 1. Numbers of races of *Podosphaera xanthii* (*Px*) and *Golovinomyces cichoracearum* (*Gc*) on melon (*C. melo*) from 1925 through the present.

Table 1. Summary of 46 reported pathogenic races of *Podosphaera xanthii* on 37 melon cultigens.

Cultigen	Race																					
	0[y]	1J[x]	1Sp[w]	1M[v]	1IV[u]	1SJ[u]	1S[u]	1Ti[t]	1Tu[t]	2US[s]	2S[u]	2F[s]	2Z[r]	2a[q]	2b[q]	3[p]	3c[q]	3d[q]	4°	5°	3.5[n]	4.5[n]	N1[x]
Iran H	s[h]	s	–	–	s	s	s	–	–	s	s	s	s	–	–	–	–	–	s	s	–	–	–
Top Mark	R	s	–	–	s	s	s	–	–	s	s	s	–	–	–	s	–	–	s	s	–	–	–
Védrantais	R	s	–	–	s	s	s	s	s	s	s	s	–	–	–	s	–	–	s	s	s	S	–
Fuyu 3	–	s	–	–	–	–	s	–	–	s	s	s	–	s	s	s	–	–	s	s	–	–	s
PMR 45	R	R	R	R	R	R	R	R	R	s	s	s	s	–	–	s	s	s	s	s	s	S	R
PMR 5	R	R	R	R	R	R	R	R	R	R	R	R	R	–	–	s	–	–	R	R	s	S	–
PMR 6	–	–	–	–	–	–	–	–	–	–	–	–	–	R	R	–	s	s	–	–	–	–	–
WMR 29	R	R	–	–	R	R	R	R	R	H	H	R	R	–	–	–	–	–	s	s	s	S	R
Edisto 47	R	R	–	–	R	R	R	R	R	s	R	R	R	–	–	R	–	–	R	s	s	R	R
PI 414723	R	R	–	–	R	R	R	R	R	s	s	R	R	–	–	R	–	–	R	R	R	I	s
MR-1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	s	–	–
PI 124111	R	R	R	R	R	R	R	–	–	R	R	R	–	–	–	R	–	–	R	R	s	–	–
PI 124112	R	R	R	–	R	R	R	R	R	R	R	R	R	–	–	R	–	–	R	R	s	–	–
Earl's Knight Natsu 2	–	R	–	–	–	–	s	–	–	s	–	–	–	–	–	–	–	–	–	s	–	–	s
Earl's Miyabi Natsu 2	–	R	–	–	–	–	s	–	–	s	–	–	–	–	–	–	–	–	–	s	–	–	R
Hainan 21	–	R	–	–	–	–	R	–	–	s	–	–	–	–	–	–	–	–	–	s	–	–	R
Quincy	–	R	–	–	–	–	s	–	–	s	–	–	–	–	–	–	–	–	–	s	–	–	s
Negro	–	–	R	–	R	R	s	–	–	–	s	s	R	–	–	–	–	–	–	–	–	–	–
AR Hale's Best Jumbo	–	–	–	–	–	–	–	s	I	–	–	R	–	–	–	–	–	–	–	R	–	–	–
Amarillo	–	–	R	–	R	s	s	–	–	–	s	–	–	–	–	–	–	–	–	–	–	–	–
Moscatel Grande	–	–	R	–	R	R	s	–	–	–	s	–	–	–	–	–	–	–	–	–	–	–	–
BG6011	–	–	–	–	–	–	–	–	–	–	–	s	R	–	–	–	–	–	–	–	–	–	–
BG 6016	–	–	–	–	–	–	–	–	–	–	–	I	R	–	–	–	–	–	–	–	–	–	–
Bellgarde	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Perlita	–	–	–	R	R	–	R	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–	–
PI 179901	–	–	–	R	R	R	R	–	–	–	s	R	–	–	–	–	–	–	–	–	–	–	–
PI 234607	–	–	–	R	R	R	R	–	–	–	R	R	–	–	–	–	–	–	–	–	–	–	–
PI 236355	–	–	–	R	s	s	s	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–
PI 313970	–	–	–	–	R	R	R	–	–	R	R	R	–	–	–	–	–	–	–	–	R	R	–
Seminole	–	–	–	R	R	–	R	–	–	–	R	R	–	–	–	–	–	–	–	–	–	–	–
Wescan	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
VA 435	–	–	–	–	–	–	–	–	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–
Noy Yizre'el	–	–	–	–	–	–	–	–	–	–	–	–	–	s	R	–	s	R	–	–	–	–	–
Harukei 3	–	–	–	–	–	–	–	–	–	–	–	–	–	s	R	–	s	R	–	–	–	–	–
Nantais Oblong	–	–	–	–	–	–	–	–	–	–	–	–	–	s	R	–	s	R	–	–	–	–	–
Solartur	–	–	–	–	–	–	–	–	–	–	–	–	–	s	R	–	s	R	–	–	–	–	–
Ames 31282[f]	–	–	–	–	–	–	–	–	–	–	–	s°	–	–	–	s°	–	–	–	s°	s°	–	–

Table 1. (continued)

Cultigen	Race																						
	N2[x]	N3[x]	N4[x]	6[m]	F[l]	G[l]	H[l]	P6[n]	S[k]	SD[j]	pxCh 1[i]	Czech 2010 races[h]											
												10	33	36	44	45	51	52	53	54 2	55 2	56 2	59
Iran H	–	–	–	–	s	s	s	–	–	–	R	s	s	s	s	s	s	s	s	s	s	s	s
Top Mark	–	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
Védrantais	–	–	–	s	s	s	s	s	–	–	s	s	s	s	s	s	s	s	s	s	s	s	s
Fuyu 3	s	s	s	–	–	–	–	–	s	–	s	–	–	–	–	–	–	–	–	–	–	–	–
PMR 45	s	R	R	s	s	s	s	s	s	s	R	s	R	s	s	s	s	s	R	s	s	s	s
PMR 5	–	–	–	R	s	s	s	R	s	s	s	s	R	s	s	s	s	s	R	R	s	R	s
PMR 6	–	–	–	–	–	–	–	–	s	s	R	R	R	R	R	s	s	R	R	R	R	R	R
WMR 29	R	R	R	s	s	s	R	s	s	s	R	R	R	s	R	s	s	R	R	s	s	R	s
Edisto 47	R	s	s	s	s	s	s	s	s	s	–	s	R	R	R	s	R	s	R	R	s	R	s
PI 414723	s	s	R	R	s	s	s	s	s	s	–	s	R	s	s	s	s	s	s	s	s	R	s
MR-1	–	–	–	–	s	R	s	R	s	s	R	R	R	R	R	s	R	R	R	R	R	R	s
PI 124111	–	–	–	–	–	–	–	–	?	?	–	R?	R	R	R	R	R	R	R	R	R	R	R
PI 124112	–	–	–	R	s	s	s	R	s	s	s	R	R	R	R	s	R	R	R	R	R	R	R
Earl's Knight Natsu 2	R	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
Earl's Miyabi Natsu 2	R	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
Hainan 21	s	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
Quincy	R	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
Negro	–	–	–	–	–	–	–	–	s	s	–	s	s	s	R	s	s?	s	s	s	s	s	s
AR Hale's Best Jumbo	–	–	–	s	–	–	–	–	s	s	–	s	s	s	s	s	s	s	s	s	s	s	s
Amarillo	–	–	–	–	–	–	–	–	s	s	–	s	s	s	s	s	s	s	s	s	s	s	s
Moscatel Grande	–	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
BG6011	–	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
BG 6016	–	–	–	–	–	–	–	–	?	s	–	–	–	–	–	–	–	–	–	–	–	–	–
Bellgarde	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Perlita	–	–	–	–	–	–	–	–	s	s	–	–	–	–	–	–	–	–	–	–	–	–	–
PI 179901	–	–	–	–	–	–	–	–	s	s	–	s	s	s	R	s	s	s	s	s	s	s	s
PI 234607	–	–	–	–	–	–	–	–	s	s	–	R	R	R	R	s	R	R	R	R	R	R	R
PI 236355	–	–	–	–	–	–	–	–	s	s	–	s	s	s	s	s	s	s	s	s	s	s	s
PI 313970	–	–	–	–	s	–	–	–	R	s	–	R	R	s	R	s	s	s	s	s	s	R	s
Seminole	–	–	–	–	–	–	–	–	s	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Wescan	–	–	–	–	–	–	–	–	s	–	–	–	–	–	–	–	–	–	–	–	–	–	–
VA 435	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Noy Yizre'el	–	–	–	–	–	–	–	–	–	–	–	R	R	R	R	s	s	R	R	R	R	s	R
Harukei 3	–	–	–	–	–	–	–	–	–	–	s	–	–	–	–	–	–	–	–	–	–	–	–
Nantais Oblong	–	–	–	–	s	s	s	–	–	–	–	s	s	s	s	s	s	s	s	s	s	s	s
Solartur	–	–	–	–	s	s	s	–	–	–	–	s	s	s	s	s	s	s	s	s	s	s	s
Ames 31282[f]	–	–	–	–	–	–	–	–	s[d]	s[d]	R	–	–	–	–								

[z]Race 1: 1J, Japan; 1Sp, Spain; 1M, Michigan; 1IV, Imperial Valley, Calif.; 1SJ, San Joaquin Valley, Calif.; 1S, Salinas; 1Ti, Tifton, Ga.; 1Tu, Tunisia. Race 2: 2US, U.S.A.; 2S, Salinas, Calif.; 2F, France; 2Z, Zaragoza

[y]Bardin et al. (1999); Bardin et al. (1997); Bertrand (1991); Křístková and Lebeda (1999a,b); Mohamed et al. (1995)

[x]Hosoya et al. (2000)

[w]Floris and Alvarez (1995)

[v]Harwood and Markarian (1968)

[u]McCreight (2006)

[t]Bertrand (2002)

[s]McCreight et al. (1987)

[r]Alvarez et al. (2000)

[q]Cohen et al. (1996); Cohen et al. (2002)

[p]Thomas (1978)

[o]Bardin (1996); Bardin et al. (1999); Cohen et al. (2002); Pitrat et al. (1998)

[n] M.Pitrat, pers. commun.; Pitrat and Besombes (2008)

[m]Bertrand (2002)

[l]Lebeda and Sedláková (2004)

[k]McCreight and Coffey (2011)

[j]Coffey et al. (2006)

[i]Liu, et al. (2010)

[h]Lebeda et al. (2012)

[g]s = susceptible, R = resistant, H = heterogeneous, I = intermediate, ? = uncertainty; "–" = not tested.

[f]Ames 31282 is the correct designation for PI 134198 in Liu et al. (2010), (K.R. Reitsma, pers. commun.); see http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1898811 and http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1812862

[e]J.Fauve, Harris Moran Clause, pers. commun.

[d]J.D. McCreight, unpubl. data

Table 2. Pathogenic races of cucurbit powdery mildew incited by *Podosphaera xanthii* in different seasons at four locations in California, and Yuma, Arizona; 2002 through 2011.

		Year									
Location	Season	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
California											
Imperial Valley	Spring	1	S	1	S	1	1	1SJ	S	1/2[z]	1
	Fall	1	–	1	–	1[y]	1[y]	–	–	–	–
Five Points	Summer	–	1SJ	–	–	–	S	–	–	–	–
	Fall	–	1	–	–	–	–	–	–	–	–
Davis		–	–	–	–	–	–	–	–	2[x]	S[x]
Woodland		–	–	–	–	–	–	–	–	2[w]	S
Arizona											
Yuma	Spring	–	1/S[v]	S	S	–	S	S	–	–	–
	Fall	–	1	1	–	1[y]	1[y]	–	–	–	–

z'PMR 45' did not germinate; variant of race 1 or 2 based on reactions of other lines.

yPlants infected with Cucurbit yellow stunting disorder virus.

xJ. Mercier, Harris Moran Clause, pers. commun.

wH. Bouzar, Sakata Seed America, pers. commun.

vPx race 1 detected in a field test at Univ. Ariz., Yuma Agric. Res. Ctr. (YARC); Px race S isolated via single spore transfer from a field sample at YARC.

Bacterial spot (*Xanthomonas cucurbitae*): An emerging disease of cucurbits in the United States

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Keywords: Bacterial leaf spot, fruit rot, disease outbreak, pumpkin, squash

Abstract

Bacterial spot, caused by *Xanthomonas cucurbitae*, was economically an insignificant disease in the United States until 2005. But, in recent years, the disease occurred widely in pumpkin fields in the Midwest. *X. cucurbitae* caused leaf spot and fruit infection. Leaf infection was observed from the time of spreading vines until harvest, and infection of fruit occurred from when fruit weights were 0.25 kg until harvest. Field surveys were conducted during 2009-2011 to determine incidence of bacterial spot in commercial pumpkin fields in the Midwest. In 2009, bacterial spot was observed in all 17 pumpkin fields visited in Illinois. In 2010, a survey in Illinois showed that bacterial spot occurred in 40 of 50 commercial pumpkin fields with symptoms on 3 to 94% of fruit in a field (average 34%). In 2011, a survey in the Midwestern states (Illinois, Indiana, Iowa, Kansas, Missouri, Nebraska, Ohio, Wisconsin) showed fruit with bacterial spot symptoms in 95 of 111 pumpkin fields, with lesions on 3 to 98% of the fruit in a field (average 79%). *X. cucurbitae* was isolated from infected leaves and fruit. The identity of the pathogen was confirmed by culturing on yeast extract-dextrose–CaCO3 agar, conducting biochemical and physiological tests, and using primers RST2 and RST3. Koch's postulates for representative isolates were carried out on leaves of the pumpkin cv. 'Howden' in a greenhouse. Koch's postulates for five of the isolates were also conducted in a field. To determine a host-range of *X. cucurbitae*, five isolates of the pathogen were mixed and inoculated onto leaves of 38 plant species in eight families (Cucurbitaceae, Brassicaceae, Chenopodiaceae, Graminae, Leguminosae, Liliaceae, Solanaceae, Umbelliferae) that are commonly grown in crop rotation with cucurbits. Only plant species in the family Cucurbitaceae developed typical symptoms of bacterial spot.

INTRODUCTION

Approximately 150,000 ha are planted to commercial cucurbit crops

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(cantaloupe, cucumber, honeydew, pumpkin, squash, watermelon) annually in the United States (US) (USDA-NASS 2012). The values of cucurbit crops in the U.S. in 2011 exceeded $1.5 billion. Commercial pumpkin and squash fields in 2011 were 20,760 and 20,320 ha, respectively, with combined values exceeding $396 million (USDA-NASS 2012). Approximately 10,000 ha of pumpkins [*Cucurbita pepo* L., *C. maxima* Duchesne, and *C. moschata* (Duchesne) Duchesne ex Poir] are grown annually in Illinois (Babadoost and Ravanlou 2012). More than 90% of commercial processing pumpkins (*C. moschata*) produced in the US are grown and processed in Illinois.

Bacterial spot (also known as bacterial leaf spot), caused by *Xanthomonas cucurbitae* (ex Bryan) Vauterin et al. [syn. *Xanthomonas campestris* pv. *cucurbitae* (Bryan) Dye] (Saddler and Bradbury 2005), has become a serious threat to production of pumpkins and squashes in the US (Babadoost and Ravanlou 2012). Bacterial spot was first described on Hubbard squash in New York in 1926 (Bryan 1958). Subsequently, the disease was reported on cucumbers, gourds, pumpkins, and summer and winter squash in Asia, Australia, Europe, and North America (Babadoost and Zitter 2009; Blancards et al. 1994; Latin and Rane 1999; Pruvost et al. 2008; Pruvost et al. 2009; William and Zitter 1996).

X. cucurbitae infects leaves and fruit of cucurbits throughout the growing season (Babadoost and Ravanlou 2012; Latin and Rane 1999; William and Zitter 1996). Lesions on leaves are small (2- to 4-mm), angular, yellow to beige spots (Fig. 1, A). Leaf infection can easily be overlooked because of the small size of the spots (Babadoost and Zitter 2009; William and Zitter 1996). The appearance and size of fruit lesions can vary, depending on rind maturity and the presence of moisture (Fig. 1, B-D). Initial lesions are small, slightly sunken, circular spots (1- to 3-mm in diameter). In immature fruit, the lesions begin as light green with darker center. In mature fruit, the lesions have a beige center and dark brown halo (Fig. 1, B). Fruit infected by *X. cucurbitae* is usually colonized by other fungi and bacteria which results in rapid collapse of the fruit (Babadoost and Zitter 2009).

During 2009-2011, *X. cucurbitae* caused severe leaf and fruit infections in pumpkin and squash fields in Illinois and other Midwestern states in the US Yield losses up to 90% forced some growers to abandon pumpkin and squash production (Babadoost and Ravanlou 2012). The objective of this study was to assess the incidence of bacterial spot in pumpkin fields in the Midwest and determine host-range of *X. cucurbitae*.

MATERIALS AND METHODS

Disease occurrence

In 2009, 2010, and 2011, surveys were conducted in 17, 50, and 65 pumpkin fields, respectively, in Illinois to determine occurrence of bacterial spot on fruit at harvest. Also, a survey was conducted in 46 pumpkin fields in Iowa, Indiana,

Kansas, Missouri, Nebraska, Ohio, and Wisconsin in 2011 to assess occurrence of bacterial spot in these states (Table 1). In each field, 12 locations in an M-shape pattern were chosen, and the severity of the disease (percentage of fruit surface with bacterial spot lesions) on five fruit at each location (a total of 60 fruit in each field) was assessed.

Pathogen isolation and identification

Six to twelve symptomatic pumpkins were collected from each field, and *X. cucurbitae* was isolated from the fruit by disinfesting an area of the fruit surface with lesions using paper tissue soaked in 95% ethanol. One or two lesions on each fruit were cut out with a sterile blade, inserted into an eppendorf tube containing 1 ml sterile, distilled water (SDW), the tubes shaken manually, and a loopful of the bacterial suspension from each tube streaked onto nutrient agar (NA). The plates were incubated at 24°C for 3 to 4 days. Single colonies of the isolated bacterium on each plate were prepared by streaking isolated colonies onto additional NA plates and selecting well-separated colonies. The colonies were identified as *X. cucurbitae* by culturing on yeast extract-dextrose–CaCO3 (YDC) agar, conducting biochemical/physiological tests, and using primers RST2 (5'AGGCCCTGGAAGGTGCCCTGGA3') and RST3 (5'ATCGCACTGCGTACCGCGCGCGA3') in a conventional PCR assay (Leite et al. 1994; Widmer et al. 1998).

Host range

A mixture of five isolates of *X. cucurbitae* from five different areas of Illinois was used for determining the host-range of this bacterium. To prepare inoculum, the isolates were streaked onto Laura Broth (LB) agar medium in Petri plates and incubated at 24 ± 1°C for 3 days. Bacterial cells were suspended in sterile-distilled water (SDW) and mixed together. Concentration of inoculum was adjusted to 10^8 cfu mL^{-1}, using a spectrophotometer (OD=0.5 at 600 nm). For inoculating plants, 0.5 ml of the bacterial suspension was infiltrated into leaf abaxial using a 10-ml syringe, and 5-10 shots were applied to each leaf. Inoculated plants were placed on a greenhouse bench at 24-28°C for 30 days. The plants were then examined for development of leaf lesions. Thirty eight crops in eight plant families, commonly grown in rotation with pumpkin were tested for their susceptibility to *X. cucurbitae*. The test crops were as follows. Cucurbitaceae: acorn squash (*Cucurbita pepo*), butternut squash (*Cucurbita moschata*), cantaloupe (*Cucumis melo*), cucumber (*Cucumis sativus*), gourd (*Lagenaria siceraria*), honeydew (*Cucumis melo*), muskmelon (*Cucumis melo*), processing pumpkin (*Cucurbita moschata*), pumpkin (*Cucurbita pepo*), pumpkin (*Cucurbita maxima*), squash (*Cucurbita argyrosperma*), squash (*Cucurbita maxima*), watermelon (*Citrullus lanatus*), and zucchini (*Cucurbita pepo*); Brassicaceae: broccoli (*Brassica oleracea*), Brussels sprout

(*Brassica oleracea*), cabbage (*Brassica oleracea*), cauliflower (*Brassica oleracea*), kale (*Brassica oleracea*), mustard (*Brassica nigra*), radish (*Raphanus sativus*), turnip (*Brassica rapa*), and wild mustard (*Synapis arvensis*); Chenopodiaceae: beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), and Swiss-chard (*Beta vulgaris* var. *cicla*); Graminae: barley (*Hordeum vulgare*), corn (*Zea mays*), oat (*Avenae* sp.), and wheat (*Triticum aestivum*); Leguminosae: bean (*Phaseolus vulgaris*), and snow pea (*Pisum sativus*); Liliaceae: onion (*Allium cepa*); Solanaceae: eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), and tomato (*Solanum lycopersicum*); and Umbelliferae: carrot (*Daucus carota*) and celery (*Apium graveolens*).

RESULTS

Disease occurrence

In 2009, bacterial spot was observed on leaves and fruit in all 17 fields surveyed. Small, water-soaked lesions first appeared on the lower sides of the leaves, which became visible as gray to yellow areas on the upper sides. The lesions gradually became angular with yellow halos (Fig. 1, A). As the lesion coalesced, chlorotic and necrotic patches develop on leaves. Lesions on young fruit were light green, with darker center. On mature fruit, yellow spots (1- to 2-mm in diameter) developed during July-September (Fig. 1, B-D). The lesions gradually became dark brown with a beige center. Infected fruit were colonized by saprophyte fungi and bacteria, resulting in development of larger lesions and collapse of fruit. Isolated bacteria produced yellow colonies on NA.

In 2010, bacterial spot occurred in 40 of 50 pumpkin fields with symptoms on 3 to 94% of fruit in a field (average 34%). In nine fields more than 50% of fruit were infected with *X. cucurbitae*. Fields with infected fruit were scattered throughout Illinois. In 2011, 95 of 111 pumpkin fields surveyed had fruit infected with *X. cucurbitae*, with symptoms on 3-98% of fruit in a field (average 27%) (Table 1). Severity of bacterial spot on fruit ranged from 1 to 20% during 2009-2011. Incidence of the disease was highest in Indiana and lowest in Iowa.

Pathogen isolation and identification

X. cucurbitae was isolated from all symptomatic leaves and fruit collected from all eight states surveyed. *X. cucurbitae* colonies produced xanthomonad-like, yellow colonies with mucoid growth on YDC agar. The isolates were Gram negative, O^+ and F^- in the oxidative and fermentative test, oxidase negative, and motile. Also, the isolates hydrolyzed starch and esculin, but did not hydrolyze nitrate, and grew on YDC agar at 33°C. Species identity was confirmed when an isolate produced 1,500 bp band using RST2 and RST3 primers in a conventional PCR assay (Leite et al. 1994; Widmer et al. 1998).

Host range

All inoculated plants in Cucurbitaceae family developed typical symptoms of bacterial spot on leaves 3-5 days after inoculation. Leaf infection began as water-soaked lesions and became chlorotic and necrotic afterward. *X. cucurbitae* was isolated from symptomatic leaves. None of the inoculated plants in Brassicaceae, Chenopodiaceae, Graminae, Leguminosae, Solanaceae, Liliaceae, and Umbelliferae families developed bacterial spot symptoms. However, some plants, including, bean, broccoli, pepper, mustard, oat, radish, snow pea, turnip, tomato, and wild mustard showed hypersensitive reaction 3-5 days after inoculation. Attempts to isolate *X. cucurbitae* from plants with hypersensitive reaction did not result in development of any bacterial colonies.

DISCUSSION

Bacterial spot, caused by *X. cucurbitae*, was economically an insignificant disease in the U.S. prior to 2005. But, in the recent years, the disease occurred widely in pumpkin fields in the Midwest. Bacterial spot was observed in all pumpkin and winter squash cultivars grown in the Midwest. Yield losses of up to more than 90% forced some growers to abandon production of pumpkin and squash. In 2011, bacterial spot was also observed in most of pumpkin and squash fields in Austria (Babadoost, unpublished data). Additional surveys may show occurrence of bacterial spot in other cucurbit crops and other cucurbit growing areas in the U.S. as well as in the world. Thus, these findings indicate that bacterial spot (*X. cucurbitae*) is an emerging disease in pumpkin and squash fields, and likely in all cucurbit crops.

Cucurbits are grown for fresh market and processing and are among the most valuable vegetable crops throughout the world. The farm-gate values of cucurbit crops in the U.S. alone exceed $1.5 billion per year. To ensure sustainable production of cucurbit crops, developing effective strategies for management of *X. cucurbitae* are essential. In order to develop effective management of bacterial spot, sufficient knowledge on the etiology of the disease is needed. There is very limited information on the biology of *X. cucurbitae* and epidemiology of the bacterial spot. Currently, we are conducting research to determine the necessary aspects of the etiology of the disease and aim to develop strategies for effective management of bacterial spot.

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Table 1. Occurrence of bacterial spot (*Xanthomonas cucurbitae*) in pumpkin fields in the Midwest in 2011.

State	No. fields surveyed	No. fields with infected fruit	Infected fruit in a field (%)	Average infected fruit in the state (%)
Illinois	65	57	3-87	24
Indiana	7	7	22-98	62
Iowa	8	4	8-28	8
Kansas	5	5	15-50	30
Missouri	6	3	7-57	14
Nebraska	5	5	7-93	54
Ohio	9	9	7-75	32
Wisconsin	6	5	3-40	18
Total	**111**	**95**	**3-98**	**27**

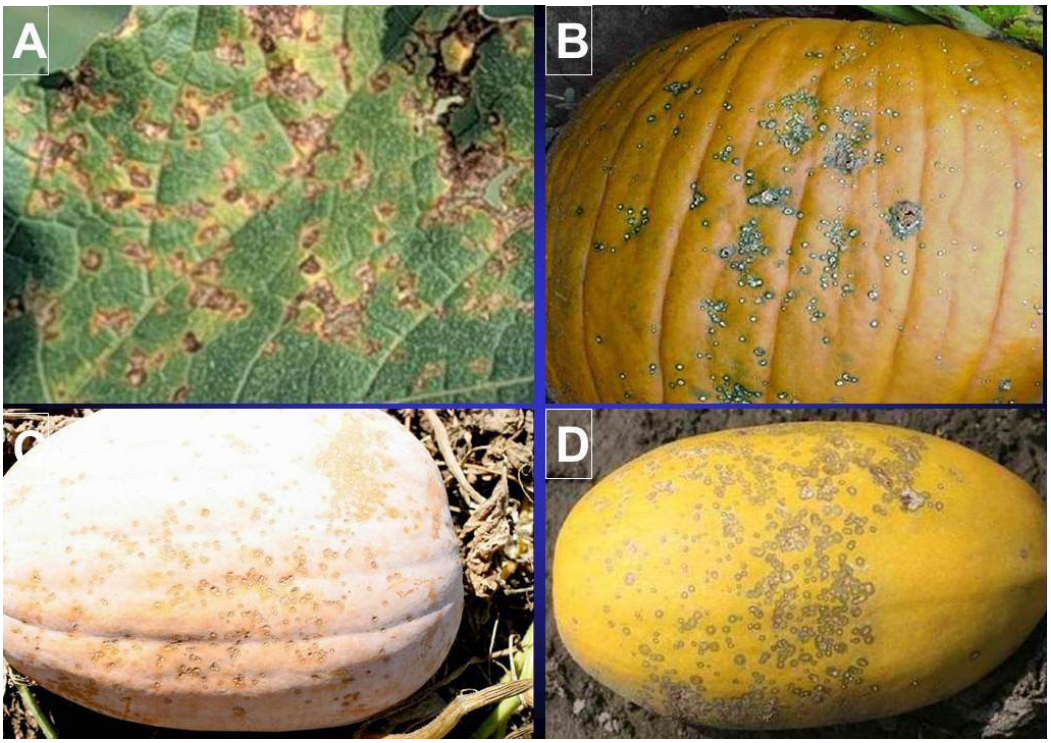

Fig. 1. Leaves and fruits of pumpkin and squash infected by *Xanthomonas cucurbitae*: A, an infected pumpkin leaf; B, an infected jack-o-lantern pumpkin fruit; C, an infected processing pumpkin fruit; D, and an infected squash fruit.

Detection of bacterial fruit blotch in *Cucurbita pepo* seeds and seed disinfection

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Keywords: *Cucurbita pepo*, bacterial fruit blotch, bacterial seed, seed disinfection

Abstract

Bacterial fruit blotch (BFB) is a devastating disease of *Cucurbita pepo* caused by *Acidovorax citruli* and can result in severe field losses. *A. citrulli* is mainly transmitted through seeds. In this study, a PCR technique was used for detecting BFB in *Cucurbita pepo* seeds, and the effect of different seed-disinfection treatments, including dipping in a solution of $CuSO_4$, bleaching powder, HCl, peroxyacetic acid, streptomycin, doxycycline, or exposure to dry heating at high-temperatures, on BFB elimination were analyzed. The dry heat treatments were ineffective in disinfecting the seeds, even at 80 ºC for 48 h. Disinfecting solutions of $CuSO_4$ and bleaching powder were effective in eliminating the bacteria from the seeds. Soaking infected seeds in a solution of 0.5% bleaching powder for five minutes effectively eliminated BFB bacteria. It is not necessary to get rid of the reagents with water after treatment, as they did not adversely affect seed germination. Thus, there are simple solutions that can help control bacterial fruit blotch in commercial fruit and seed production of *C. pepo*.

INTRODUCTION

Bacterial fruit blotch disease of cucurbit crops caused by *Acidovorax citrulli* occurs in almost all areas that cucurbits are grown and especially in the tropics and subtropics (Kubota et al. 2012). This pathogen is a serious threat to hybrid seed production of squash, pumpkins, watermelons, and melons (Kubota et al. 2012; Tomita et al. 2006).

Cucurbita pepo is the most economically important species within the *Cucurbita* genus. Its great economic value is based mainly on the culinary use of the immature fruits as vegetables, often referred to collectively as “summer squashes”. BFB is a seed-borne disease that often causes seedling damping-off type symptoms and seedling death in *C. pepo* (Rane and Latin 1992). BFB used to occur rarely in *C. pepo*, but now the disease is considered widespread throughout most of China,

caused by infected seeds. However, until now most methods of control of this pathogen have not been effective (Song et al. 2007). Ultimately, the best strategies to reduce the threat of BFB are to produce pathogen-free seeds. Many physical and chemical treatments were tested in attempts to minimize the presence of the pathogen in melon and watermelon seeds (Kubota et al. 2012; Hara et al. 2006). The usual methods employed to control seed contamination by the BFB pathogen were not effective in *C. pepo*.

In this report, we precisely analysed the relationship between effective disinfection and different seed treatments, including chemical treatments and dry heat of various temperatures and duration, on seeds of *C. pepo*. Germination of seeds was also tested after disinfection treatments.

MATERIALS AND METHODS

Seed treatments and evaluation of the treatments for disinfection

Hybrid seeds of 'Jing Hu 4' (*C. pepo*, provided by Beijing Vegetable Research Center, Beijing Academy of Agricultural and Forestry Sciences, China), contaminated by the BFB pathogen, were used in this study.

Solutions of $CuSO_4$ (0.1 to 1 percent), bleaching powder (0.5 to 2 percent), HCl (1 percent), peroxyacetic acid (0.1 percent), streptomycin (1g/L), doxycycline (0.1 percent) and periods of 5, 10, 15, 20 and 30 min were combined and applied to the seeds. Temperatures of 65, 70, 75, 80 ºC for 48 hours were applied to tested seeds in electric ovens (WFO-601SD, Tokyo Rikakikai Co. Ltd., Tokyo or DN-81, Yamato Scientific Co. Ltd., Tokyo, Japan). All seeds were predried in ovens at 40 or 60ºC for 24 hours before applying the dry heat treatments above.

Presence of the BFB pathogen was tested using the method described by Song et al. (2007). Three hundred seeds were dipped in 100 mL sterile water and then incubated for 4 h at 30 ºC with 220 r/min. Then the liquid samples were centrifuged for 10 min at 6000×g and the precipitate was collected and resuspended in 500 µL sterile distilled water. The presence of the BFB pathogen was detected by polymerase chain reaction (PCR) using the extracts as template with sense primers BFBf: 5′ GACCAGCCACACTGGGAC 3′ and antisense primers BFBr: 5′ CTGCCGTACTCCAGCGAT 3′. PCR cycling parameters were 95 ºC for 3 min followed by 35 cycles of denaturation for 30 s at 94 ºC, annealing for 30 s at 55 ºC and extension for 35 s at 72 ºC, followed by a final extension at 72 ºC for 5 min. PCR products were separated on 1% agarose gels containing ethidium bromide and visualized and photographed under UV light. All samples were replicated three times.

Germination test

The seeds were germinated in Petri dishes (11 cm diameter), containing two sheets of Whatman No. 1 filter paper moistened initially with water, after soaking

in tap-water for 4 h at 30 ºC. Each petri dish contained 50 seeds. Seeds were kept in a germination chamber at 30 ºC in darkness. Each treatment was replicated 3 times.

Number of germinated seeds was checked daily. A seed was considered to have germinated when a 5 mm radicle had emerged from the seed coat. Germination rate and germination "energy" were quantified with the following formula:

Germination rate= a/c×100%;
Germination energy= b/c×100%;

where a is for total number of seeds germinated during a 7-day period, b for total number of seeds germinated during 3-day period, c for total number of seeds to germinate.

RESULTS AND DISCUSSION

Effect of different chemical treatments on seed disinfection of *C. pepo*

The results show that solutions of $CuSO_4$ (1%) and bleaching powder (2%) were effective in eliminating the contaminating bacteria from seeds (Fig. 1). No BFB pathogen was detected by PCR in these treatments. HCl (1%), peroxyacetic acid (0.1%), and doxycycline (0.1%) were relatively ineffective in reducing bacterial contamination of squash seeds by BFB.

To save time and expense, and reduce environmental pollution, we further tested the effect of reducing the chemical concentration and treated period on seed disinfection (Fig. 2). The results indicated that bleaching powder is more effective than $CuSO_4$ in BFB pathogen elimination. Even soaking the bacteria-contaminated seeds in a solution of 0.5% bleaching powder for 5 minutes can completely eliminate the BFB bacterium from infested seed.

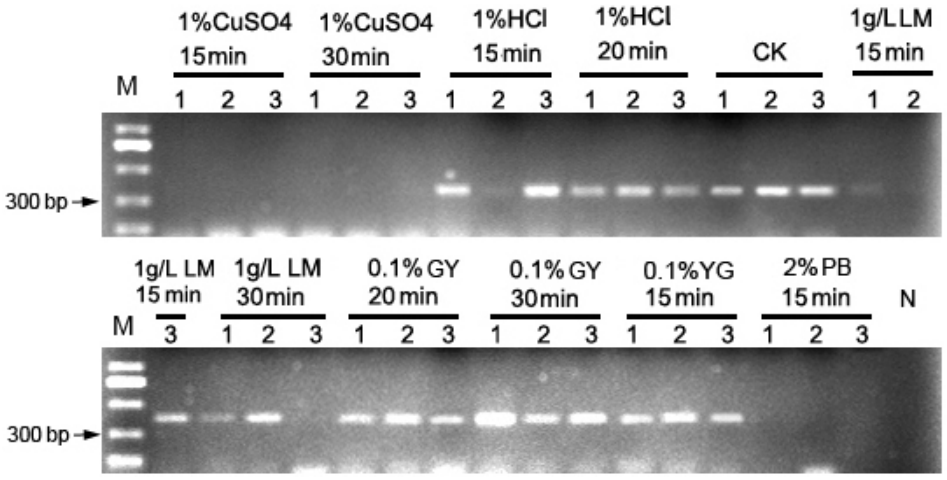

Fig. 1. Effect of different chemical treatments on seed disinfection: M, DNA marker; CK, positive control treated with distilled water; LM, streptomycin; GY,

peroxyacetic acid; YG, doxycycline; PB, bleaching powder; N, negative control.

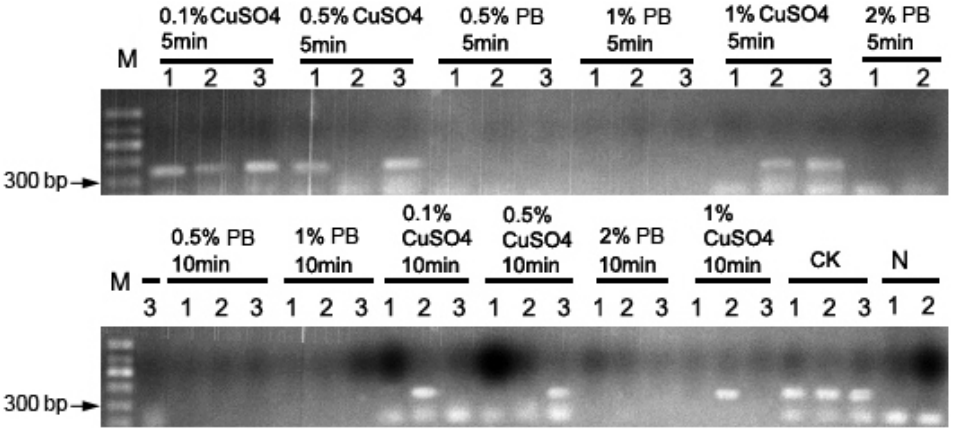

Fig. 2. Effect of concentration and periods of CuSO4 and bleaching powder solutions on seed disinfection: M, DNA marker; PB, bleaching powder; CK, positive control treated with distilled water; N, negative control.

Effect of different dry heat treatments on seed disinfection of *C. pepo*

Some studies have focused on BFB-pathogen elimination from infested seeds with dry heat treatments (Kubota et al. 2012). Our results show that the BFB pathogen is highly tolerant to dry heat, and cannot be completely eliminated even at temperatures as high as 80ºC (Table 1). Thus, there were no feasible conditions for squash seed BFB-pathogen disinfection by heat treatment without adversely affecting germination, consistent with the results of Kubota et al. (2012) and Wall (1989).

Table 1. Effect of dry heat on seed disinfestation.

Temperature and periods	Number of treated seeds	Number of tested seeds by PCR	Results
40 ºC 24 h，65 ºC 48 h	6×300	3×300	+
40 ºC 24 h，70 ºC 48 h	6×300	3×300	+
40 ºC 24 h，75 ºC 48 h	6×300	3×300	+
40 ºC 24 h，80 ºC 48 h	6×300	3×300	+
60 ºC 24 h，70 ºC 48 h	6×300	3×300	+
60 ºC 24 h，75 ºC 48 h	6×300	3×300	+
60 ºC 24 h，80 ºC 48 h	6×300	3×300	+
Not treated	6×300	3×300	+

+ = presence of BFB pathogen in three replicates

Seed Germination

Bleaching powder solution did not adversely affect seed germination rate and germination energy even if the seeds were not rinsed with tap-water after treatment

(Table 2). Thus, it is not necessary to eliminate with water any remaining bleaching powder solution.

Table 2 Effect of bleaching powder solution (PBS) on seed germination

Treatments	Number of seeds	Germination (%)	Germination energy(%)
0.5% PBS 5min, rinsedwith distilled water	3×100	99.7	99.7
0.5%PBS 5 min, not rinsed	3×100	100.0	100.0
0.5% PBS 10 min, rinsed with distilled water	3×100	99.3	99.3
0.5% PBS 10 min, not rinsed	3×100	100.0	100.0
1% PBS 5 min, rinsed with distilled water	3×100	100.0	100.0
1% PBS 5min, not rinsed	3×100	100.0	100.0
1% PBS 10 min, rinsed with distilled water	3×100	100.0	100.0
1% PBS 10 min, not rinsed	3×100	100.0	100.0
CK	3×100	100.0	100.0

CONCLUSION

Bleaching powder was effective in eliminating the bacterial fruit blotch pathogen from squash seeds, and more effective than antibiotics and other chemical reagents such as $CuSO_4$ and HCl. Also, it is cost-effective and less harmful to the environment and human health. Soaking the bacteria-contaminated seeds of *C. pepo* in a solution of 0.5% bleaching powder for 5 minutes effectively eliminated the BFB bacterium and it was not necessary to rinse the seeds after treatment with bleaching powder solution, as whatever might have remained of the bleach did not adversely affect seed germination.

ACKNOWLEDGEMENTS

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Resistant rootstocks for managing root-knot nematodes (*Meloidogyne incognita*) in grafted watermelon and melon

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Keywords: *Citrullus lanatus* var. *citroides*, *Cucumis metuliferus*, African horned cucumber, wild watermelon, cucurbit, hybrid squash, tinda

Abstract

Rootstock lines of wild watermelon (*Citrullus lanatus* subsp. *lanatus* var. *citroides*) with resistance to root-knot nematodes (RKN) were developed at the U.S. Vegetable Laboratory. Rootstock lines RKVL 301, RKVL 316, and RKVL 318 (RKVL = Root Knot Vegetable Laboratory) were compared to wild commercial cucurbit rootstocks and tinda (*Praecitrullus fistulosus*) for grafted seedless triploid watermelon (*C. lanatus* subsp. *vulgaris* var. *vulgaris*) 'Tri-X 313' in fields infested with southern RKN (*Meloidogyne incognita*) in Charleston, SC. RKVL wild watermelon rootstocks had lower ($P<0.05$) root galling (range: 9% to 16%) than non-grafted 'Tri-X 313' (41%), 'Emphasis' (86%), 'Strong Tosa' (99%), and wild tinda (96%) rootstocks. The RKVL 318 rootstock produced higher fruit yield (29.5 kg/plot of 6 plants) ($P<0.05$) than all other rootstocks (mean = 11.0 kg/plot), except for self-grafted 'Tri-X 313' watermelon (21.5 kg/plot). The three RKVL wild watermelon lines should be useful rootstocks for grafted watermelon, and useful germplasm sources for developing RKN-resistant watermelon breeding lines and cultivars. In greenhouse tests, we evaluated reaction of 23 commercial cucurbit rootstocks and germplasm accessions including African horned cucumber (*Cucumis metuliferus*), pumpkin (*Cucurbita moschata*), hybrid squash (*C. maxima* × *C. moschata*), and melon. Melon, pumpkin, and squash exhibited the most severe root galling (range: 4.2 – 5.0 using a 1 to 5 rating scale where 1=no galls and 5=more than 80% of root system galled) in the greenhouse test. African horned cucumber was moderately resistant to susceptible (galling range 2.6 – 3.2). Several accessions of different cucurbit species were evaluated as rootstocks for grafted 'Athena' melon in a field infested with RKN (*M. incognita*) in Charleston, SC. All C*ucurbita* spp. and *C. melo* entries were highly susceptible to *M. incognita* in the field tests as evidenced

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by high numbers of *M. incognita* eggs in roots (up to 3600 eggs/gram fresh root). African horned cucumber rootstocks supported the lowest RKN reproduction. All rootstocks were compatible with the scion 'Athena' melon. Additional studies are underway for developing African horned cucumber rootstocks that could be useful for managing RKN in grafted melon.

INTRODUCTION

The southern root-knot nematode (RKN) (*Meloidogyne incognita*) is a serious constraint to U.S. melon and watermelon production and can significantly reduce yields of both of these important cucurbit crops worldwide (Davis 2007; Sumner and Johnson 1973; Thies 1996). Until recently, the primary method for controlling root-knot nematodes in vegetable crops, including melon and watermelon, has been pre-plant fumigation of soil with methyl bromide (Thies et al. 2010). However, the recent ban on methyl bromide, as well as the loss of other nematicides and fumigant biocides from the U.S. market because of human health risks and ground water contamination (Chitwood 2003; USEPA 2011), has stimulated high interest in the development of alternative technologies, such as use of resistant rootstocks for grafting, for managing RKN in melon and watermelon.

Grafting vegetables onto resistant or nonhost rootstocks to reduce damage by soil-borne diseases or pests has been utilized in eastern Asia for many years (Cohen et al. 2007). Grafting has been used as an effective alternative to long-term breeding programs aimed at incorporating resistance to soil-borne diseases, especially Fusarium wilt, into elite vegetable cultivars (Miguel et al. 2004). Rootstocks were also shown to be valuable in enhancing tolerance to abiotic stresses and in improving fruit yield and quality (Cohen et al. 2007; Core 2005; Edelstein and Ben-Hur, 2006).

Squash hybrid (*Cucurbita maxima* × *C. moschata*) and bottle gourd (*Lagenaria siceraria*) rootstocks are among the most commonly used rootstocks for grafting watermelon and melon. These species are not susceptible to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* and they have vigorous roots systems which can increase absorption of soil water and nutrients for the grafted scion (Miguel et al. 2004; Edelstein and Ben-Hur, 2006). However, the squash hybrid rootstocks and bottle gourd rootstocks are highly susceptible to root-knot nematodes (Thies et al. 2010), and thus these rootstocks are not suitable for grafting when soils are infested with root-knot nematodes. In the same studies, Thies and colleagues (2010) identified several improved lines derived from RKN-resistant wild watermelon PIs that may be useful as rootstocks for grafted watermelon. There is a need to evaluate the response of cucurbit rootstocks and germplasm to RKN and identify germplasm useful for further development of RKN-resistant rootstocks for grafting watermelon and melon cultivars.

The objectives of this study were to: 1) evaluate the performance of improved *C. lanatus* subsp. *lanatus* var. *citroides* rootstock lines versus commercial bottle gourd and hybrid squash rootstocks, and wild tinda rootstocks for managing RKN in grafted watermelon; 2) evaluate response of several cucurbit species as rootstocks for grafted melon in RKN-inoculated tests in the greenhouse and in field soils heavily infested with *M. incognita*.

MATERIALS AND METHODS

Watermelon Rootstock Study, Charleston, SC

Eleven rootstock/seedless watermelon scion ('Tri-X 313') combinations, a non-grafted control ('Tri-X 313'), and a self-grafted control ('Tri-X 313') were evaluated in a field infested with the southern RKN, *Meloidogyne incognita*, at the U.S. Vegetable Laboratory, Charleston, S.C. Rootstocks were grafted to the scion triploid watermelon 'Tri-X 313' and grafted plants were transplanted on raised white plastic mulch beds on 4 June 2009. Seedless watermelon 'Tri-X 313' was grafted on to five wild watermelon (*Citrullus lanatus* subsp. *lanatus* var. *citroides*) germplasm lines developed at the U.S. Vegetable Laboratory (RKVL 301, RKVL 302, RKVL 303, RKVL 316, and RKVL 318), one bottle gourd (*Lagenaria siceraria* 'Emphasis') cultivar, one squash (*Cucurbita maxima* × *C. moschata* 'Strong Tosa') hybrid, one commercial watermelon rootstock cultivar (*C. lanatus* subsp. *lanatus* var. *citroides* 'Ojakkyo'), and three wild tinda (*Praecitrullus fistulosus*) rootstocks (P0004, P0005, and P0006). 'Tri-X 313' self-grafted and 'Tri-X 313' non-grafted also were included as checks in the study. The experimental design was a randomized complete block with six replicates of six plants per replicate.

Watermelon fruit were harvested and fruit weight recorded on twice weekly from 30 July through 17 August 2009. Then roots of all plants were dug and evaluated for percent of root system galled and covered with egg masses, and percent of fibrous roots. Nematode eggs were extracted from the roots using 1% NaOCl (Hussey and Barker, 1973) and eggs were counted using a stereomicroscope. Root galling, egg mass, and fibrous root percentages were arcsine transformed and eggs per gram fresh root were $\log_{10}(x + 1)$ transformed to normalize data. Analysis of variance was conducted on transformed data using the GLM procedure of SAS v.9.1 for Windows (SAS Institute Inc., Cary, NC) and means were separated using Fisher's Protected Least Significant Difference (LSD).

Evaluation of melon rootstocks for response to Meloidogyne incognita in a greenhouse test

Twenty-three commercial cucurbit rootstocks were evaluated for resistance to the *Meloidogyne incognita* in the greenhouse (Table 2). *Meloidogyne incognita* race 3 was cultured on tomato (*Lycopersicon esculentum* L.) in the greenhouse and nematode egg inoculum was prepared as previously described (Thies and Levi,

2003; 2007) for inoculation of the cucurbit rootstocks. Twenty-three current commercial cucurbit rootstocks and selected PIs were evaluated for reaction to *M. incognita* in replicated greenhouse tests, as described by Thies and Levi (2003; 2007). Commercial *Cucurbita maxima* × *C. moschata* 'Strong Tosa' was included as a known susceptible reference control. The experimental design was a randomized complete block with six replicates and five plants per replicate (n = 30). Seeds were sown in plastic trays containing 50 individual 0.2-L cells filled with Metro-Mix 360 and placed in a greenhouse maintained at 26°C to 31°C. Seedlings were inoculated at the first true leaf stage with 2,500 eggs of *M. incognita* race 3. Eight weeks later, roots were evaluated for galling severity and egg mass production using a 1 to 5 scale in which 1 = 0% to 3%, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 79%, and 5 ≥ 80% of root system galled (Thies and Fery 2000). Data were analyzed using the GLM procedure of SAS for Windows, v.9.1 (SAS Institute, 2002), and means were separated using Fisher's protected least significant difference test. Non-transformed data are shown in Table 2.

Evaluation of melon rootstocks for response to root-knot nematode in a field experiment, Charleston, SC

Several accessions of cucurbit species that had been previously reported as compatible rootstocks for grafted melon (*C. melo*) were evaluated for reproduction of *M. incognita* in a field that was heavily infested with *M. incognita* in Charleston, South Carolina. The accessions evaluated included wild germplasm and elite rootstock cultivars of hybrid squash (*Cucurbita maxima* × *C. moschata*), cushaw (*Cucurbita argyrosperma*), Malabar gourd (*Cucurbita ficifolia*), pumpkin (*Cucurbita moschata*), squash (*C. maxima*), winter melon (*Benincasa hispida*), African horned cucumber (*Cucumis metuliferus*), and melon (*Cucumis melo*).

The scion and rootstock seeds were sown in 50-cell trays. Three weeks later, 'Athena' melon scions were grafted to each of the rootstocks (Table 3) using the one-cotyledon method (Hassell et al. 2008). On 16 July 2008, grafted plants were transplanted into single-row plots on raised white plastic mulch beds on 2.0-m centers. The experimental design was a randomized complete block with six replications and each plot consisted of a single row of six grafted plants spaced 60-cm apart. Approximately 12 weeks after planting, roots of all plants in each plot were lifted from the soil and washed. Root systems from each plot were bulked, weighed, and cut into 1- to 2-cm pieces, and eggs were extracted in 1.0% NaOCl (Hussey and Barker, 1973). Eggs were counted using a stereomicroscope. Eggs per gram fresh root were log10 (x + 1) transformed for analysis of variance to normalize data. Analysis of variance was conducted using the GLM procedure of SAS v.9.1 for Windows (SAS Institute Inc., Cary, NC) and means were separated using Fisher's protected least significant difference (LSD).

RESULTS AND DISCUSSION

Watermelon Rootstock Study, Charleston, SC

Root-knot nematode galling was extensive in 'Strong Tosa' squash hybrid (*Cucurbita maxima* × *C. moschata*) (100% galled) and 'Emphasis' bottlegourd (*L. siceraria*) (86% root system galled) rootstocks (Table 1). The tinda (*P. fistulosus*) rootstocks were also severely galled by RKN (91% to 100% of root systems galled. The five wild watermelon rootstocks had less root galling ($P \leq 0.05$) (range: 9% to 16%) than non-grafted 'Tri-X 313' (41%), 'Emphasis', 'Strong Tosa', and the wild tinda accessions. The commercial watermelon rootstock 'Ojakkyo' exhibited heavier root galling (26%) than the five wild watermelon rootstocks, however, the differences were not significant.

The wild watermelon rootstocks, RKVL 302 and RKVL 318, had fewer ($P \leq 0.05$) eggs of *M. incognita* per gram fresh root than 'Tri-X 313' (non-grafted and self-grafted), 'Emphasis', 'Strong Tosa', P0004, P0005, and P0006. 'Emphasis' and 'Strong Tosa' had the highest reproduction of *M. incognita* (835 and 3137 eggs per gram fresh root, respectively). RKVL 301, RKVL 303, RKVL 316, and RKVL 318 had more ($P \leq 0.05$) fibrous roots than 'Tri-X 313' (non-grafted and self-grafted), 'Emphasis', 'Strong Tosa', P0004, P0005, and P0006 (Table 1). Large amounts of fibrous roots are often associated with the ability of a host plant to tolerate attack by plant-parasitic nematodes.

The wild watermelon rootstock RKVL 318 produced more fruit (12 per plot) ($P \leq 0.05$) than any other entry and also produced the greatest yield (29.5 kg per plot) ($P \leq 0.05$) compared to all other entries except self-grafted 'Tri-X 313' (21.5 kg per plot). 'Strong Tosa' and the three *P. fistulosus* rootstocks had the lowest yields which was associated with the extensive root galling, root damage, and poor fibrous root systems (range: <1% to 35% fibrous roots) observed for these rootstocks.

The five wild watermelon rootstocks showed resistance to southern RKN in this test. The wild watermelon rootstock RKVL 318 produced the most fruit and highest yield of any of rootstock entries in the test. These results indicate that these wild watermelon rootstocks (*C. lanatus* subsp. *lanatus* var. *citroides*) should be useful for managing RKN in grafted watermelon.

Evaluation of melon rootstocks in a greenhouse test

In the greenhouse study, melon (*C. melo*), pumpkin (*C. moschata*), and squash (*Cucurbita* spp.) exhibited the most severe root galling with root gall indices ranging from 4.25 to 5.00 and egg mass indices ranging from 3.79 to 4.88 (Table 2). The African horned cucumber (*C. metuliferus*) accessions exhibited the least root galling (range: 2.6 to 3.3) and egg mass production (range: 1.1 to 1.2) (Table 2). Previous studies indicated that *C. metuliferus* accessions have higher resistance to RKN (*M. incognita*) compared to *C. melo* (Fassuliotis and Rau 1963) or moderate resistance to RKN compared to squash (*Cucurbita moschata*) rootstocks (Sigüenza et al. 2005).

Evaluation of melon rootstocks for response to root-knot nematode in a field experiment, Charleston, SC

Most of the cucurbit rootstocks evaluated in the field studies supported high numbers of *M. incognita* eggs in the roots (Table 3). *Cucurbita argyrosperma* and *C. moschata* supported significantly greater numbers of *M. incognita* eggs per gram root (6793 and 7044, respectively) than all other rootstocks. *Cucurbita maxima* PI 548692 and 'Dinero' melon also had high numbers of *M. incognita* eggs per gram root (1849 and 1016, respectively), although not significantly different from numbers of *M. incognita* eggs per gram root for *C. ficifolia* Grif 9448 (773 eggs per gram root), *B. hispida* PI 391545 (659), *C. melo* 'Athena' self-grafted (767) and 'Athena' non-grafted (453). *Cucumis metuliferus* PI 482452 and PI 482443 had numerically lower numbers of eggs per gram root (370 and 323 eggs per gram of root, respectively) than *C. maxima*, *C. ficifolia*, *C. melo*, and *B. hispida*; however, the numbers of eggs per gram roots were not significantly different among these entries.

All of rootstock genotypes were compatible for grafting with 'Athena' melon, however, the African horned cucumber accessions were the only entries that exhibited resistance, e.g. low root galling and low reproduction of *M. incognita.* Studies are in progress to develop African horned cucumber rootstocks that could be useful for managing RKN in grafted melon.

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Table 1. Percentages of root systems galled, numbers of M. incognita eggs per gram fresh root, and watermelon fruit yields and numbers for 'Tri-X 313' seedless watermelon grafted on rootstocks of wild watermelon lines and wild tinda lines, and selected commercial rootstocks. Charleston, SC.

Rootstock accession	Percentage root system galled[a]		Eggs per g fresh root[b]		Total fruit weight (kg/plot)[c]		No. fruit per plot[c]	
Wild watermelon (*Citrullus lanatus* var. *citroides*)								
RKVL 301	9	a[d]	24	ab	20.0	d	7	cd
RKVL 302	10	a	21	a	14.8	cd	5	bd
RKVL 303	16	ab	58	abc	16.6	cd	6	cd
RKVL 316	14	ab	31	ab	18.8	cd	8	d
RKVL 318	13	ab	16	a	29.5	e	12	e
'Ojakkyo'	26	ac	34	abc	19.8	d	7	cd
Bottle gourd (*Lagenaria siceraria*)								
'Emphasis'	86	d	835	fg	17.2	cd	7	cd
Hybrid squash (*Cucurbita maxima* × *C. moschata*)								
'Strong Tosa'	99	e	3137	g	11.6	abc	3	abc
Tinda (*Praecitrullus fistulosus*)								
P0004	98	de	284	def	6.2	a	2	a
P0005	91	de	128	cde	4.7	a	2	a
P0006	100	f	448	ef	7.0	ab	3	ab
Watermelon checks (*C. lanatus* var. *lanatus*)								
'Tri-X 313' non-grafted	41	c	140	cde	15.7	cd	6	cd
'Tri-X 313' self-grafted	31	bc	91	bcd	21.5	de	8	d

[a]Data was arcsine transformed before analysis; non-transformed data are shown.
[b]Data was $\log_{10}(x+1)$ transformed before analysis; non-transformed data are shown.
[c]Six plants per plot (12' x 15' = approx. 30 square ft per plant).
[d]Means within a column followed by the same letter are not significantly different ($P<0.05$) according to Fisher's Protected LSD.

Table 2. Root gall and egg mass indices for cucurbit rootstocks inoculated with southern root-knot nematode, *Meloidogyne incognita*, in a greenhouse test.

Cucurbit taxon/entry	Gall index[a]	Egg mass index[a]
Cucurbita maxima*× *Cucurbita moschata		
Jing Xin No. 3	5.00 a	4.88 a
Tetsukabuto	4.95 ab	4.84 a
Strong Tosa	4.90 ab	4.62 abc
Jing Xin No. 2	4.89 ab	4.77 ab
TI-140	4.84 ab	4.66 abc
Jing Xin No. 4	4.72 ab	4.40 a-d
RS 1330	4.68 abc	4.58 abc
Shintosa Camel	4.62 abc	4.34 a-d
No. 1	4.60 abc	4.18 bcd
Kazako	4.59 abc	4.39 a-d
Carnivor	4.25 c	3.79 d
C. moschata		
BS1	4.75 ab	4.48 abc
TZ 148	4.68 abc	4.55 abc
AQ	4.63 abc	4.34 a-d
53009	4.54 bc	3.82 d
Cucumis melo		
'Dinero'	4.95 ab	4.59 a
WR-15006	4.71 ab	4.13 cd
Cucumis metulifer		
PI 526242	3.26 d	1.18 f
PI 441995	3.05 de	1.21 f
PI 482455	2.77 e	1.12 f
PI 500424	2.68 e	1.07 f
PI 422520	2.62 e	1.11 f

[a] Gall index: 1 = 0% to 3% root system galled or covered with egg masses, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 79%, and 5 = 80 % to 100% root system galled or covered with egg masses.[b]Means within a column followed by the same letter are not significantly different ($P<0.05$) according to Fisher's Protected LSD.

Table 3. Numbers of *Meloidogyne incognita* eggs per gram fresh root for grafted 'Athena' cantaloupe scion / cucurbit rootstock combinations, grown in a field infested with southern root-knot nematode (*M. incognita*), Charleston, SC.

Cucurbit taxon/entry	No. $\log_{10}$ (x+1) eggs per gram root (no. eggs per g root , non-transformed)
Cucurbita argyrosperma	
Plant Introduction (PI) 511908	3.7637 (6793) a[a]
Cucurbita moschata	
PI 543218	3.5652 (7044) a
Cucurbita maxima	
PI 548692	2.8439 (1849) b
Cucurbita ficifolia	
Grif 9448	2.8293 (773) b
Cucumis melo	
'Dinero'	2.7229 (1016) b
'Athena' self-grafted	2.8386 (767) b
'Athena' non-grafted	2.5412 (453) bc
Cucurbita hispida	
PI 391545	2.6715 (659) b
Cucumis metuliferus	
PI 482452	2.5107 (370) bc
PI 482443	2.5030 (323) bc

[a]Means within a column followed by the same letter are not significantly different ($P<0.05$) according to Fisher's Protected LSD.

Pilot study of the interaction of watery saliva proteins of *Aphis gossypii* Glover with the soluble protein fraction of *Cucumis melo* L. leaves

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Keywords: Far western-blot, incompatible interaction, melon, aphid, resistance, susceptibility.

Abstract

In the species *Cucumis melo L*. several sources of resistance against aphids have been reported but all of them seem to be controlled by the same gene called *Vat*. Recently, the presence of this gene has been associated with specific hypersensitive response (HR) mechanisms against *A. gossypii* infestation. This active defence response seems to be only triggered after the secretion of aphid watery saliva into the cytoplasm. Presumably, aphid saliva proteins act as pathogen associated molecular pattern (PAMP) and are detected by specific plant proteins, which trigger plant defence cascades. Thus far, interactions between aphid watery saliva proteins and plant proteins have hardly been investigated. Here, the Far Western Blot technique was used to study *in vitro* interaction revealing that, at least, 15 plant proteins interact with 5 different aphid saliva proteins. Further work is needed to determine the specificity and the biological function of each interaction to unravel the complex relation between aphids and plants.

INTRODUCTION

Aphids have specialized mouth parts that enable penetration of sieve tubes and ingestion of phloem sap. By doing so, aphids establish a long-lasting relation with their host plants (Miles 1999; Smith and Boyko 2007). Prior to ingestion, aphids advance their stylets through the intercellular spaces while secreting gel saliva that develops a sheath around the stylet (Tjallingii and Hogen Esch 1993). Numerous cells are probed along the stylet pathway during which watery saliva is secreted into the respective cells and with this, viruses can be inoculated (Chen et al. 1997).

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In the compatible interaction with plants, gel and watery saliva seem to have an important role in the protection of the aphid stylet against plant defence responses. Gel saliva forms a proteinaceous salivary sheath around the stylet which prevents direct contact between the stylet and the plant tissues. In addition, gel saliva functions as a lubricant during stylet progression. Sheath components may also be important in the detoxification of plant defence compounds (Cherqui and Tjallingii 2000; Will and van Bel 2006). Watery saliva is composed of soluble proteins involved in the compatible interaction between aphids and plants. These proteins seem to degrade defence compounds synthesized by the plants such as reactive oxygen species (ROS) or phenols (Cherqui and Tjallingii 2000; Cooper et al. 2010). Aphid saliva proteins have been also described to function as calcium chelators (Will et al. 2007; Carolan et al. 2009) which is considered as essential second messenger in active defence responses against plant pathogens and pests (Lecourieux et al. 2006).

By contrast, aphid saliva proteins are also good candidates to act as elicitors of plant defence responses in the incompatible interaction. A role of saliva proteins triggering defence responses has been reported for various plant species. Saliva glycoproteins injected into different barley genotypes induced higher levels of peroxidase activity in the resistant genotypes (Lintle and van der Westhuizen 2002) than in susceptible genotypes. More recent studies have reported the induction of diverse defence responses, e.g. induction of polyphenol oxidase, in wheat plants after injection of the watery saliva proteins (Ma et al. 2010).

Aphid resistance in melon is controlled by one single dominant gene, called *Vat*, which belongs to the superfamily of the resistance genes (R genes) (Dogimont et al. 2007). This gene encodes a protein with nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains. The NBS domain functions as molecular switch in pathogenic signaling pathways, while the LRR domain functions in binding of pest or pathogen proteins (De Young and Innes 2007). Sarria et al. (2009) observed that *A. gossypii* probing activities induced a very fast hypersensitive response (HR) in epidermal and mesophyll cells of *Vat*-plants. The electrical penetration graph (EPG) technique revealed that probing activities of this aphid induce callose deposition only in *Vat*-plants. These findings indicate that watery saliva, or rather one or more of its components, elicits plant defense responses in *Vat*-plants.

This report summarizes pilot experiments on the *in vitro* interaction between aphid saliva and plant proteins, viz. PAMPs (Pathogen-Associated Molecular Patterns) and receptor candidates in *Vat*-plants, which could be involved in triggering plant defense in response to aphid infestation.

MATERIALS AND METHODS

Aphids and plant material

A. gossypii was collected from a melon crop in a greenhouse (Almería, Spain). One only nymph from a female aphid was used to create the clone. Aphid colonies were reared on plants of the susceptible Spanish cultivar 'ANC-57' of the species *C. melo* L in glasshouse, at 25 °C (light) and 18 °C (dark) with a photoperiod of 16:8 h (light:dark).

Experiments were carried out with the melon genotype 'AR 5'. This genotype is an aphid-resistant cantaloupe cultivar carrying the *Vat* gene introgressed from PI 414723 (McCreight et al. 1984). Plants were grown under the same environmental conditions as described for aphid rearing.

Watery saliva collection

A. gossypii phototaxis was used to collect the aphids. Leaves heavily infested with aphids were detached from the plant and they were put inside a black box with a few holes in its lateral walls. The mouth of transparent glass flasks were introduced through these holes and the box was kept in an artificially illuminated room overnight. Aphids moved to the light, fell down into the flasks and were collected after twelve hours. Subsequently, aphids were fed on artificial diets as described by Will et al. (2007). During 24h of incubation, aphids pierced their stylets through the Parafilm™ cover to probe and ingest the diet. Around 1000 aphids per set were used to obtain the desired amount of aphid saliva proteins. Prior to ingestion, aphids secreted watery saliva into the diet, similar to *in planta* behavior. After incubation the saliva-containing diet solution was collected under sterile conditions and frozen at -40 °C until its use.

Saliva of 20000 aphids was concentrated using Vivaspin 20 (GE Healthcare) (3000 MWCO) units from 120 ml to a final volume of 100 µL. The buffer exchange was accomplished with phosphate buffered saline (PBS) to remove the aminoacids and the sugar components from the mixture. The saliva collection was carried out in triplicate (different saliva samples).

Saliva labeling

Saliva proteins dissolved in PBS were labeled by the fluorochrome DyLight 633 NHS-Ester (DLNHS) (Thermo SCIENTIFIC) with the maximum absorption/emission at 638/658 nm. This compound reacts with primary amines, forming a stable covalent amide bond, labelling proteins nonspecifically. For the binding reaction, 800 µL of PBS was added to aphid saliva samples to prevent protein precipitation. To adjust the pH between 7.5 and 8.5 to reach an efficient binding between DLNHS and proteins, 100 µL of 1M Na_2CO_3 and the fluorochrome were added to the mixture which was incubated for two hours at room temperature. After the binding reaction,

labeled proteins were separated from free DLNHS buffer exchange using Vivaspin 20 (>10 KDa pore). Finally, PBS was added to labeled saliva samples to a volume of 10 mL and stored at -40 °C until their use in interaction experiments with plant proteins separated by 2D SDS-PAGE.

Plant protein extraction

Leaf proteins were extracted according to Rampitsch et al. (2006) with some modifications. Leaves were ground in liquid nitrogen, and subsequently suspended in 10 % TCA-acetone and 0.07 % dithiothreitol (DTT) to precipitate for 24 h at -20 °C. The sample was centrifuged at 18000 rpm and the pellet was washed with cold acetone containing 0.07 % DTT (4 times). Residual acetone was removed from the pellet by using a vacuum concentrator. Subsequently, RuBisCo proteins (big and small subunits) were depleted from the sample which permitted a better separation of the low-abundant plant proteins (protocol to be published). Pellets of three plant protein samples, each from different plants were dissolved in 250 µL of lysis buffer R2D2 (Mechin et al. 2003).

Plant protein separation

The combination of isoelectric focusing (IEF) with SDS-PAGE was employed for plant protein separation. IEF was carried out on 7 cm IPG strips pH 5-8 (Bio-Rad) which enabled separation of the plant proteins.

IPG strips were rehydrated with 70 µg of protein in 130 µL of R2D2 buffer during 12 h in a PROTEAN IEF cell (Bio-Rad) at 20°C. Proteins were focused in a PROTEAN IEF cell in accordance with the IPG strip manual (Bio-Rad catalogue 163-2099). Subsequently, plant proteins were separated by size in custom-made 12% SDS-PAGE gels in a Mini Protean 3 cell.

Far Western Blot technique was used to assess the binding properties of the aphid saliva proteins as well as identification of the interacting plant proteins.

Protein transfer

Plant proteins from the 2D gel were blotted onto nitrocellulose membrane (Protran BA 85, pore size 0.45 µm; Whatman) by using Towbin buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3) (Towbin et al. 1979) at 35 V overnight at 4 °C.

To test the efficiency of protein transfer, proteins on the blotting membrane were stained with Ponceau S (Roche Diagnostic, Mannheim, Germany). PBS was used to distain.

In order to achieve the quality of the 2D-SDS PAGE, gels were stained with Coomassie (Roti-Blue, Carl Roth GmbH) when needed.

Renaturation and blocking

Protein renaturation is considered to be the bottle-neck in protein interaction studies because the correct restoration of the secondary and tertiary conformation is essential for proper interaction. The protocol described by Zeng et al. (1996) was followed to renaturate the immobilized proteins on the blot membrane.

Nitrocellulose membranes were incubated during 10 hours with renaturating solution (5% BSA, 1% casein, 1% gelatin, 5% non-fat dry milk, 0.02-0.5% Tween 20, 0.05-0.1% Triton X-100) at 4°C. Beside renaturation of blotted proteins, the solution blocked the membrane to prevent binding of labeled aphid saliva proteins to the areas without plant proteins during the interaction experiment. Subsequently, membranes were washed with 10 mL of PBS three times and immediately used in interaction experiments.

Protein interaction

Blot membranes were incubated with 10 mL of the mixture of DLNHS-tagged saliva proteins in PBS at room temperature for 10 h. Membranes were then washed with PBS to remove excess of unbound DLNHS-tagged saliva proteins on surface. Fluorescence of the labeled proteins was detected in a VersaDoc MP 4000 (Bio-Rad), equipped with epi-illuminator and chemiluminescence detector. The exposure time was set at 30 minutes. Three repetitions were done with different plant and saliva protein samples.

After observation, the three blot membranes were dried for further analyses. Fluorescent spots were picked from the membrane and washed each with 100 µL of R2D2 lysis buffer to recover the aphid saliva and the plant proteins from the nitrocellulose membrane. Subsequently, proteins were precipitated with 400 µl of TCA-Acetone. The sample from each spot was centrifuged at maximum speed and the pellet was washed with pure acetone. Finally, pellet was dried using a vacuum concentrator, solubilized in 1X Laemmli sample buffer. The gel was then dissolved and analyzed by using 15 % 1D SDS-PAGE. The obtained gels were stained with Oriole fluorescent gel stain (Bio-Rad) according to manufacturer instructions.

RESULTS AND DISCUSSION

Plant protein blotting

The number of spots in 2D SDS-PAGE gels and on blot membranes after protein transfer from the 2D gels were compared to assess blotting efficiency. Proteins within the complete range of detected molecular weights were successfully transferred but only the high-abundant proteins could be observed in both, in the 2D SDS-PAGE gel and on the nitrocellulose membrane. A reliably quantitative comparison of the Ponceau S-stained blot membranes (Fig. 1 A) and Coomassie-stained gels (Fig. 1 B) could not be made because of the lower sensitivity of Ponceau S. However, Ponceau S distribution on the blot membranes indicated that protein transfer was efficient over the entire gel area (Fig. 1).

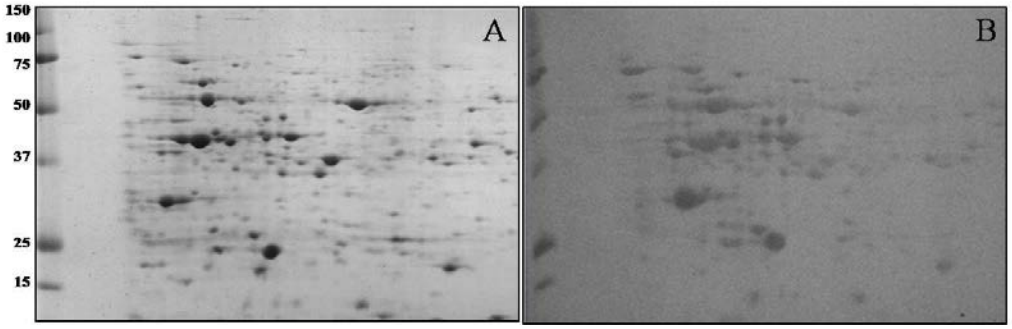

Fig. 1. Comparison between the melon plant protein pattern after 2D SDS-PAGE (A) and after subsequent transfer onto a nitrocellulose blot membrane (B).

Far Western assays of aphid saliva protein

Several spots of plant proteins were detected on western blot membrane (Fig. 2B) after incubating the membrane with a solution that contained DLNHS-labeled saliva proteins. No significant differences were observed among the three replications. No fluorescence was detected when western blot membrane with plant proteins was incubated with PBS solution without labeled saliva proteins. Main interacting spots were identified and numbered for further analyses.

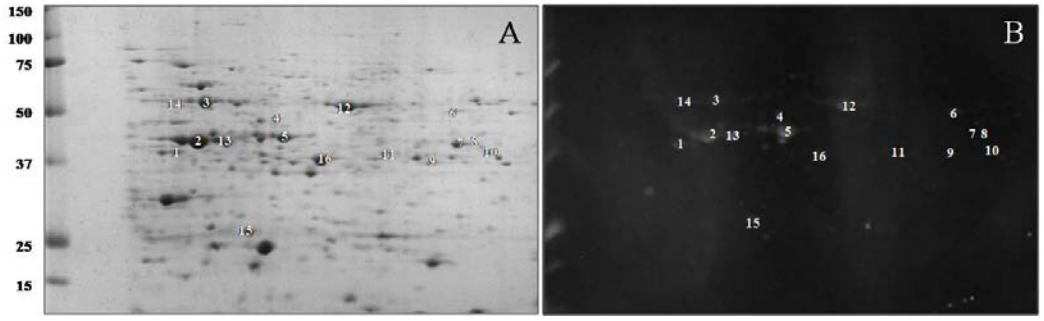

Fig. 2. 2D SDS-PAGE of melon leaf proteins stained with Coomassie blue (A) and a representative far western blot (B). Spots that were detected after far western blotting and incubation with aphid saliva proteins were numbered in A and B.

After picking the fluorescent spots, proteins were extracted from the filter and separated by 15% SDS-PAGE (Fig. 3). The study of the gels revealed many bands in the lanes, some of them were common in all the lanes while few of them were specific of the interacting spot.

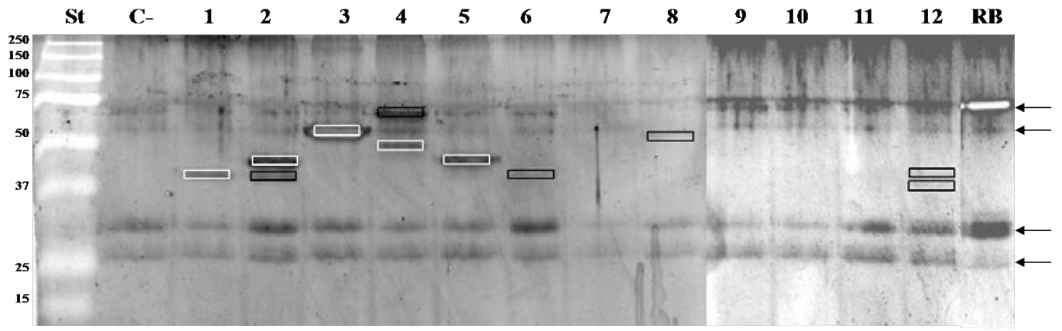

Fig. 3. 1D SDS-PAGE of the recovered fluorescent spots, stained with Oriole (BioRAD). Legend: St indicates the molecular weight marker proteins (Precision plus protein standard, Bio-Rad). Lane C- (negative control) belongs to a spot taken in one area of the membrane that did not interact with aphid saliva. The number of each lane indicates the interacting spots. Lane RB contains diluted (1:500) renaturation buffer and arrows indicate detected protein bands that also appear in all the lanes. White rectangle around bands indicates plant proteins. Black rectangles around bands indicate bands containing aphid saliva proteins which were bound to a plant protein in far western blot experiments.

Protein bands that are commonly present in lanes C- and 1-12 (Fig. 3) appear to be related to proteins that are part of the renaturation buffer (Fig. 3, lane RB (arrows)). Potential plant proteins in lanes 1-12 (Fig. 3, white rectangles around bands) were allocated by their molecular size, observed in the 2D SDS-PAGE (Fig. 2A). Bands that do not show the expected molecular weight does presumably represent aphid saliva protein that bound to a plant protein in the far western blot experiment (Fig. 3, black rectangles around bands). In some lanes, no protein bands with exception of the bands that belong to renaturation buffer were detected. In these cases, it can be suggested that the quantity of proteins was insufficient for detection.

This pilot study was conducted to identify a potential elicitor of the HR triggered by *A. gossypii* cell probing activities in melon genotypes that carry the *Vat* gene. However, at least 15 plant proteins seem to interact specifically with 5 different aphid saliva proteins. These results do not point to the existence of just one key interaction that switch on the active defence response observed in *Vat* genotypes by Sarria et al. (2009) but reveal a complex network of interactions between aphids and plants proteins. Most of those proteins could be likely associated to the general plant defence response triggered by aphid infestation in compatible relations between aphids and plants. To identify the specific protein acting as elicitor of the HR in *Vat* genotypes the same experiment using a *non-Vat* genotype should be done. The comparison between the two protein profiles would allow us to better understand the plant-insect interactions.

Successful phloem feeding requires overcoming a number of barriers located at the epidermal and mesophyll levels (Powell et al. 2006; Giordanengo et al. 2010) as well as several phloem-related plant properties and reactions (Tjallingii 2006). Several scientific papers have revealed that aphid saliva is able to prevent plant response and modify plant metabolism to allow successful phloem feeding. The existing scientific papers has been focused in the enzymatic activities of the aphid saliva that appear to be associated with the overcoming of these barriers at the epidermal and mesophyll levels (Jiang and Miles 1996; Urbanska et al. 1998; Miles 1999), the role of watery saliva as a calcium scavenger to prevent its effect as a second messenger of the plant defence responses (Will et al. 2007) and the ability of the saliva to form a physical barrier against the plant defence responses (Tjallingii 2006; Will and van Bel 2006). The role of protein-protein interaction in the aphid-plant relation has never been studied.

All these studies have revealed that protein mixture secreted by aphids is complex. In some aphid species, more than 20 different protein types have been reported in the watery saliva (Will et al. 2007; Harmel et al. 2008; Will et al. 2009). The study of the molecular, protein and metabolic pathways involved in the compatible and incompatible interactions between pests and pathogens has allowed the identification of candidate genes involved in the resistance against them. This is the case for the identification of modifications of transcription factors involved in virus resistance, as *eIF4E* against MNSV in melon (Nieto et al. 2006) or against ZYMV in watermelon (Ling et al. 2009) or the case of *Mlo* genes for powdery mildew resistance in barley or tomato (Buschges et al. 1997; Bai et al. 2008). In the same way, the knowledge of the role of the plant proteins interacting with the aphid biology and *vice versa* could be usable for the development of durable resistance in *C. melo* and other plant species.

ACKNOWLEDGEMENTS

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Recovery-type resistance to *Zucchini Yellow MosaicVirus* in oilseed pumpkin

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Keywords: ascorbate peroxidase, lipoxygenase, oilseed pumpkin, recovery type of resistance, *Zucchini yellow mosaic virus*

Abstract

In Central Europe (Austria, Germany, Hungary), the most serious threat to the production of oilseed pumpkin is *Zucchini yellow mosaic virus* (ZYMV). One of the effective methods to control this disease is the use of resistant or tolerant cultivars. Genes for resistance were introgressed into oilseed pumpkin breeding lines by T. Lelley in 2000, and these lines were tested several times with a Hungarian isolate of ZYMV (ZYMV-10). The highly tolerant lines, showing mild symptoms after ZYMV inoculation, were selfed and selected for eight years according to their growth habit, fruit and seed shape, color as well as weight. When selected breeding lines were inoculated with ZYMV-10 in 2010, a novel"recovery" type of resistance was observed. First disease symptoms appeared 4–5 days after inoculation on the leaves of these recovery-type plants. These infected leaves were necrotized later but the upper, uninoculated leaves showed no symptoms. The recovered plants remained symptomless or, in some leaves, showed very mild vein-clearing symptoms, but apical leaves were symptomless. The presence of ZYMV could be detected in leaves showing symptoms, but not or with difficulty in symptomless leaves. ZYMV inoculations induced ascorbate peroxidase and lipoxygenase enzyme activities, both in recovery type and in otherwise tolerant oilseed pumpkin cultivars. Recovery-type plants were crossed with normal tolerant plants. The F1 generation showed recovery-type symptoms after ZYMV inoculation, indicating that this trait is governed by dominant gene(s).

INTRODUCTION

Cucurbita pepo L., which encompasses many cultigens of pumpkins and squash, is one of the more economically important vegetable crop species and is grown on arable land throughout the world. In many regions, the most serious threat

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to production is *Zucchini Yellow Mosaic Virus* (ZYMV), which can cause severe economic losses. ZYMV was first described by Lisa et al. (1981) in northern Italy and soon it was identified in the Mediterranean region, Central Europe, Japan China, Chile, Australia, USA and Canada (Lecoq and Pitrat 1984; Nameth et al. 1986; Greber et al. 1987; Tóbiás et al. 1995; Prieto et al. 2001). ZYMV is a relatively new, but very aggressive member of the potyvirus group that has spread rapidly throughout the world and is efficiently transmitted from plant to plant by several aphid species in a non-persistent manner (Lisa and Lecoq 1984), and long-distance transmission can occur via infected seeds (Schrijnwerkers et al. 1991; Tóbiás and Palkovics 2003). Symptoms associated with ZYMV infection are mosaic, severe leaf and fruit deformation, and stunting. Since its first appearance, ZYMV has become widely distributed and caused devastating epidemics in a range of cucurbit crops, including oilseed pumpkin (Riedle-Bauer, 1998; Tóbiás and Tulipán 2001). One effective method for control of this virus is the use of resistant cultivars. Several resistant accessions were identified in *Cucurbita moschata* Duchesne*, C. ficifolia* Bouché, and *C. ecuadorensis* Cutler & Whitaker, but only *C. moschata* has been crossed successfully with *C. pepo* (Pachner et al. 2011).

Genes for resistance to ZYMV derived from *Cucurbita moschata* 'Nigerian Local' and 'Menina' (Pachner et al. 2011) were used to increase resistance in breeding materials of oilseed pumpkin lines selected by H. Kulhmann. In the first two years, the resistant lines were inoculated with the ZYMV-10 strain, representing a Hungarian ZYMV isolate, and virus symptoms were evaluated. Some lines were tested also for virus concentration by ELISA. Lines showing mild symptoms and low virus titer were selected and used in the breeding programme. Over the next eight years, the breeding materials were selected according to other characters (plant growth habit, morphological characters and color of fruit, seed, etc.). In 2010, the breeding materials were tested by ZYMV inoculation again and, interestingly, a novel "recovery type" of resistance was observed in some lines. In order to study the genetic basis of the recovery-type of resistance, several crosses were made.

Virus infections often result in the rapid accumulation of reactive oxygen species (oxidative burst), in lipid peroxidation, and in the activation of antioxidative defense reactions (Fodor et al. 1997; Gullner et al. 2010). Lipoxygenase (LOX, E.C. 1.13.11.12) isoenzymes catalyze the peroxidation of free and membrane lipid-bound polyunsaturated fatty acids by molecular oxygen. The produced fatty acid hydroperoxides are further converted enzymatically into a wide variety of biologically active oxylipins, which often show antimicrobial effects (Mosblech et al. 2009; Gullner et al. 2010). Ascorbate peroxidase (APX, E.C. 1.11.1.11) isoenzymes play a pivotal role in the detoxification of hydrogen peroxide in plant cells (Nakano and Asada 1981; Fodor et al. 1997). Little information is available about the role of these stress-inducible enzymes in oilseed pumpkin tissues, particularly following

ZYMV inoculation. To gain a deeper insight into the defense reactions of virus-infected oilseed pumpkin plants, the foliar lipoxygenase and ascorbate peroxidase activities were also measured following ZYMV inoculation.

MATERIALS AND METHODS

Seeds of oilseed pumpkin breeding lines and testcross generations were sown in an aphid-free greenhouse in April and May under natural illumination. In October and November, natural illumination was supplemented with HgLI lamps, maintaining a day length of 14 h. Plants of 25 breeding lines were inoculated on cotyledons when the first true leaf began to expand.

Carborundum-dusted cotyledons were inoculated by rubbing with crude extract of leaves infected with ZYMV-10 (Tóbiás et al. 1998) and homogenized, in a 1:1 ratio, with 0.1 M sodium phosphate buffer of pH 7.2. Symptom development was observed from 2 to 40 days after inoculation. Mock inoculations without ZYMV were carried out similarly. Testcrosses were made in the field in a carefully controlled manner by securing shut the staminate and pistillate flowers one day prior to the artificial pollination. After hand-pollination, the pistillate flower was resecured with a ventilated plastic bag.

Total APX and LOX enzyme activities were observed in cell-free extracts of oilseed pumpkin leaves by spectrophotometry. APX activity was determined according to the method of Nakano and Asada (1981), except that the concentrations of H_2O_2 and ascorbic acid were 0.5 and 0.25 mM in the assay mixture, respectively. LOX activities were determined at 234 nm by using linoleic acid as a substrate, as described by Gullner et al. (2010).

RESULTS AND DISCUSSION

The oilseed pumpkin lines exhibited distinct, characteristic symptoms after inoculation with the Hungarian isolate ZYMV-10. Interestingly, some lines exhibited a recovery process following viral inoculation. Recovery-type plants showed vein clearing and downward curling of the first true leaf 4–5 days after inoculation (Fig. 1A). In some plants, chlorotic spots were also observed on the cotyledons. Subsequently, 7–8 days after inoculation, the first true leaf became chlorotic and then necrotized (Fig. 1B). The second true leaf was stunted but from the third true leaf onwards the plants were symptomless (Fig. 1C). On another oilseed pumpkin line (non-recovery-type plants), only chlorotic spots appeared 7–9 days after inoculation on the first true leaf (Fig. 1D), but the plants developed a systemic mosaic by 14 days after inoculation (Fig. 1E). Of the 25 breeding lines tested, six were of the recovery type, 15 were of the non-recovery type, and four lines had both types of symptoms. After crossing the recovery- and non-recovery-type plants, the F_1-generation plants were of the recovery type. However, there were differences

among plants in the severity of necrotization (Fig. 2A, B, C). Nonetheless, all plants later became symptomless (Fig. 2D). Our results indicate that the recovery type of resistance in oilseed pumpkin is a dominant trait and most probably more than one gene is involved, consistent with results described for zucchini squash (Paris and Cohen 2000). Additional experiments are needed to evaluate the possible usefulness in the field of this recovery type of resistance.

To better understand the biochemical mechanisms underlying the recovery-type ZYMV resistance, we investigated the total extractable activities of two typical plant defense enzymes, APX and LOX, in oilseed pumpkin leaves at different time points following ZYMV inoculation. Firstly, LOX activities were determined in the cotyledons of a recovery-type oilseed pumpkin line as well as in those of a non-recovery line. ZYMV inoculations resulted in marked increases of LOX activity in both lines as compared to mock-inoculated plants (Fig. 3). APX activities were measured also in the cotyledons following ZYMV inoculations or mock treatments. ZYMV inoculation led to a substantial increase of APX activity in the leaves of recovery-type plants (Fig. 4). In non-recovery-type plants, the ZYMV inoculation also resulted in increased APX activities, but the rate of enzyme induction was significantly lower than in the case of recovery-type plants (Fig. 4). The strongly increased antioxidative capacity caused by elevated APX activities was probably due to an oxidative stress in the cotyledons of recovery-type oilseed pumpkins.

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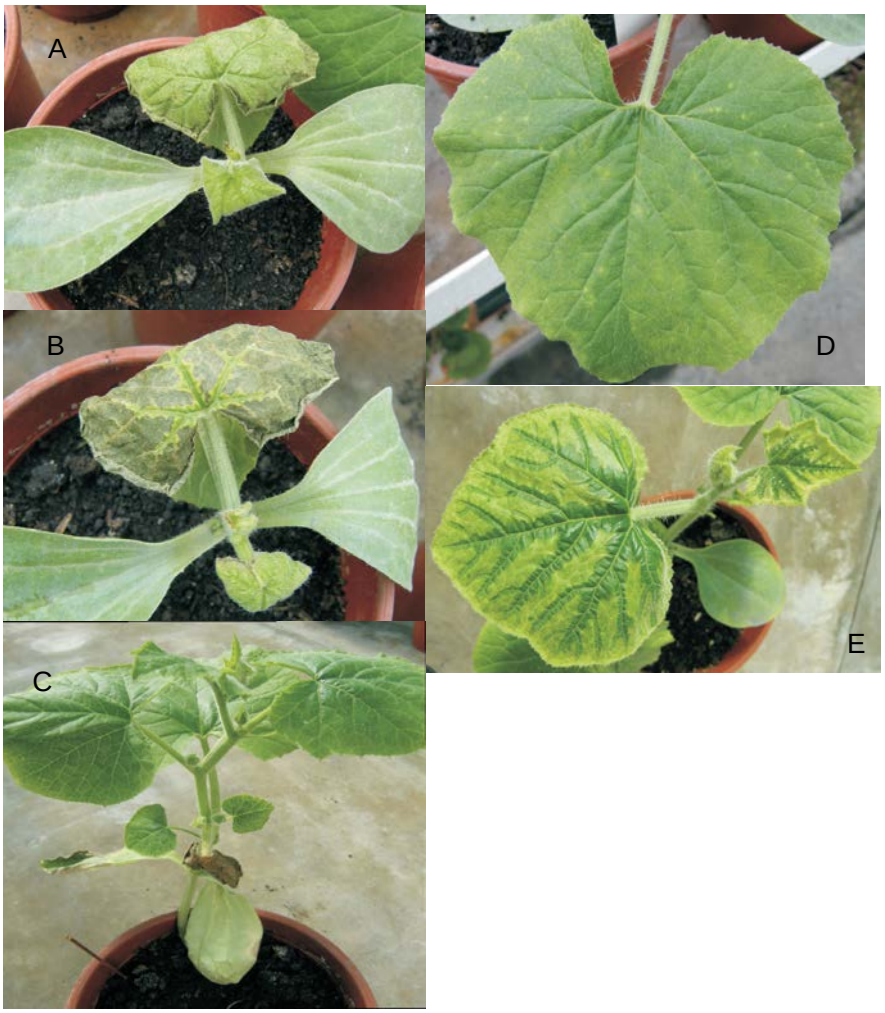

Fig. 1. Symptoms of recovery-type (A, B, C) and non-recovery-type (D, E) plants after ZYMV-10 inoculation. A = 5 days, B = 8 days and C = 20 days after inoculation, D = 8 days, E = 14 after inoculation.

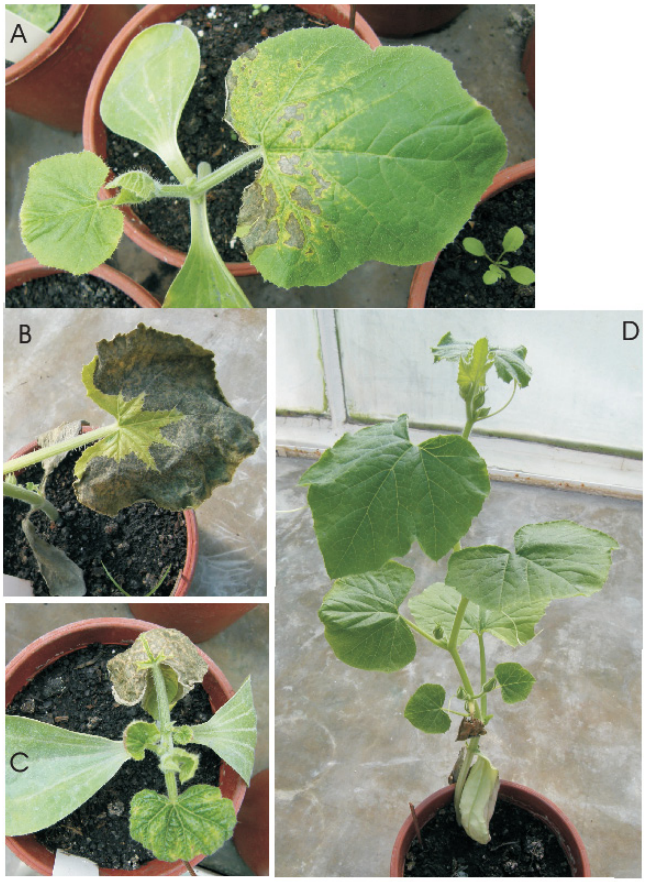

Fig. 2. Variation in necrotization and stunting of the first true leaves of recovery-type plants (A, B, C), which became symptomless by 21 days after inoculation (D).

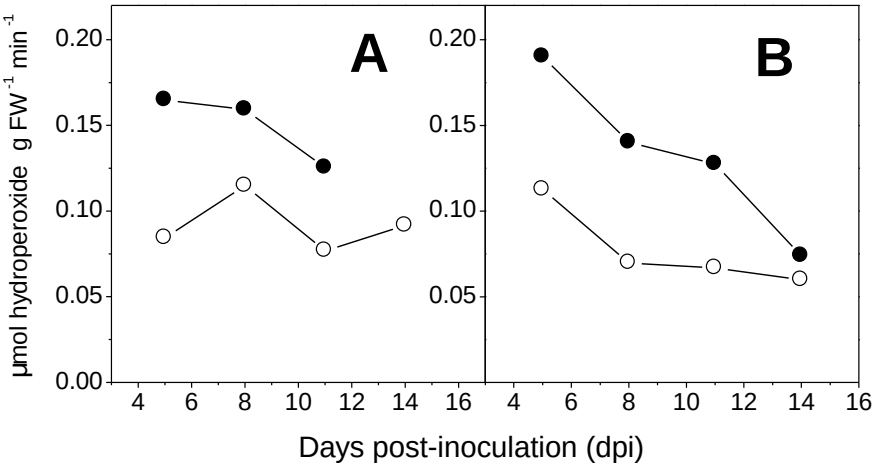

Fig. 3. Total extractable lipoxygenase (LOX) enzyme activity in the first true leaves of two different oilseed pumpkin lines following *Zucchini yellow mosaic virus* (ZYMV) inoculation or mock-inoculation on the cotyledons. A = recovery-type oilseed pumpkin line. B = non-recovery-type oilseed pumpkin line. Empty circles = mock-inoculated. Full circles = ZYMV-inoculated.

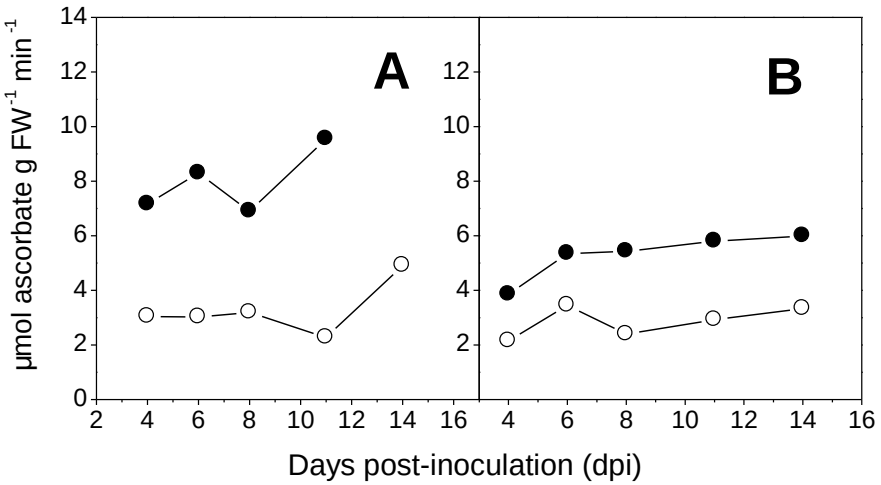

Fig. 4. Total extractable ascorbate peroxidase (APX) enzyme activity in cotyledons of two different oilseed pumpkin lines following Zucchini yellow mosaic virus (ZYMV) inoculation or mock-inoculation on the cotyledons. A = recovery-type oilseed pumpkin line. B = non-recovery-type oilseed pumpkin line. Empty circles = mock-inoculated. Full circles = ZYMV-inoculated.

Background and development of a model to predict the fate of an escaped virus resistant transgene from *Cucurbita pepo*

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Keywords: cucumber beetles, *Erwinia tracheiphila*, Texas gourd, watermelon mosaic virus, zucchini yellow mosaic virus

Abstract

Gene flow between cultivated and wild squash in the United States may result in the introduction of a virus resistant transgene (VRT) into wild populations. We have extensively studied the interactions among *Cucurbita pepo* ssp. *texana* (the Texas gourd), transgenic introgessives with the Texas gourd, the primary herbivores (cucumber beetles and aphids) and the pathogens the herbivores transmit (the bacterium, *Erwinia tracheiphila*, and common mosaic viruses). The goals of this manuscript are to summarize our findings over the past 10 years, to describe a model that we are developing that predicts the fate of the VRT as it introgresses into wild populations of *Cucurbita*, and to describe our ongoing studies to parameterize and validate our model so that it can be used to manage the VRT should it escape and become problematic.

INTRODUCTION

Gene exchange between crops and their wild relatives is common and difficult to contain (Ellstrand 2003). Unlike most traits of cultivated species, there are concerns that transgenes conferring resistance to herbivores or pathogens could enhance the fitness and weediness of wild species and/or have indirect impacts on non-target species such as pollinators, herbivores, predators, soil fauna, and other plants in the community (Ellstrand 2003; Pilson and Prenderville 2004). The introgression of resistance transgenes into populations of native species could also modify the genetic diversity of these populations (Ellstrand 2003) and it has the potential to alter the species composition of the community should these species become more competitive/aggressive (Pilson and Prenderville 2004; Fuchs and Gonsalves 2007).

Gene flow from cultivated to free-living taxa of *Cucurbita* is common

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and well-documented (Kirkpatrick and Wilson 1988). Relative to wild taxa of *Cucurbita*, cultivated varieties tend to have larger seeds, shorter internodes, and larger fruits with a soft pericarp and sweet (not bitter) flesh. Normally, these traits are a selective disadvantage in the wild and tend to be rapidly purged from wild populations. However, in 1994, the United States Department of Agriculture (USDA) deregulated a transgenic yellow crookneck squash (Asgrow, ZW-20) that was engineered to express a dual coat protein (CP) construct of watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) that conferred resistance to those viruses (USDA 1994). In 1996, a second transgenic cultivar (CZW-3) with CP-based resistance to WMV, ZYMV, and cucumber mosaic virus (CMV) was deregulated (USDA 1996). In both transgenic cultivars a marker gene, neomycin phosphotransferase II (*nptII*) was co-transferred. By the late 1990s, several cultivars with the transgene were developed, marketed, and grown commercially throughout the United States but especially in the Southeastern United States, where it greatly reduced pesticide use and increased yield (Fuchs and Gonsalves 2007). There is now concern that the virus resistant transgene (VRT) will escape and introgress into wild populations with potentially adverse effects on the environment.

Since 2002, we have extensively studied the interactions among *Cucurbita pepo* ssp. *texana* (the Texas gourd), transgenic introgessives with the Texas gourd, the primary herbivores (cucumber beetles and aphids) and the pathogens the herbivores transmit (the bacterium, *Erwinia tracheiphila*, and common mosaic viruses). The goals of this manuscript are to summarize our findings over the past 10 years, to describe a model that we are developing that predicts the fate of the VRT as it introgresses into wild populations of *Cucurbita*, and to describe our ongoing studies to parameterize and validate our model so that it can be used to manage the VRT should it escape and become problematic.

The Cucurbita pathosystem

The wild gourd, *Cucurbita pepo* ssp. *texana* (Cucurbitaceae) is an annual monoecious vine with indeterminate growth and reproduction. It is native to Texas and to states along the Mississippi River from Southern Illinois southward (sometimes called ssp. *ozarkana*). It is completely inter-fertile with the cultivated pumpkins and squashes (*C. pepo* ssp. *pepo* and ssp. *ovifera*) and several annual *Cucurbita* taxa from Mexico (*C. pepo* ssp. *fraterna, C. moschata, C. ficifolia, C. agyrosperma* ssp. *sororia and* ssp. *agyrosperma*) (Arriaga et al. 2006). After a period of vegetative growth (5-7 nodes), wild gourds produce one large yellow flower (either male or female) in the axil of each leaf. The flowers last for only one morning and are pollinated by bees, especially squash bees. The fruits of the Texas gourd are round to oval with a volume of 175-450 ml and typically contain 150-300 seeds that weigh 20-40 mg (Winsor et al. 2000).

The leaves and other organs of the wild gourd produce cucurbitacins (oxygenated tetracyclic triterpenes). Cucurbitacins are among the bitterest compounds known (Metcalf and Rhodes 1990). However, cucumber beetles (*Diabrotica* spp. and *Acalymma* spp.) are adapted to feed on cucurbitacins in the leaves of *Cucurbita* species and are found throughout the native ranges of *Cucurbita* species (Robinson and Decker-Walters 1997). The cucumber beetles chemically modify the cucurbitacins, use them for their own protection, and the males also transfer some of the modified cucurbitacins to the females in their seminal fluid which is used to chemically protect the eggs (Ferguson et al. 1985; Metcalf and Rhodes 1990). Cucumber beetles are attracted to cucurbitacins in the foliage of *Cucurbita* and, when flowers are present, it has been shown that floral volatiles not only attract bees (the pollinators), but also cucumber beetles over relatively large distances (Lampman and Metcalf 1988). These beetles cause a characteristic pattern of holes (1-1.5 cm in diameter) in the portions of the leaves serviced by the smallest veins and beetle damage has been shown to substantially reduce yield in cultivated cucurbits and reproductive output in the wild gourd (Du et al. 2008; Stephenson et al. 2004). After feeding on the leaves, the beetles aggregate in the flowers to mate.

The full impact of herbivory by cucumber beetles on wild gourds, however, also includes increased exposure to a pathogen, *Erwinia tracheiphila* (Enterobacteriaceae),the causative agent of bacterial wilt disease. Cucumber beetles are the only known vector of *Erwinia* which overwinters in their guts. Transmission occurs when fecal pellets containing *Erwinia* land on leaf wounds at the sites of feeding damage (Fleischer et al. 1999). Recently, we have shown that there is a second, and perhaps more common, mode of transmission (Sasu et al. 2010a). When the beetles aggregate in the flowers to mate their fecal pellets containing *Erwinia* fall onto or near the floral nectaries and the bacteria enters *via* the nectaries. Once inside the plant the bacteria proliferate in the xylem where they secrete a mucilaginous, exopolysaccharide matrix that cuts off the water supply resulting in wilting. Wilt symptoms typically develop 10-15 days after infection and the disease is nearly always fatal once symptoms appear (Stephenson et al. 2004). Bacterial wilt disease is the most economically important disease of cultivated cucurbits (cucumbers, melons, pumpkins and squash) in the Eastern United States (Fleischer et al. 1999).

Several generalist aphids are known to infest *Cucurbita*. These aphids also vector the four most common viral diseases of cucurbits (cucumber mosaic virus [CMV], papaya ringspot virus [PRSV], watermelon mosaic virus [WMV], and zucchini yellow mosaic virus [ZYMV]). ZYMV and WMV are the two most common viral diseases of cucurbits at our field sites in Central Pennsylvania. Both are single-stranded, positive-sense RNA viruses of the family Potyviridae and are transmitted *via* aphids in a non-persistent manner. Transmission occurs when the

stylet of the aphid probes a new plant (analogous to a dirty needle). These viral diseases produce symptoms that include blisters, necrotic lesions, branches with short internodes and small highly serrate leaves, and other leaf deformities.

From 2002-2005 we performed a series of large, field scale, experimental studies of the Texas gourd pathosystem. These studies revealed that: (a) viral diseases have no impact on survivorship during the growing season, but decrease male and female flower number, fruit number and *in vitro* pollen tube growth; (b) large plants, measured as absolute size, or flower number, have a higher incidence of wilt disease than smaller, viral infected plants; (c) more beetles congregate per flower in the flowers of healthy (non-viral infected) plants than in the flowers of viral infected plants; (d) there is significantly less beetle damage on virus infected plants than on non-diseased plants; (e) herbivory by cucumber beetles reduces reproductive output through both the male and female functions; (f) pollen from viral diseased plants and from beetle damaged plants sired significantly fewer seeds in competition with pollen from healthy plants (that is, herbivory and viral diseases reduce both the quantity and quality of pollen); (g) the adverse effects of beetle herbivory and viral infections are greater for female function than for male function, indicating that the impact of environmental stress can differentially affect the two sexual functions; (h) both resistance and tolerance to cucumber beetles varies with ontogeny; and (i) there is heritable variation in the ontogeny of resistance and tolerance to cucumber beetles (Stephenson et al. 2004; Hayes et al. 2004; Avila-Sakar and Stephenson 2006; Stephenson et al. 2006; Ferrari et al. 2006; 2007; Simmons et al. 2008; Du et al. 2008).

Effects of the VRT during introgression

In the Liberator III transgenic cultivar, the VRT is hemizygous and, importantly, the *NPTII* gene conferring resistance to neomycin has not been deactivated and is still tightly linked to the coat protein genes of the three viruses. Consequently, we were able to introgress the transgene into 20 families of Texas gourds (using the wild gourd as the recurrent parent) because the presence of the NPTII protein in hybrid progeny can be detected.

From 2006-2008 we performed a series of large, field-scale experiments to determine the effects of the VRT on plant fitness relative to Texas gourds and non-transgenic introgressives (non-VRT plants), and to identify the amount of herbivory by cucumber beetles and the incidence of viral diseases and bacterial wilt diseases on the VRT, non-VRT and Texas gourd plants. In each year there were four 0.4 ha fields each with 180 plants (25% VRT; 25% non-VRT; 50% Texas gourds). These studies (Sasu et al. 2009; 2010b) revealed: (a) that the VRT effectively prevents viral infection in the VRT-introgressives (F1, backcross BC1, BC2, BC3 and BC4), (b) that ZYMV/WMV came into our fields naturally in mid-July of each

year and rapidly spread through the susceptible plants (Texas gourds, and non-VRT introgressives), (c) that the VRT plants had greater reproductive output than the susceptible plants, (d) and that the VRT plants sired significantly more of the offspring than would be predicted by chance alone.

Moreover, we found that there was no significant difference in the amount of beetle damage or incidence of wilt disease among the VRT introgressives, the non-VRT introgressives and the Texas gourds from late May (planting) until mid-July (initiation of the viral epidemic). However, after mid-July we found that: (a) the VRT plants experienced significantly more beetle damage than the susceptible plants, (b) that more beetles aggregated in the flowers of virus-free plants (mostly VRT plants) than in the flowers of virus infected plants, (c) that the *Erwinia* infection rate for VRT plants was approximately twice as great as the infection rate for susceptible plants from mid-July until mid-September (first killing frost), and (d) plants with a viral disease were very unlikely to contract wilt disease compared to virus free plants (Sasu et al. 2009; 2010b). These data suggest that the cucumber beetles prefer to eat virus free plants and to aggregate in the flowers of virus free plants compared to virus infected plants. Consequently, as viral disease spreads through a population of Texas gourd plants containing VRT introgressives, the cucumber beetles become increasingly concentrated onto the flowers and leaves of healthy plants (mostly VRT plants) and consequently the VRT plants are more likely to contract the deadly wilt disease.

In 2009, we repeated our field scale experiment except this time we used an aphid specific insecticide to prevent aphid infestation and the spread of viruses transmitted by the aphids (Sasu et al. 2010b). We found that in the virus free fields, the VRT plants did not experience greater beetle damage after mid-July nor did they experience a greater incidence of wilt disease compared to the susceptible plants in the same fields. Moreover, we found that the reproductive output of the VRT plants in the "with virus fields" (no aphid specific insecticide) was only 60% of that in the virus free fields because in the "with virus fields" the VRT plants experienced greater herbivory after mid-July and a higher incidence of wilt disease than did the susceptible plants. That is, the concentration of the beetles (and the disease they transmit) onto the healthy plants following the spread of ZYMV mitigated some of the beneficial effects of the transgene on fitness. Recent laboratory experiments using GC/MS reveal that the flowers of ZYMV infected plants produce significantly less of the volatile organic compounds that attract beetles to flowers than do healthy plants. Moreover, in Y-tube choice tests we found that the cucumber beetles overwhelmingly prefer the odors of flowers from healthy plants compared to virus infected plants.

Model development and preliminary simulations

Our research over the past 10 years has resulted in a deep understanding of the web of plant-herbivore-pathogen interactions that form the *Cucurbita* pathosystem. Our research has also shown that many components of the fitness of introgressives bearing the VRT depend strongly on the suite of interactions among multiple herbivores/vectors and pathogens. Our data show that the VRT provides a fitness advantage and would increase in frequency from one generation to the next under our experimental conditions (i.e., 25% frequency of VRT plants, *Erwinia* present throughout the growing season and viral diseases present only in the last half of the growing season). However, our data also show that beetle herbivory, flower production, and the numbers of beetles that aggregate in the flowers to mate are all greater on healthy plants than on viral infected plants. In short, as viral diseases spread through our fields, cucumber beetles (and the damage they cause and the disease that they vector) becomes concentrated onto plants with the VRT. In fact, our four years of field data with Texas gourds, non-transgenic and transgenic introgressives revealed that the proportion of transgenic plants that contracted wilt disease increased as the incidence of viral disease increased on the non-transgenics. Moreover, even when beetle damage to the leaves does not result in the transmission of *Erwinia*, it does significantly depress reproductive output especially when there are developing fruits on a vine (Du et al. 2008). Consequently, the evolutionary trajectory of the VRT in wild communities of *Cucurbita* and the associated pathosystem becomes difficult to predict because there are indirect ecological costs associated with the transgene. The fitness of the VRT may depend upon 1) the arrival times and transmission rate of both the target and the non-target diseases in the population, 2) the proportion of plants in the population with and without the VRT (which will determine the increase in beetle concentration on VRT plants as non-VRT plants become infected with virus and perhaps also affect the transmission rate of the virus), and 3) the population density of the cucumber beetles.

To predict the evolutionary fate of an escaped VRT into a wild population of *Cucurbita*, we are developing a stochastic, individual-based model to study the spread of both viral and *Erwinia* infection in fields (180 plants in a regular grid) within a season. The probability of infection for each plant, at each time step, was governed by a risk function that decayed with distance to infectious source plants. We represented behavioral avoidance of virus infected plants by beetles (concentration effect = θ; 0 = no effect, 1 = complete avoidance of virus infected plants) as positive correlation between the risk of *Erwinia* infection in susceptible plants and the total number of virus infected plants. Viral infection was assumed to suppress flower production and *Erwinia* infection was assumed to lead to mortality 3 weeks post infection. Mating was assumed to occur at random among flowers (i.e. plants with more flowers were more likely to sire offspring/ be fertilized). Total seed production from each plant within each season went into a "seed bank" from which the plants in the following year were drawn, uniformly at random. Simulations were initialized with 25% transgenic plants in the first year and we tracked the proportion of transgenic offspring at the end of the growing season over the course of 50 years (generations) (Fig. 1).

The results of our preliminary simulation study reveal that (a) in the absence of *Erwinia*, a viral epidemic will favor the rapid spread of the transgene (if virus occurs early in the growing season, fixation of the VRT will occur rapidly). In the absence of virus, the VRT will disappear by drift or be present at low levels in the population (if there are no direct costs of the VRT to fitness). When both diseases are present there is a range of outcomes including fixation, extinction, and stable polymorphism of the VRT in the population. If the virus is present early in the growing season, the stunting caused by viral infection resutls in variation in host quality and the behavioral avoidance of virus infected plants leads to a biased exposure of the VRT plants to *Erwinia*; if this occurs early relative to plant reproduction, the fatal bacterial wilt disease leads to reduced fitness of VRT plants.

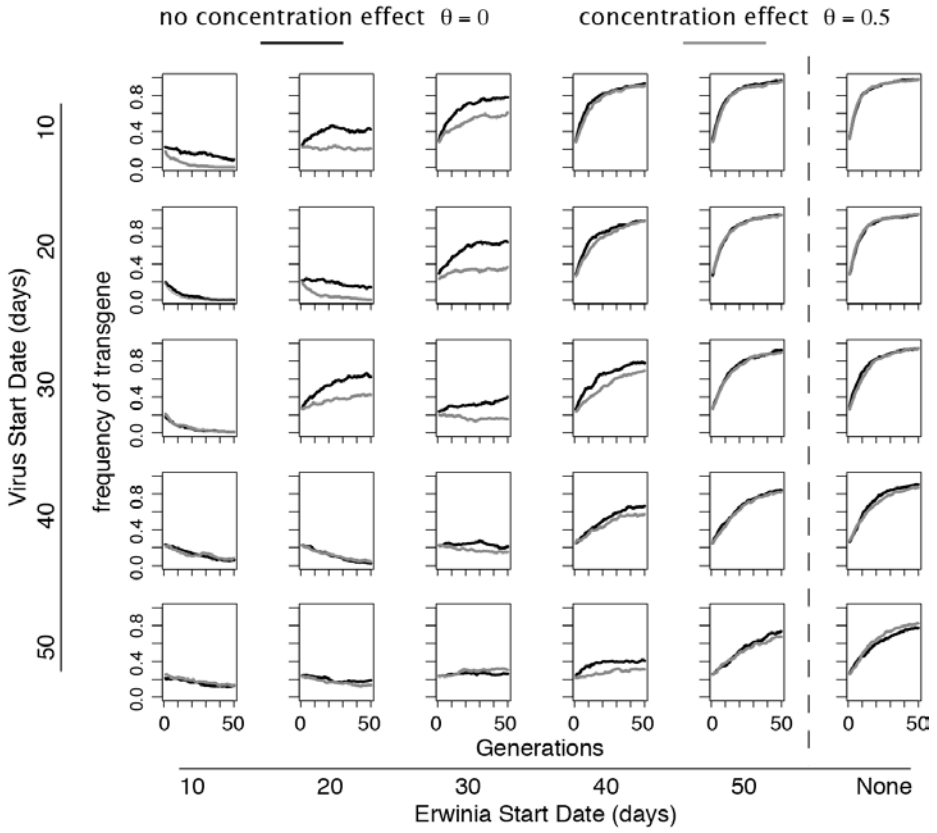

Fig. 1. Results of a 50 generation simulation across a range of virus and *Erwinia* start dates (from seedling emergence) in population in which 25% of the plants are transgenic and across two beetle concentration effects (black indicates $\theta = 0$, no behavioral avoidance of virus infected plants; grey indicates $\theta = 0.5$, partial avoidance of virus infected plants). Each panel gives the frequency of the transgene at the start of each season. Rows and columns of panels indicate the day of the introduction of virus/*Erwinia*, respectively, in a 100 day growing season.

Ongoing studies

We are currently conducting a series of large scale field experiments designed to parameterize the model (e.g., determine θ across a range of beetle densities and across a range of VRT frequencies), and to test the predictions of the model (e.g, introduce both virus and *Erwinia* into the fields early in the growing season). Our goal is to develop a fully parameterized and robust model that can be used by land managers to control the VRT should it escape into wild populations and become problematic.

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Single nucleotide polymorphism in genes accounting for ethylene biosynthesis and perception in melon, and its possible involvements in fruit ripening character

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Abstract

Melon is an attractive model for investigating fruit ripening. In melon varieties, there are two different types of fruit ripening process, climacteric and non-climacteric. Generally, climacteric phenotype exhibits short shelf life while non-climacteric one shows relatively long shelf life. Ethylene is a key hormone involved in fruit ripening process and the ripening phenotypes mainly depend on the ability of ethylene production and perception.

In order to detect SNPs involved in the ripening phenotype and the shelf life, we previously performed EcoTILLING of genes encoding ethylene biosynthesis (*CmACS2, CmACO1,2,3*) and ethylene receptor (*CmETR1,2, CmERS1*) with 46 melon cultivars, and several SNPs were detected. However, the mutation type, such as silence, missense and nonsense was not shown in each SNP from the attributes of the EcoTILLING technology. In this study we attempt to elucidate the mutation type of these SNPs by sequence analysis and then evaluated relationship between the SNPs and ripening character.

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Study of the *Fom-2* resistance gene using composite melon plants

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Abstract

Composite plants are obtained by *Agrobacterium rhizogenes*-mediated transformation of seedlings whose root system has been removed. The seedlings develop an adventitious, chimeric root system where some of the roots are transformed with the gene under study. Composite plants do not require tissue culture and allow the study of a large numbers of transformants in a short time. To study the function of *Fusarium oxysporum* f.sp. *melonis* (FOM) resistance genes, we set up a composite plant system for melons and obtained good transformation efficiency. In melon, a dominant gene, *Fom-2*, specifying resistance to FOM race 1 and 0 was identified by genetic linkage analysis (Joobeur et al. 2004), but it has not been functionally validated. We have cloned a 1450 bp-fragment upstream the *Fom-2* ORF, and fused the putative promoter with the GUS reporter gene. Composite melon plants that expressed the construct demonstrated promoter activity of the cloned sequence. Specific expression was observed in tissues adjacent to the vascular system, where the defense response is likely to occur, but no further induction by the fungus was seen. The function of the entire *Fom-2* gene in conferring FOM resistance was explored in the same system. In most experiments, partial resistance to race 1 of the fungus was conferred to composite plants of susceptible genotypes that expressed *Fom-2* under the control of its native promoter.

INTRODUCTION

The soil-borne pathogen, *Fusarium oxysporum* f. sp. *melonis* (FOM) exclusively attacks melon (*Cucumis melo* L.), causing a severe wilt disease. Four races of the fungus, designated FOM 0, FOM 1, FOM 2, and FOM 1.2, have been characterized (Risser et al. 1976). Melon genotypes that are resistant to the different races have been identified, and monogenic dominant inheritance was described against races 0, 1, and 2. The *Fom-2* gene, controlling resistance to races 0 and

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1, has been positionally cloned by Joobeur et al. (2004), and found to encode a nucleotide-binding site–leucine rich repeat (NB–LRR) protein of the coiled-coil class. The authors did not validate the gene by transgenic analysis.

A major difficulty in obtaining transgenic melons is the low transformation efficiency. The composite plants method developed by Hansen et al. (1992) and by Collier et al. (2005), uses *Agrobacterium rhizogenes* strains that harbor a binary vector to induce the growth of transgenic roots, while the shoot remains non-transformed. The method does not require axenic tissue culture and provides a convenient tool for studying gene expression in the roots of transformation-recalcitrant plants. In this study, we have used composite melon plants to study the promoter of *Fom-2* and to examine the gene's ability to confer resistance to FOM race 1.

MATERIALS AND METHODS

Plant and fungal material

The following melon genotypes were used: Dulce, Védrantais and Perlita FR (FOM1 and FOM 1.2 susceptible, FOM2 and FOM0 resistant); Ein-Dor, Charentais and Noy Yizrael (susceptible to all strains); Eyal (FOM1, 2, 0 resistant, FOM1.2 susceptible). For composite plant preparation, seedlings were germinated in 0.5 L pots in a 3:1 soil and perlite mix. Fungi were grown at 25°C in 250 ml liquid PDA medium (0.5% PDA and 0.5% Yeast extract; Difco Laboratories) on a rotary shaker at 180 rpm, for 5 days. Hydroponically grown composite plants were inoculated by adding conidial suspension to the hydroponic medium to a final concentration of 10^6 conidia /ml. In other experiments, composite plantlets in rockwool cubes were mechanically wounded by root trimming, soaked in 10^6 a suspension of conidia/ml for 5 min and planted in 250 ml pots with autoclaved soil.

Fom-2 promoter: GUS construct

A 1450 bp DNA fragment upstream the *Fom-2* start codon was cloned in binary vector pME-524 (Edelman et al. 2000), replacing the 35S promoter present in the GUS cassette. The promoter fragment was amplified from clone 174L04 of a melon BAC library (Luo et al. 2001) of the FOM1, FOM2 resistant melon MR1, using the following primers (inserted restriction sites are underlined):

Pro-7620R-*HindIII* 5'AT<u>AAGCTT</u>GCTCGCATTGGCATTGATTTTACC 3'

ATG-6170F-*XbaI* 5'AT<u>TCTAGA</u>GTCCATAGGAAATCACCCATTGTG 3'

The PCR product was digested using *HindIII* and *XbaI* and ligated into pME524 using Roche T4 DNA ligase.

Fom-2 expression construct

The above putative promoter fragment was cloned along with the *Fom-2* ORF

and 550 bps of 3' sequence into binary vector pCAMBIA2300. The promoter, ORF and terminator sequence was PCR-amplified from clone 174L04 of the MR1 BAC library using the following primers:

Term-2420F 5'ATCCATGGATGGCGCGCCGACAGAGGAAGCCGCACAGAAATG 3'

Pro-7620R 5'ATCCATGGATGGCGCGCCGCTCGCATTGGCATTGATTTTACC 3'.

The 5200 bp-amplicon was cloned into pCR2.1-TOPO vector, was released using *XhoI* and *SacI*, and cloned into pCAMBIA2300 using *SalI* and *SacI* cloning sites. The constructs were transferred into *A. rhizogenes* strain K599 using the freeze/thaw method (Höfgen and Willmitzer 1988).

Composite plant system and analysis

The engineered *A. rhizogenes* strain was cultured overnight at 28-30ºC on LB-kanamycin medium. Bacteria were centrifuged at 3300 g for 10 min, the supernatant discarded, and the pellet re-suspended in 0.25 x MS solution to $O.D._{600}$ 0.2-0.5. Rockwool cubes (3 cm length), were autoclaved and placed on open Petri dishes, 3-4 cubes per plate. A hole was made using a 1 ml plastic tip and soaked with 4 ml bacterial suspension. Ten day-old melon seedlings were clipped at the hypocotyl region and placed individually in the rockwool cubes. The Petri dishes were arranged in plastic trays that were left uncovered for several hours, till the plants began to lose turgor. The trays were then covered with nylon sheet for approx. 1 week until new roots emerged from the cube, and transferred to the hydroponic system. Such system (where roots grow in medium-filled boxes aerated by an air pump) provided a controlled environment and easy access to the roots (Berezin et al. 2011). Histochemical GUS assay was performed according to Jefferson (1989). For statistical analyses we used the SPSS 17.0 program.

RESULTS AND DISCUSSION

Composite melon plants

In order to calibrate the composite plants method for melon plants, we tested the transformation rate of several melon genotypes using a 35S::GUS binary vector, pME524, transferred to *A. rhizogenes* strain K599. The proportion of plants having at least one GUS-expressing root was 73-80% (Table 1). An example of a chimeric root system is shown in Fig. 1A.

Expression pattern of the *Fom-2* promoter

We tested the functionality of the putative *Fom-2* promoter, cloned upstream the GUS reporter gene. FOM1-susceptible 'Dulce' and resistant 'Eyal' plants were transformed using the *Fom-2* promoter::GUS and the 35S::GUS constructs, and the expression patterns were examined (Fig. 1B, C). The 35S promoter directed GUS expression in all root tissues, including root cap, epidermis with root hairs and cortex

(not shown). The DNA fragment that we cloned acted as a functional promoter, directing GUS expression in transformed roots in a tissue specific pattern. GUS activity was localized in a "sleeve" of tissue (endodermis and xylem-parenchyma) around the root vascular system. This represents the site of Fusarium penetration to the xylem that is probably guarded by the R-protein. Adding the fungus to the roots did not visibly change the expression pattern (not shown).

***Fom-2* over-expression**

We asked whether partial transformation of susceptible plant roots will protect the chimeric root system from the fungus. Susceptible Dulce and resistant Eyal seedlings were transformed with the *Fom-2* expression construct, and pCAMBIA2300 empty vector as a control. An additional negative control consisted of non-transformed plants that were rooted in rockwool cubes without *A. rhizogenes*. Upon the appearance of adventitious roots, plants were transferred to liquid growing medium, and normal-looking roots that did not exhibit a transgenic phenotype (*i.e.*, thicker and less gravitropic roots) were removed. Seven days after the transfer (1 day for soil-grown experiment), the plants were inoculated with FOM1 by adding conidial suspension to a final conc. of 10^6 conidia/ml. The progress of wilt symptoms was monitored up to one month post inoculation (Fig. 2A, C). Two weeks after inoculation, untreated control plants suffered 70% mortality, while only 20% of the *Agrobacterium*-treated ones died. After another week, all the untreated plants died, and plants transformed with the *Fom-2* expression construct had a clear advantage over those transformed with an empty pCAMBIA2300 vector that persisted till the end of the experiment with, respectively, 58% *vs*. 89.28% mortality. The AUDPC values (area under disease progression curve) were 54.4% of the susceptible control in *Fom-2* plants and 93% in empty-vector plants. To test the statistical significance of the treatment, individual plants were taken as replicates and scored at several time points, as either live (1) or dead (0; Compton 1994). Then, an independence χ^2 test was performed, against the null hypothesis that treatments had no effect. The first test was performed on all three treatments, pCAMBIA2300 (n=28), *Fom-2* (n=26) and control (n=10) and showed that the *A. rhizogenes* treatments had a significant effect compared to untreated controls (χ^2=10.275, df=2, $p<0.05$). Next, each treatment was compared to the control separately. At the conclusion of the trial, the advantage of the *Fom-2* treatment over the empty vector was significant (χ^2=7, df=1, $p<0.05$). The experiment was repeated with soil-grown plants (Fig. 2B). Under these conditions, disease progression appeared more moderate than in the hydroponic medium, and after two weeks the control plants suffered only 60% mortality. Here too, we witnessed a significant advantage of *Fom-2* transformation over the empty vector, as measured at 19 and 23 DPI (Fig. 2B).

To repeat these experiments, a total of 10 independent trials were performed,

including additional susceptible genotypes, with variable results. In all cases we witnessed an advantage of empty vector transformation, compared to the non-inoculated control, eight cases were statistically significant (Table 2). Such effect could result from a general defense response to *Agrobacterium.* Eight experiments showed an advantage of the *Fom-2* vector compared to the empty vector, five of them statistically significant; in two experiments, empty-vector plants were more resistant.

Composite plants were used to study the *Fom-2* gene function. This method has proven to be very effective, enabling us to get up to 80% transformation rate in many genotypes. We isolated the *Fom-2* gene promoter and were able to demonstrate its functionality. We showed that *Fom-2* has a specific expression pattern in root tissue that could be related to protection of the vascular system. A very similar pattern of expression was reported by Mes et al. (2000) for the *I-2* gene that controls *F. oxysporum* resistance in tomato.

In order to test whether roots transformed with *Fom-2* gene can induce a systemic defense reaction in susceptible plants and protect them against FOM, we generated composite plants expressing the entire gene in part of their roots. We were able to induce partial resistance, probably because of the chimeric nature of the root system. Transformation with *Fom-2* did not prevent the symptoms of Fusarium wilt disease but reduced mortality in a quantitative manner. The fungus could invade the plant through the non-transformed, genetically susceptible roots. The fact that in most experiments it appeared moderated could suggest that a systemic mechanism is being activated by the transgenic roots, possibly causing a defense response in the entire plant.

To validate gene function one would need to demonstrate that the partial resistance imparted by the gene is race-specific, but preliminary experiments designed to test this have been inconclusive. Variation among experiments could be due to variable transformation rates, environmental or fungal parameters. Improvement of the composite plants method by selecting against wild-type roots could be attempted, to strengthen the results obtained in this study.

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Table 1. Transformation rates of different melon genotypes. GUS positive plants had at least one GUS expressing root.

Genotype	Plants transformed	GUS positive plants	Transformation rate
Eyal	130	102	0.78
Dulce	120	95	0.79
Perlita FR	40	32	0.80
Ein-Dor	53	40	0.75
Védrantais	60	44	0.73
Charentais	20	15	0.75

Table 2. Summary of ten experiments with composite plants transformed with *Fom-2* expression construct compared to empty vector transformation and non-transformed control. '+' Difference in mortality of susceptible genotype upon FOM1 inoculation, between the indicated treatments. '-' opposite effect than indicated; '*' - Statistical significance ($p<0.05$) at least at one time point of the disease progression curve

Genotype	Growing medium	Empty vector > control	*Fom-2* > Empty vector
Dulce	Hydroponic	+*	+*
Dulce	Hydroponic	+*	+*
Dulce	Hydroponic	+*	+*
Dulce	Hydroponic	+*	-*
Dulce	Soil	+	+*
Dulce	Soil	+*	+
Dulce	Soil	+	-
Ein-Dor	Hydroponic	+*	+*
Ein-Dor	Soil	+*	+
Noi-Yzrael	Hydroponic	+*	+

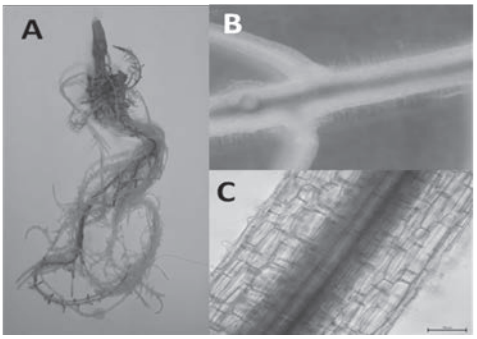

Fig. 1. GUS staining patterns (dark hue) in composite plant roots. A. Chimeric roots of Eyal melon plant transformed with the 35S::GUS binary vector pME524. B, C – transgenic roots expressing GUS under the *Fom-2* promoter fragment in the tissue surrounding the root vascular bundle.

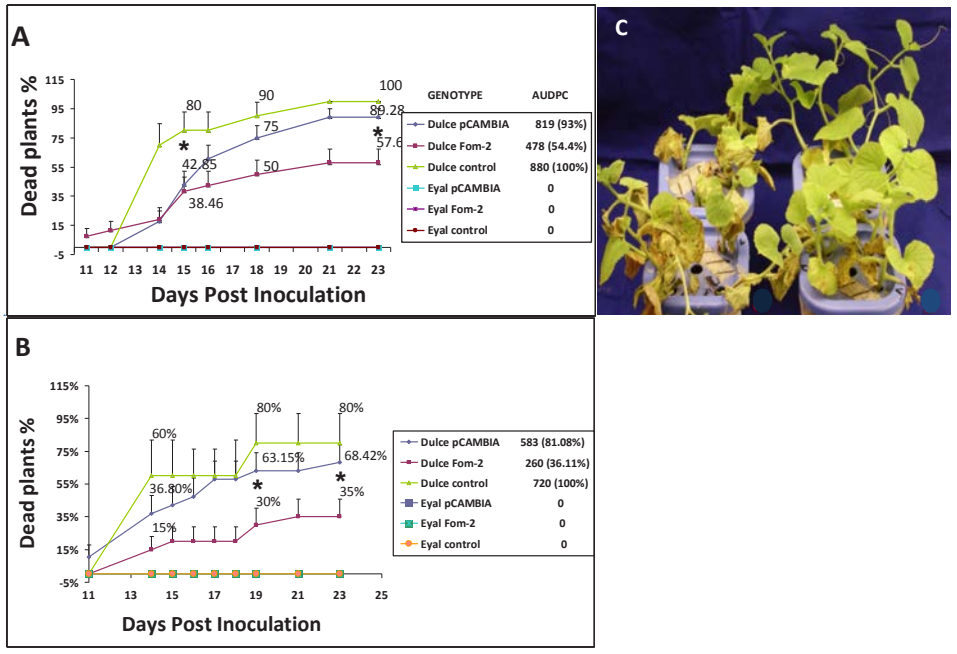

Fig. 2. Disease progression in composite Dulce and Eyal composite plants. Graphs compare plants that express *Fom-2* under its own promoter, plants transformed with empty pCAMBIA2300, and non-transformed plants. A. Experiment performed in hydroponic medium. B. Experiment performed in soil. For each experiment, the area under the disease progression curve (AUDPC) was calculated and expressed as a percentage of the control group that was the most susceptible. Mortality among resistant Eyal plants was null. χ^2 test was performed for a few of the time points. '*' indicates statistical significance ($p<0.05$) of the difference between two adjacent curves. C. Photograph of hydroponics experiment. Right: composite Dulce plants transformed with *Fom-2*. Left: composite Dulce plants transformed with empty vector at the end of the experiment; none of the control plants survived at this point.

Unravelling the molecular basis of an old enemy: CMV resistance in melon (*Cucumis melo* L.)

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Abstract

The study of the complex resistance to *Cucumber mosaic virus* (CMV) present in the Korean melon accession 'Sonwang Charmi, PI 161375' (SC) has been addressed using two melon populations, a near isogenic line (NIL) collection and a dihaploid line (DHL) collection, both generated from a cross between SC as resistant parental line and the cultivar Piel de Sapo (PS) as susceptible parental line. Previously, the NIL collection allowed us to describe a single recessive gene, *cmv1*, which confers full resistance to CMV strains P9 and P104.82, but not to strains M6 and TL. Screening of the whole DHL population followed by QTL analysis revealed that the genetic control of the resistance to the strains M6 and TL is complex and requires the involvement of three QTLs that interact, one of them probably being *cmv1* itself. Therefore, the resistance to CMV in melon SC is qualitative for some strains and quantitative for others. For this later resistance, *cmv1* is necessary and explains most of the phenotypic variance, but is not sufficient, and needs the interaction with the other two loci. The molecular cloning of the major gene, *cmv1*, has progressed up to an interval of 140 Kb.

INTRODUCTION

Cucumber mosaic virus is able to infect more than 1000 plant species, causing severe damage in the most economically important families, such as Solanaceae and Cucurbitaceae. Resistance to CMV is frequently partial and polygenic, with

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several QTLs involved acting with different effects (Caranta et al. 2002). In Cucurbits, however, the resistance has been described mostly as recessive (Risser et al. 1977). Specifically, in melon the resistance of the Korean accession 'Sonwang Charmi, PI 161375' (SC) was initially described as oligogenic, recessive (Karchi et al. 1975) and quantitative (Dogimont et al. 2000), with a major QTL present in the linkage group XII. This QTL was characterized in our laboratory using a collection of near isogenic lines (NILs) with introgressions of the exotic accession SC into the genome of the susceptible cultivar Piel de Sapo (PS) (Eduardo et al. 2005). The gene, named *cmv1*, resulted to be a major gene for resistance to some CMV strains, providing qualitative, instead of quantitative resistance, since it was necessary and sufficient to confer full resistance to strains P9 and P104.82. Other strains, such as TL and M6, were able to overcome it and therefore, other loci must be present in the melon genome are needed to confer full resistance (Essafi et al, 2009). We described three QTLs involved in this second resistance, mapping one of them in the region of LGXII where *cmv1* maps (Martin-Hernández et al. 2010). Here we have addressed the molecular cloning of *cmv1* and the characterization of the other QTLs.

MATERIALS AND METHODS

Plant and virus material

The *Cucumis melo* genotypes used were the cultivar Piel de Sapo T111 (PS) and the Korean accession PI 161375 cultivar Sonwang charmi (SC) as susceptible and resistant controls, respectively. Two populations of melon plants generated using SC and PS as parental lines were used for the screenings, a near isogenic line (NIL) collection (Eduardo et al. 2005) and a double haploid line (DHL) population (Gonzalo et al. 2005). For the cloning of *cmv1*, an F2 population from the cross between the NIL SC12-1 and the susceptible parental PS was generated. For the inoculation experiments, plants were grown in growth chambers in long day conditions at 22ºC for 16 h in the presence of 20,000 lux of light and 18ºC for 8 h in the dark. CMV strain M6 was described previously (Diaz et al. 2003).

Inoculations and virus detection

Viral inoculations were performed as described previously (Essafi et al. 2009). Plants were observed at the onset of symptoms production and also after 16 to 20 days post-inoculation. At this point, for the screening of the DHL population, symptoms were scored with a scale from 0 to 5 depending on the phenotype. 0 was no symptoms, 1 was very mild mosaic only in the first or second leaves, 2 was mild mosaic in all leaves, 3 was stronger mosaic in all leaves, 4 very strong, with some curled leaves, 5 was very strong, with very small curled leaves and small plant. PS was used always as positive infection control, producing normally a score of 4 and SC was used as negative control, with a score of 0. Viral infection was con-

firmed via RT-PCR as described in Essafi et al. (2009) using primers specific of M6 and FNY strains F309-3'R (5'TGGTCTCCTTTTGGAGGCC) and F309-1600F (5' TTCGAGTTAATCCTTTGCCG).

Data analysis

The molecular markers and genetic map were obtained from previous work (Fernandez-Silva et al. 2008). QTL analysis was performed by Composite Interval Mapping using WinQTLcart (Wang et al. 2010). The experiment-wise LOD threshold for a signification level $p<0.05$ was calculated by permutation analysis with the same software. The genotype for each QTL of individual DHL was estimated according to marker genotypes. Differences in resistance among DHL carrying 0, 1, 2 or 3 resistance QTLs were investigated by ANOVA and Tukey´s mean contrast using JMP 5.1 (SAS Institute).

RESULTS AND DISCUSSION

Approximation to the molecular cloning of *cmv1*

We have generated an F_2 population of 1400 individuals from the cross between the NIL SC12-1 and the susceptible PS parental line. 780 individuals were screened with flanking markers CMN61_44 and CMN21_55 that were mapping in an interval of 3 cM (Essafi et al. 2009). The recombinants were phenotyped by inoculation with the strain P104.82 of CMV. The region where *cmv1* maps has now been narrowed down to 140 Kb between the markers *sc4_344* and *sc4_358* on the upper arm of chromosome XII (Fig. 1). There are three candidate genes in this region. We are generating more recombinants to precisely identify *cmv1*. Although until now the recessive resistance genes identified have been eukaryotic translation initiation factors (eIFs), there is no eIF residing in this interval, which suggest that *cmv1* is involved in different mechanism of defence not related to translation.

Resistance to the other CMV strains

There are some CMV strains able to overcome the resistance provided by *cmv1*, but not able to overcome the resistance of the parental line SC (Essafi et al. 2009). Therefore, the gene or genes involved in this second resistance must map in other regions of the melon genome. We had previously performed a screening of a DHL collection obtained from the cross between the same parental cultivars: SC and PS. During that screening we found 8 resistant lines out of 64 screened, compatible with the existence of three QTLs involved (Martin-Hernandez et al. 2010). Out of the three QTLs, one was located in LG XII, co-localizing with *cmv1* (Essafi et al. 2009) and had a major effect in resistance. The other two, with minor effects, were in LG III and LG X. In all cases, the QTLs coming from SC were associated with increased resistance (Martin-Hernandez et al. 2010). We have now increased

the number of DHLs screened and found 11 resistant lines out of 69 DHLs. As shown in Fig. 2, after classification of the DHL regarding the number of resistance QTLs according to their marker genotype, ANOVA showed that DHL carrying the SC alleles at the QTLs were significantly ($p<0.001$) more resistant that those that carried two or less SC alleles at the QTLs. Manual inspection of DHL genotypes revealed that not all the resistant DHLs carry SC alleles for all the three loci detected by QTL analysis. In fact, two of them carried only the QTLs in LG III and XII and one the QTLs in LG X and XII. None of them carried only LG III and X, as expected if *cmv1* is necessary for this resistance (Table 1). Moreover, as seen in table 1, the F1 of the cross between two resistant DHL (DHL 29x41) was fully susceptible, suggesting that there may be another locus involved, with minor role, that in the crossing may become heterozygous. The F1 of the backcross of DHLs harbouring 3 QTLs and the susceptible parental PS was susceptible as expected from a recessive resistance. Previously Dogimont et al. (2000) had also found several minor QTLs involved in resistance to strains P9 and TL in a RILs population generated between the cultivar Vedrantais and SC as parental lines. However, none of them coincide with those identified by us. Only one of them was located in LG III- but did not co-localize with marker CMCTN66a (Martin-Hernandez et al. 2010) - and none was placed in LG X. Additionally, the minor QTLs do not play any role in resistance against strain P9 in our hands, since for this strain, only *cmv1* was required.

In melon, resistance to CMV is mostly recessive (Risser et al. 1977). In agreement with this, we had previously demonstrated that the resistance conferred by *cmv1* was recessive (Essafi et al. 2009). As seen in Table 1, the two new QTLs show also recessive resistance, confirming the recessive character of the resistance to CMV in melon. Recessive resistances against several plant viruses have been described and all genes found till now have resulted to be involved in translation, either as translation initiation factors (Robaglia and Caranta 2006) or as factors required for translation reinitiation such as a TOR-like factor (Schepetilnikov et al. 2011). The described recessive resistant phenotypes at the molecular level are arrested viral translation/replication and impaired cell-to-cell movement. Translation initiation factors have found to be involved in cell-to-cell movement in Arabidopsis since a mutation in eIF4E impairs the translation of the movement protein, but not the translation of other viral proteins (Yoshii et al. 2004). The resistance to CMV in melon SC that we describe here suggests that there is a locus, *cmv1*, necessary to confer total resistance against some strains, and other loci, located in LG III and X, that interact with *cmv1* to confer full resistance to other set of strains. None of the *cmv1* candidates is an eIF and there is no eIF mapping in the regions of LG III and X where the other two QTLs map. This indicates that the resistance to CMV in melon SC will be due to a mechanism other than translation.

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Table 1. DHL resistant to CMV strain M6 and F1 of crosses among some of them. SC, PI 161375 allele. PS, Piel de Sapo allele. R, resistant, S, susceptible, to CMV M6 strain

DHL or cross	QTLIII	QTLX	QTLXII	Phenotype upon M6 inoculation
29	SC	SC	SC	R
41	SC	SC	SC	R
46	SC	SC	SC	R
69	SC	SC	SC	R
85	SC	SC	SC	R
128	SC	SC	SC	R
142	SC	SC	SC	R
2012	SC	SC	SC	R
140	SC	PS	SC	R
1046	SC	PS	SC	R
1123	PS	SC	SC	R
29x41	SC	SC	SC	S
128x2012	SC	SC	SC	R
85X128	SC	SC	SC	R
85xPS	SC/PS	SC/PS	SC/PS	S
128xPS	SC/PS	SC/PS	SC/PS	S
2012xPS	SC/PS	SC/PS	SC/PS	S
PS	PS	PS	PS	S
SC	SC	SC	SC	R

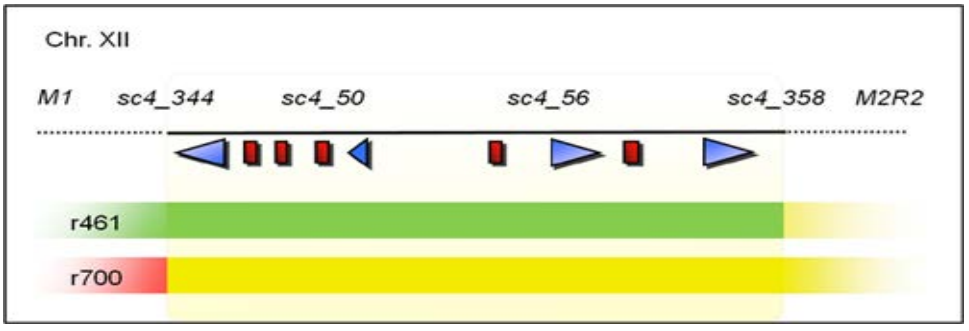

Fig. 1. Schematic representation of the genomic region of *cmv1*. The arrows indicate protein coding regions with high confidence, whereas the boxes show potentially coding regions partly of transposable elements. The approximate position of relevant markers is given. r461 (resistant phenotype) and r700 (susceptible phenotype) are the relevant recombinants delimiting the interval.

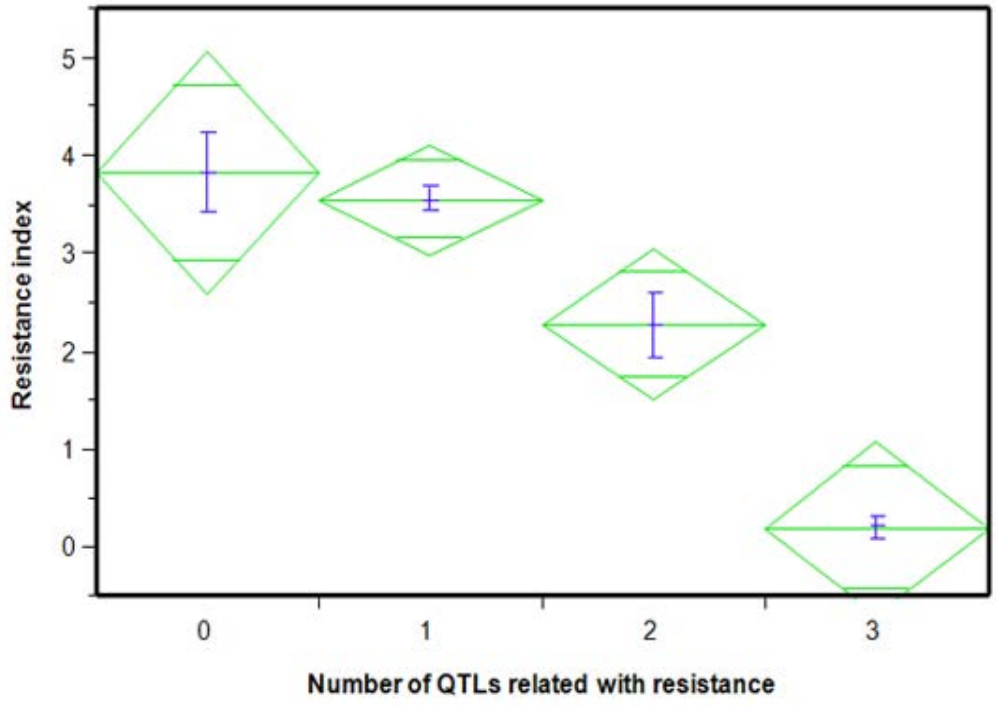

Fig. 2. Resistance index means of DHL carrying 0, 1, 2 or 3 resistance QTLs after inoculation with CMV M6 strain.

How microsatellite diversity helps to understand the domestication history of melon

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Keywords: *Cucumis melo*, genetic structure, phylogeny, SSR, wild melons

Abstract

Melon (*Cucumis melo* L.) population structure remains incomplete because of the sampling weakness in some botanical groups and for wild melons. The purpose of this study was to assess the genetic subdivisions in melon germplasm using a more representative sampling of the worldwide diversity and to investigate the localization of melon domestication and diversification. To reach this objective, a set of 20 microsatellite markers was used to genotype 713 accessions including 635 cultivated and feral melons, 66 wild melons and 12 relative *Cucumis* sp. A two-level structure in melon was revealed using population genetics statistics, clustering methods with genetic distances and Bayesian assignment method. Accessions split into two groups, fitting very well the subspecies *melo* and *agrestis*. *Agrestis* group was clearly substructured according to geographical origins, with African, Far-Eastern and Indian subgroups. The substructure of *melo* group was less resolute and the four clusters obtained grouped together several botanical groups originating from Europe, America, Middle East or Central Asia. African and Asian wild *Cucumis melo* were assigned to two distinct clusters in *agrestis* group, each with cultivated melons from the same respective geographical origin, suggesting that melon was domesticated twice. The domestication of wild African melon would have had a weak impact on the diversification of cultivated melon, producing only Sudanese *tibish* and *seinat* types. The domestication of wild Asian melon, probably in India, would have first produced Indian cultivated melons and then be at the origin of Far-Eastern melons. Introduction of Eastern melons in the West and successive breeding activities may have then produced *melo* ssp. These findings are consistent with recent data on the *Cucumis* genus structure and origin, and with the high frequency of resistance genes found in melon Indian accessions.

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INTRODUCTION

Melon (*Cucumis melo* L.) is an important crop cultivated world wide. It belongs to the genus *Cucumis* (Kirkbride 1993). *C. melo* is a diploid (2n=24), allogamous and insect-pollinated species. Widely considered as originating from East Africa, recent phylogenetic data on the *Cucumis* genus demonstrated that *C. melo* originates from Asia, together with its closely related species *C. sativus* (Sebastian et al. 2010; Telford et al. 2011).

Wild melons are rampant, highly branched vines with small leaves and flowers. Fruits are small; they have thin, bitter and non sweet flesh enclosing many small seeds. Wild melons have a wide geographical distribution and are found in Africa, in the Indian subcontinent and have been also described in Australia (Kirkbride 1993; Telford et al. 2011). Domesticated melons typically have larger fruits and seeds and an exceptional diversity of shapes and colours, which corresponds to a diversity of use. Some cultigroups are grown for eating the immature non-sweet fruits and others for the mature sweet fruits. The seeds of some melons are consumed; some melons are cultivated for their aromatic or decorative characteristics (Pitrat et al. 2000).

C. melo was described to split into two subspecies *C. melo* subsp. *melo* and *C. melo* subsp. a*grestis*. Various infraspecific classifications have been proposed, mainly based on fruit shape and colour and flesh type. According to Pitrat (Pitrat et al. 2000), sixteen botanical groups were defined: five botanical groups in *C. melo* subsp. *agrestis* (*acidulus, chinensis, conomon, makuwa* and *momordica*) and eleven in. *C. melo* subsp. *melo (adana, ameri, cantaloupensis, chandalak, chate, chito, dudaim, inodorus, flexuosus, reticulatus and tibish).* No genetic barriers preventing crosses between the two subspecies, between the botanical groups or with wild melons have been reported.

The genetic diversity of *C. melo* has been described using various molecular markers, including RAPDs, RFLPs and microsatellite markers (SSR). Until now, most studies have focused on either a set of accessions from a single geographical origin or a limited number of accessions (Tanaka et al. 2007; Phan et al. 2010; Soltani et al. 2010; Aierken et al. 2011; Fergany et al. 2011; Yildiz et al. 2011, for some recent ones).

The aim of the present work was to further elucidate the genetic structure and evolutionary history in *C. melo* by using SSR markers on a wide-based collection of melon consisting of more than 700 accessions. In the present paper, we use a set of 20 microsatellite markers to survey the genetic diversity occurring in a collection spanning most of the diversity collected around the world (Table 1). The collection comprises 66 wild accessions and 635 cultivated and feral accessions, from all the 16 botanical groups defined by Pitrat (Pitrat et al. 2000). Our main objective was (1) to quantify the genetic diversity available in this collection using 'neutral'

molecular markers (2) to test for the presence of an underlying genetic structure (3) to localise the processes of domestication and diversification of cultivated melons.

MATERIALS AND METHODS

Plant material

A collection comprising 701 accessions was chosen from the 2,400 *C. melo* accessions maintained at INRA Avignon. Six hundred and thirty-five cultivated and feral accessions were chosen to be representative of all geographical origins and botanical groups (Table 1). Their assignments to the botanical groups were made according to Pitrat et al. (2000). Sixty-six wild accessions were included originating from Africa, India and Central Asia. Accessions from 12 *Cucumis* species were added as outgroups. Most of the accessions have been collected long before this study and maintained by self-pollination at INRA Avignon.

DNA extraction and detection of SSR loci

Genomic DNA was isolated from fresh young leaves using the DNA extraction kit (Qiagen). Eight seedlings per accession were sampled in bulk. Twenty melon SSR markers, previously developed and used in genetic diversity studies were chosen (Díaz et al. 2011). The SSR markers have been mapped and were distributed in all 12 linkage groups, one or two per linkage group.

Data analysis

Genetic distance matrix between pairs of accessions was estimated from an index of dissimilarity based on a simple matching implemented in DARwin 5.0.156. It was used to perform a principal coordinate analysis (PCoA) and to construct a neighbour-joining tree using the DARwin software. The model-based program Structure (Pritchard et al. 2000) was used to infer population structure using a model allowing for admixture and correlated allele frequencies, with a burn-in length of 100,000 iterations and a run length of 100,000 iterations. The tested values of the number of groups K ranged from one to 30, with 10 replicate runs for each K. The most likely number of groups was determined using the magnitude of ΔK (Evanno et al. 2005). Inter-population genetic differentiation was measured by pairwise *Fst* calculated using GENETIX 4.05 (Belkhir et al. 2004). The intra-population level of gene diversity was measured by the expected heterozygosity using GENETIX. A home-made script computed the number of alleles, non-rare alleles (frequency higher than 5%) and private alleles (specific to one population) using R.

RESULTS

Microsatellite diversity

The 20 SSR loci were polymorphic and yielded a total of 272 alleles in *C.*

melo. The average number of alleles per locus was 13.6 and 3.5 when removing rare alleles (i.e. with a frequency lower than 0.05). Combining data from the 20 microsatellite loci, we found 660 different multilocus genotypes, including 637 unique genotypes.

Genetic structure of the collection

We tested the existence of genetic structure in the melon collection. We determined the number (K) of diverged groups or clusters using the software Structure. The first peak of ΔK-value was obtained when testing the K=2 hypothesis, indicating the presence of two main genetic groups. The majority of the accessions were clearly assigned to one of the two groups, but some accessions appeared with high admixture level between the two groups. Principal coordinates analysis (PCoA) also allowed to distinguish two groups with a transitional zone. One group (I) essentially included accessions of the botanical groups *adana, ameri, cantalupensis, chandalak, chate, flexuosus, inodorus* and *reticulatus*, corresponding to the *C. melo* subsp. *melo.* The other group (II) essentially included accessions of the botanical groups *acidulus, chinensis, conomon, makuwa* and *momordica*, which belong to the *C. melo* subsp. *agrestis*. *Chito* and *tibish* and *seinat* accessions, which have been reported to belong to *C. melo* subsp. *melo* (Pitrat et al. 2000) indubitably clustered in the group II (Table 2). 58 out of the 66 wild accessions were assigned to the group II. The eight wild melon accessions included in the group I all had wild global look but presented some typical characters of the cultivated melons, orange or sweet flesh for example; they likely resulted from gene flow with cultivated melons.

Further results of the Structure analysis showed that each group could be divided into subgroups: four subgroups (A to D) in group I and three subgroups in group II (E to G). The three subgroups E to G were clearly distinguishable in the PCoA results while the four subgroups in group I overlapped. In the same way very few accessions of the subgroups E to G were determined as admixtured while admixture was frequent between accessions of the four subgroups A to D.

The three subgroups E to G clearly corresponded with the geographical origin of accessions. The group E consisted of accessions from India, including accessions of the botanical groups *acidulus* and *momordica*, unknown-type Indian cultivated melons and 27 Asian wild melons. It also included the eight accessions of the botanical group *chito* of this study, which were collected in South America and West Indies. The group F consisted of cultivated (16 *tibish* accessions) and wild melons (30 accessions) from Africa. The group G consisted of accessions from Far East, belonging to the botanical groups, *chinensis, conomon* and *makuwa.*

The sense of the four subgroups A to D was more horticultural than geographical. Subgroup A mainly consisted of *inodorus* melons from Europe,

Middle East, America and Africa; it also included the *ameri* melons recognized as cultigroup *ananas*. The group B mainly consisted of European, American and African accessions of the *reticulatus* and *cantalupensis* botanical groups. The group C grouped accessions of the botanical groups *chate, chandalak, adana* and *ameri* -except *ananas* cultigroup- and 19 *flexuosus* of which 13 were Sudanese melons. The group D included melons which showed high admixture level between groups I and II. These melons are mainly unknown types from India and *flexuosus* from India, Maghreb and Middle East.

Phylogenetic analysis

Phylogenetic analysis supported the genetic structuration of melon in the seven genetic groups defined by the Structure analysis. The three subgroups E, F, G of the group II (‘*agrestis*’) were well isolated from each other whereas the four subgroups of the group I (‘*melo*’) were less distinct. According to the tree root defined with other *Cucumis* species, the African subgroup F represented a distinctive population. The Far East accessions of the subgroup G and the American feral *chito* descended from the subgroup E grouping Indian melons. The subgroup D was intermediate between the three other ‘*melo’* subgroups and the subgroup E, proving that melons of the subsp. *melo* came from Indian ‘*agrestis’* melons. Relationships between the three ‘*melo’* subgroups, A, B and C were less obvious.

Wild melons were split into two groups, an African one and an Asian one. The distribution of cultivated melons on the phylogeny in relation to wild melons suggested that cultivated melons had two distinct origins: African *tibish* and *seinat* came from the African wild pool whereas all others cultivated melons came from the Asian wild pool.

Genetic diversity and differentiation among groups

Genetic diversity was higher within the group II ‘*agrestis’* (He=0.61) than within the group I ‘*melo’* (He=0.45) in spite of lower sample size (Fig. 1). The subgroups E and D which comprise a large number of Indian accessions had a much higher genetic diversity than other subgroups (He_E=0.68; He_D=0.60). Genetic diversity of the subgroup F, which comprises African accessions, was also high (He=0.49). On the other hand, subgroup G, which grouped Far East accessions, was genetically poor (He= 0.18) and shared all the alleles with the group E.

Pairwise *Fst* confirmed the proximity between the subgroups A, B and C of the group I and the intermediate place of the subgroup D between the subgroups A, B, C, and the subgroups E, F, G of the group II. Asian and African wild melons were respectively extremely close to Indian (subgroup E) and African (subgroup F) cultivated melons.

DISCUSSION

Infraspecific genetic differentiation of *C. melo*

A worldwide collection of melon, currently maintained and evaluated at INRA Avignon (France) is publicly available. Using 20 highly polymorphic SSR markers, we confirmed the uniqueness of most accessions of the collection and revealed a large amount of genetic diversity and allelic variation within the melon species. The average number of alleles per locus was 13.6, whereas it was only 3.0 in a broadly based collection of *Cucurbita* comprising 104 accessions and using a very large set of SSRs (134) (Gong et al. 2012).

The subdivision of the 701 melon accessions in two main clusters corroborated the taxonomical subdivision of *C. melo* into the two subspecies *C. melo* subsp. *melo* and *C. melo* subsp. *agrestis*. Western melons notably compound the main part of the '*melo*' group while Eastern melons notably compound the majority of the '*agrestis*' group, revealing a West-East geographical differentiation of cultivated melons. The botanical group *chito*, attributed to the subsp. *melo* (Pitrat et al. 2000), clearly clustered in the '*agrestis*' genetic group, as previously found (Stepansky et al. 1999). Sudanese accessions of the recently described botanical group *tibish* and *seinat* were also classified into the subsp. *melo* and clustered in the '*agrestis*' genetic group. These conflicts between botanical classification and genetic structure stress the limits of basing the botanical classification on characters which are difficult to evaluate and may have a continuous variability, as the pilosity of the female hypanthium (Pitrat et al. 2000).

In addition to this *melo / agrestis* structuration, a substructure of seven genetic subgroups was revealed by analyses at subspecies level. Within the '*agrestis*' group, the subdivision in three subgroups is highly consistent with geographical distribution: the group E in India, the group F in Africa and the group G in Far East. We can infer from the genetic proximity of *chito f*eral accessions from America with Indian melons of the group E that *chito* melons originated from India, as previously suggested (Decker-Walters et al. 2002). The substructure of '*melo'* group in four clusters was less resolute, likely due to important gene flow from modern breeding programs.

The seven genetic groups did not exactly coincide with the botanical groups based on horticultural traits. Distinct botanical groups clustered in the same genetic subgroup and could not be differentiated: *reticulatus* and *cantalupensis*; *chinensis, conomon* and *makuwa*; *acidulus*, *momordica* and *chito*; *adana*, *ameri*, *chate* and *chandalak*. Inversely, *flexuosus, ameri, dudaim* and *cantalupensis* were included in several genetic subgroups. *Flexuosus* accessions (or snakemelons) from Sudan and Saudi Arabia, together with elongated melons of *chate* and *adana*, clustered in the genetic group C, whereas *flexuosus* from India and North Africa clustered in the intermediate genetic group D. This suggests, as reported recently for long-fruited

cultivars in *C. pepo* subsp. *pepo* (Gong et al. 2012), that independent selection events for long fruits, occurred in *C. melo* subsp. *melo*. A great diversity among *flexuosus* accessions was previously reported (Yildiz et al. 2011; Soltani et al. 2010). Most *cantalupensis* were assigned to the subgroup B of '*melo*'. Brazilian *cantalupensis* clustered in the subgroup A with most *inodorus* melons. The six ornamental and aromatic *dudaim* accessions included in this study fall in three distinct genetic subgroups (D, E and G) from both '*melo*' and '*agrestis*' groups. Larger sampling would be required to understand the origin and spreading of the *dudaim* botanical group.

Melon may have been domesticated twice, in Asia and in Africa

The occurrence of a single domestication event or several independent domestication events can be settled by comparing the phylogenetic relationships between a representative sample of cultivated species and their wild forms or relatives. We showed that wild melons did not constitute a group distinct from the cultivated melons. Wild African melons clustered with the African subgroup of *C. melo* subsp. *agrestis*, while wild Asian melons clustered within the Indian subgroup of subsp. *agrestis*, suggesting two independent domestication events, in Africa and in Asia, from the *agrestis* subsp. wild gene pool. Two domestication events were also reported for *Cucurbita pepo*, in Mexico 10,000 years ago and in Eastern North Africa 5,000 years ago (Sanjur et al. 2002).

Our results are consistent with the fact that all the *melo* subsp. cultivated diversity may derive from the domestication of melons in Asia, by human selection and spreading. In contrast, domestication of melons in Africa may have had a marginal impact, only contributing to the *tibish* and *seinat* cultivated group, essentially grown in East Africa. However, the diversity of *tibish* and *seinat* is large (He=0.49) and may be especially relevant for the study of horticultural traits related to the domestication process and for pest and disease resistances.

Identifying what were the first cultivated melon types in Asia represents a hard challenge. The large genetic diversity observed in Indian melon germplasm and the occurrence of wild melons in the genetic Indian subgroup (E) suggest that India could be a domestication centre for melon. Traces of cultivation of melons in India are attested from 2,000 BC (Bates and Robinson 1995) but further archaeological data would be required. Far East subgroup, including *conomon*, *makuwa* and *chinensis* melons have a large phenotypic diversity but a low genetic diversity and a large differentiation with all the other subgroups. This suggests Far-eastern melons would have experienced a severe bottleneck and subsequent genetic drift. They would originate from the same Indian gene pool, probably small-seed Indian melons as this trait was fixed in all Far-eastern melons. In parallel to the spread of melon cultivation towards Far-East, Indian melons would have been introduced towards the West, leading to the development of *melo* subsp.

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Table 1. Number and geographical origin of the studied accessions.

Type	Africa	America	Central Asia	Europe	Far East	India-Pakistan	Middle East	Un-known	Total
feral and cultivated	88	70	46	171	108	65	75	12	635
wild melon	36		3			17	5	5	66
other *Cucumis sp.*								12	12
Total	124	70	49	171	108	82	80	29	713

Table 2. Table of contingency between botanical groups to which the accessions belong and genetic group assignment to one of the two groups determined by Structure.

Botanical groups	I *'melo'*	II *'agrestis'*	total
acidulus	1	9	10
adana	2		2
ameri	37		37
cantalupensis	76		76
chandalak	16		16
chate	4		4
chinensis		7	7
chito		8	8
conomon		9	9
dudaim	3	3	6
flexuosus	54	3	57
inodorus	100		100
makuwa	6	44	50
momordica	7	10	17
reticulatus	59	1	60
tibish and s*einat*	1	16	17
unknown	128	22	150
wild melon	8	58	66
total	502	190	692

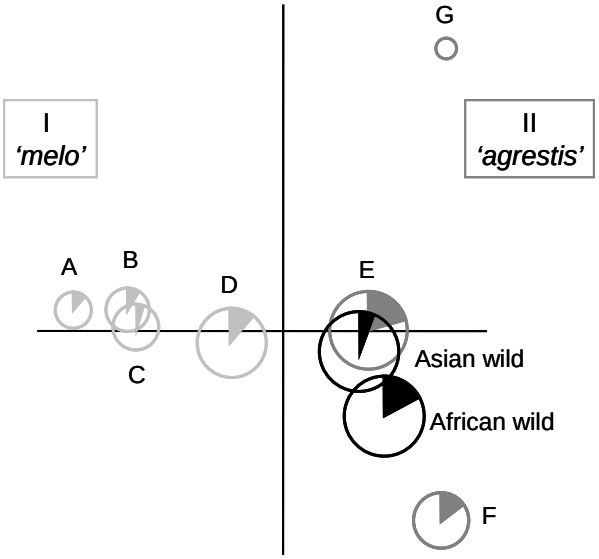

Fig. 1. Principal coordinates analysis (PCoA) for seven cultivated melon groups and two wild melon groups based on pairwise *Fst* generated from microsatellite data. The circle diameter indicates genetic diversity (He). Filled arcs reflect the proportion of private alleles in each group. Cultivated groups are differentiated by K2 color code from Structure: light grey for group I and dark grey for group II. Wild melon groups are black.

African watermelons and their uses

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Abstract

Watermelon, *Citrullus lanatus*, originates from Africa, presumably the Kalahari Desert. The domestication process has lead to the development of various types of watermelon landraces with specific traits suitable for different uses. The common dessert type watermelon with sweet, red fleshed fruits exist in the form of landraces in Africa. However, cooking and seed type landraces with white to yellow fleshed fruits also exist in local communities. Production is usually based on farm-saved seed. The cooking type watermelons are used as a multipurpose vegetable in many African countries, and are especially cultivated in resource poor, remote areas as a food security crop. Fruits can be stored up to a year and cooked in periods with food scarcity. Seed types are used to make snacks, flour and oil in local communities, especially in West Africa. Some of the seed type landraces, selected through generations by farmers under desert conditions, have been estimated to produce a seed yield of more than 350 kg/ha in farmers' fields in Mali. These white fleshed seed types have been shown to be genetically distinct from the red fleshed dessert types. Locally adapted landraces of African watermelons have immediate potential to enhance food supply and nutrition in arid, heat- and drought-prone areas. Where intercropping is practised, watermelon may also be a good companion crop to mitigate the risk of complete crop failure under unpredictable growing conditions. Landraces of watermelons also have a potential for development of improved, added-value speciality products to generate income. In addition, African genetic resources of watermelon and other *Citrullus* species, possess traits of interest for future breeding programmes for Africa and beyond.

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Watermelon originates from Africa

Watermelon, *Citrullus lanatus* is believed to originate from Africa, presumably the Kalahari Desert (Robinson and Decker-Walters 1997; van der Vossen et al. 2004). The species is currently divided in two botanical varieties. *C. lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai includes the well known, widely distributed dessert type watermelons, and seed type watermelons cultivated in West Africa. *C. lanatus var. citroides* (L. H. Bailey) comprises the citron and 'tsamma' types, which are used for cooking and seed processing, respectively, or as animal fodder, common in Eastern and Southern Africa (Jarret et al. 1997; Robinson and Decker-Walters 1997; GRIN 2012). The division in the two botanical varieties is supported by chloroplast genome analyses of *Citrullus* species, which identified distinct haplotypes within *C. lanatus* associated with var. *lanatus* and var. *citroides*, respectively (Dane et al. 2004), and by chloroplast and and mitochondrial DNA analysis (Levi and Thomas 2005). Phylogenetic analyses with AFLP and SSR markers also support this classification in the two botanical varieties (Nimmakayala et al. 2010). The two taxa possibly evolved independently from *Citrullus ecirrhosus*, a perennial species from Namibia, which may have derived from *Citrullus colocynthis*, also named bitter apple (Dane and Liu 2007). *C. ecirrhosus* and the fourth *Citrullus* species, *Citrullus rehmii*, are wild species endemic to the desert regions of Namibia, whereas *C. colocynthis* is known from Northern Africa (Robinson and Decker-Walters 1997). Distribution and diversity of wild taxa of *Citrullus* species in South West Africa points towards the possibility that domestication was initiated there (Dane et al. 2004), but archaeological findings of 5000 year old *Citrullus* seeds in Libya also propose that domestication may have happened in Northen Africa (Wasylikowa and van der Veen 2004). The present geographic distribution pattern of *Citrullus* in Africa may reflect a pattern of survival, more than the actual origin and ancient distribution (Dane et al. 2004; Wasylikowa and van der Veen 2004). From Africa, *C. lanatus* colonised the rest of the world via the Middle East, China and Russia, reaching America in the sixteenth century (Robinson and Decker-Walters 1997).

Watermelon production in the world and in Africa

During the last 50 years, the world production area of watermelon has increased with 62%, from 1.96 million ha in 1961 to 3.16million ha in 2010 (FAOSTAT 2012). The yield tripled during the same period, resulting in a fivefold total production increase (from 17.8 to 89.0 million tonnes). The top 10 world watermelon producers include China, accounting for 63 % of the production in 2010, followed by Turkey,

Iran, Brazil, USA, Egypt, Uzbekistan, Russia, Mexico and Algeria.

Africa accounted for 7.6% of the watermelon acreage in 2010. However, the acreage has nearly quadrupled during the last 50 years, from 64.822 ha in 1961 to 241.458 ha in 2010 (FAOSTAT 2012). The yield increase was 14% during the period, so in total the production has quadrupled on the continent during the last 50 years. The top 10 producers in 2010 were Egypt, Algeria, Tunisia, Morocco, Mali, Libya, Senegal, South Africa, Sudan and Kenya (FAOSTAT 2012). The production reported typically includes the cash crop dessert type watermelons, which are popular as a refreshing dessert and thirst quencher. However, statistics do not differentiate among types, and may therefore also to some extent include types used for seed extraction and cooking. However, most non-dessert types of watermelon are produced by farmers as subsistence crops, and are most likely not captured in production statistics.

Watermelon landrace types

The domestication process has lead to the development of watermelon landraces in Africa, and these still persist, are cultivated and used for various purposes in local communities (Achigan-Dako et al. 2008; Jensen et al. 2011; Maggs-Kölling et al. 2000; Munisse et al. 2011; Mujaju and Nybom 2011; Nantoumé et al. 2012). Such landraces have specific, local names (Munisse et al. 2011; Nantoumé et al. 2012), and they are often cultivated in areas with wild *Citrullus* forms that may intercross (Wehner 2008) and lead to deterioration of the landraces. However, continuous farmer selection seems to counteract the gene flow and maintain landraces. The African watermelon landraces can typically be distinguished in three major use types: dessert; seed, and cooking types (Fig. 1), but some landraces may serve several uses. Landrace names may be derived from the locality of origin, and may also reflect specific phenotypic characteristics, such as for instance seed characteristics for the seed type landraces.

Dessert type landraces and improved cultivars

Dessert type landraces usually have sweet flesh with a red tinge or deeper red colour, but they may also have white flesh. Their most common use is as a refreshing dessert and thirst quencher, either consumed freshly cut, or as a juice. In local communities it is common to sell watermelon by slice as a dessert snack. Mali, with the largest cash crop production in Sub-Saharan Africa, reported 23.847ha in 2010 and a production of 430.995 tonnes (FAOSTAT 2012). Here, cash crop

production is common in areas with fairly good infrastructure and market access to consumers in cities, and production is based on a combination of local landraces and introduced, improved cultivars (Nantoumé et al. 2012). However, dessert types are also cultivated in more remote areas by subsistence farmers for family consumption and local sale. Landraces, not influenced by imported, improved varieties are more common in such remote areas.

Seed type landraces

Seed type watermelons commonly have pale to white flesh, and have been selected by farmers to be particularly rich in seed. Seeds may have characteristics similar to those in the dessert types, or have seeds with a pale to yellow, soft seed hull, which sometimes are surrounded by a fleshy pericarp when formed in the fruits. These are the so called 'egusi' seed types, which are common in West African countries (Achigan-Dako et al. 2008; Nantoumé et al. 2012). Egusi is a generic term, used in the Yoruba language spoken in West Africa, also for other cucurbit species grown for their seeds. In Benin, egusi crops rank among the 10 most important crops, and watermelon is the most important egusi species (Achigan-Dako et al. 2008). Seed type watermelons are also reported from Southern Africa, for instance Namibia (Maggs-Kölling and Christiansen 2003), and Mozambique (Munisse et al. 2011) and these typically have seeds with a harder seed hull. Seed type watermelons in West Africa botanically belong to *C. lanatus* var. *lanatus*, whereas the seed types reported in Southern Africa typically belong to *C. lanatus* var. *citroides.*

Seed extraction is mainly a task carried out by women. The seeds, rich in protein and oil, are commonly extracted manually, when the fruits are well mature. In Mali, this may be done by crushing the fruit on a stone to release the seeds, followed by seed drying in the sun. Seeds, which are covered by a fleshy pericarp, may be left to ferment some time during the seed extraction process to release the seeds (Jensen et al. 2011). In Benin, it is common practice to leave the fruits to rot, before the seeds are extracted, washed in a river, and dried in the sun (Achigan-Dako et al. 2008). In Mali, the seeds may be pounded into flour and used as the main ingredient to cook and thicken sauces, or to prepare various energy rich snack products. In some areas, specific landrace seed types are used to produce specific snacks, for instance by a simple roasting of the seed, or by a more sophisticated process involving cooking of the seed with ash to loosen the seed coat, followed by drying and finally roasting to 'pop' off the seed coat and release the seed kernels (Jensen et al. 2011). The seeds may also be used to produce oil by pressing the seeds

or by boiling the flour.

Seed kernels of *C. lanatus* contain around 48-66% oil and 27-32% protein (Loukou et al. 2007). A high content of unsaturated fatty acids makes the oil a good source of edible oil for human consumption (El-Adawy and Taha 2001). Also, kernel based flour contain a considerable amount of minerals and the protein content and functional properties makes it a good protein source and a good functional agent, for instance for baking (El-Adawy and Taha 2001). In some areas, the seeds and the seed derived products provide a good nutrient supplement to starch-rich, cereal based diets (maize, sorghum and millet). The value of the seed is recognised since it is used as a precious gift to relatives and neighbours. Both flesh and seed may also play a medicinal role (Achigan-Dako et al. 2008; Munisse et al. 2011). Seed type watermelons are also an important resource for income generation in some communities. Women are typically in charge of processing and sale of flour and snack derived products, which have a relatively good shelf life. However, there is still an untapped potential for improved, value-added product development and income generation (Jensen et al. 2011).

Cooking type landraces

Cooking type landraces typically have cream to yellow, rather firm fruit flesh. These watermelons are used as a multipurpose vegetable for cooking in many African countries, and both leaves and fruits are used. They play a special and important role in resource poor, remote areas as a food security crop, for instance in some regions in Mozambique (Munisse et al. 2011) and Tanzania. When the watermelon is cooked, it may typically be consumed as a separate vegetable dish, or mixed with other vegetables or cereals. Sometimes the cooked items are mashed and served as porridge. *C. lanatus* var. *citroides* types in Zimbabwe are used for cooking and fodder in some villages, whereas they are only used for fodder in other areas (Mujaju and Nybom 2011). The use probably relates to the characteristics of the material, and whether it is a landrace or a wild type. Bitterness, for instance, make the fruits less desirable for cooking. The use for fodder for animals is common, and includes flesh of the seed types, when the seeds have been harvested. An intriguing characteristic is that cooking type watermelons have a long shelf life. Local cooking type landraces may store in the shade outside the house for up to 12 months (Maggs-Kölling and Christiansen 2003). In this way, it is a food source that can be used when other staple food sources are scarce or unavailable.

Genetic differentiation among botanical varieties and landrace types

Morphological descriptors (36 traits) have been used to characterise cooking, seed and dessert type landraces and cultivars, and wild *Citrullus lanatus* accessions in Namibia. Commercial dessert types clustered with landrace dessert types, while a seed type and the cooking types clustered together with wild types (Maggs-Kölling et al. 2000), supporting the division in the two botanical varieties *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* The division in the two varieties was also found when 22 accessions of South-African landraces were analysed with SSR markers, and the study showed comparable levels of diversity within *C. lanatus* var. *lanatus* and var. *citroides* (Mujaju et al. 2011).

SSR based STRUCTURE analyses of *C. lanatus* var. *lanatus* accessions from Mali revealed that the accessions could be separated in two genetic groups, related to the flesh colour (red and white) of the watermelon fruits (Nantoumé et al. *submitted*). Additional STRUCTURE analyses of the material proposed eight genetic groups. One group included again the red fleshed (dessert) types with local and foreign origin, while the remaining seven genetic structure groups comprised the white fleshed landrace types used for seed processing and cooking, and one dessert type. Some of these seed and cooking types, presumably all *C. lanatus* var. *lanatus*, were to a large extent different and assigned to one group each. Geographical isolation, seed exchange and in particular farmer driven selection seem to have shaped the white fleshed landrace types (Nantoumé et al. *submitted*).

Seed sources

Farmers mainly use their own farm-saved seed for sowing. In Benin, around 80% of the farmers use seed of their own landraces for the following season. The remaining farmers either buy seed on local markets or receive them as gifts (Achigan-Dako et al. 2008). In Mali, Mozambique and Zimbabwe, more than 85 % of the farmers use their own farm-saved seed for the next growing season (Mujaju and Nybom 2011; Munisse et al. 2011; Nantomé et al. 2012). Witchcraft is believed to be associated with seed exchange and will result in no crop yield, for which reason seed exchange is not practised in some communities (Mujaju and Nybom 2011).

Cropping systems

African watermelon cultivation is prevailing in drought-prone, semiarid to arid areas. In very dry areas, it may be the only crop, which will grow and produce

a yield (Jensen et al. 2011; Nantoumé et al. 2011). Watermelon crops are mainly rainfed with sowing around the beginning of a rainy season (Mujaju and Nybom 2011) or sown along river bed following retraction of the water (Nantoumé et al. 2011). Cultivation is dominated by low or no-input systems, except for the cash crop production of red fleshed, dessert types. For cash crops, the production mainly rely on fertiliser in the form of organic manure, and to a limited extent on chemical fertilisers, with little or no pesticide use, such as for instance in Mozambique and Mali (Munisse et al. 2011; Nantoumé et al. 2012). Often, both dessert and seed types (Nantoumé et al. 2012) or dessert and cooking types (Mujaju and Fatih 2011) are cultivated in neighbouring fields. Fields of different landraces are often separated by rows of millet or maize, but the distance between landraces differ depending on the farmer (Mujaju and Nybom 2011).

Smallholder farmers frequently use intercropping as a strategy to diversify food production and as an insurance against complete crop failure, in particular in semiarid and arid areas with unpredictable precipitation. In Zimbabwe, intercropping is practised in maize, sorghum and millet crops with watermelon as the companion crop (Mujaju and Nybom 2011). In Mozambique, intercropping trials, carried out in farmers' fields, revealed that dessert watermelon as the companion crop in maize suffered a yield reduction (58%) in comparison to cultivation as a sole crop, whereas maize suffered less yield reduction (29%). However, watermelon also responded well to better conditions, and it still produced even under dry conditions where maize and sorghum failed to develop a harvestable crop (Munisse et al. 2012). Yield depressing effects of maize and cassava have also been reported from on-station trials with egusi watermelon in Nigeria, and the effect was especially pronounced when cassava developed a high leaf area index (Ikeorgu 1991). Thus, watermelon is especially a good companion crop for intercropping with cereals in drought-prone areas to avoid complete crop failures.

Egusi watermelon crops are grown in monoculture in home gardens in Benin, whereas they are often intercropped with maize, yam, cassava, sorghum or cotton in the field (Achigan-Dako et al. 2008). In some fields, egusi is grown in a crop rotation scheme, because they are known to control weeds and to improve the soil (Achigan-Dako et al. 2008). However, under more extreme, drought-prone conditions on the verge of the desert, African watermelon landraces are grown as a sole crop, since no other crops can thrive and produce a harvestable product (Nantoumé et al. 2012).

Yield potential and other traits of interest

In Namibia, cooking watermelons have been shown to out-yield seed and dessert type watermelons in terms of an average fruit weight of more than 6 kg and a yield up to 119 t/ha in on-station trials (Maggs-Kölling and Christiansen 2003). Landraces of dessert types sometimes produce smaller, but more fruits than commercial cultivars (Maggs-Kölling and Christiansen 2003). Three local seed type landraces, selected through generations by farmers under desert conditions, were estimated to produce a seed yield of 364 kg/ha under farmers' field conditions in Mali, where temperatures often exceeded 35°C. This proves that locally adapted plant genetic resources of African watermelons have potential to enhance food supply in arid, heat- and drought-prone areas (Nantoumé et al. 2011).

Dessert types (Munisse et al. 2012) and seed types (Nantoumé et al. 2011) produce fruits in arid areas under high temperature conditions, even where and when no other crops can grow. Production under such conditions demonstrates that watermelons have a good tolerance to heat and drought and special abilities to acquire water from the soil. Screening of USDA watermelon accessions for drought tolerance at the seedling stage under controlled conditions revealed that the most drought tolerant *C. lanatus* accessions originated from Africa (Zhang et al. 2011). The necessity to use and ensure conservation of *Citrullus* landraces and wild relatives is highly relevant in the light of climate change, since good heat and drought tolerance is a trait of crucial importance for watermelon production, as well as food production in general.

African genetic resources of watermelon also have potential to secure new sources of disease and insect pest resistance for future breeding programmes. Resistance to Gummy Stem Blight caused by *Didymella bryoniae* (Gusmini et al. 2005) and resistance to Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *niveum* race 2 (Wechter et al. 2012) have been identified in *C. lanatus* var. *citroides* accessions with African origin. Resistance to whitefly (*Bemisia tabaci*) identified in *C. colocynthis* (Simmons and Levi 2002) further support that genetic resources, also of the other *Citrullus* species, will be important to solve future production constraints in watermelon production.

CONCLUSION

Local African landraces of dessert, seed and cooking type watermelons exist and are used in Africa. They have an immediate potential to enhance food security, nutrition and income generation if further attention to the crop and its uses is

provided to local communities. Also, African genetic resources of watermelon and *Citrullus* relatives exhibit heat and drought tolerance, and make an important gene pool to search for disease and pest resistance. These traits are of interest for future breeding programmes for Africa and beyond, especially seen in the light of climate change.

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Fig 1. Watermelon use types: Row a: Dessert type: Cash crop field in Tanzania, fruits sold by 'slice' on a local market in Mali, and seeds. Row b: Seed type: Farmers' field in the desert in Mali, a seed type with a fleshy pericarp surrounding the seeds, and seeds. Row c: Cooking type: Intercropped with sorghum in Tanzania, fruit being cut, and seeds.

Introgression of genes conferring the bush habit of growth and variation in fruit rind color into white nest egg gourd

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Abstract

The 'Nest Egg Gourd' [*Cucurbita pepo* L. ssp. *ovifera* (L.) D.S. Decker] is a small (40 to 100 g), hard-rind, white, ornamental gourd, about the size and shape of a chicken egg. In 1996, I embarked on a breeding project to develop egg gourd cultigens with an array of fruit color patterns, earlier maturity, and a bush growth habit. The source of the bush gene was a small-fruited (200 to 300 g) ornamental pumpkin breeding line, NH23-12-4. As a result of selections made in the F_2 generation together with one to two subsequent backcross generations, plants with a bush growth habit and the desired egg phenotype were obtained in 1999. Genes and alleles for color and pattern variation (*l-1^{BSt}*, *L-2, Wf, wf*, and *B*) were introgressed into egg genotypes from a striped, bicolor spoon gourd through a series of complex crosses. By 2002, the following bush inbred egg gourd lines were developed with early fruit maturity, a concentrated fruit set, and an array of fruit colors and patterns: G344-22, dark green (*L-1/ L-1, L-2/ L-2, b/ b, wf/wf*); G344-13, dark orange (*L-1/L-1, L-2/ L-2, B/B, wf/wf*); G424-25-3, narrow blue-gray/wide green stripes (*l-1st/ l-1st L-2/L-2 b/b Wf/Wf*); and G424-1-10, narrow white/ wide–gray-white stripe (*l-1st/ l-1-st, l-2/ l-2, b/b, Wf/Wf*). Inbreds were then developed with yellow and orange stripes (*l-1st/l-1st, L-2/L-2, B/B, w/w*) and white and yellow stripes (*l-1st/l-1st, L-2/L-2, B/B, W/W*), and expressing bicolor rind coloration. The pumpkin cultivar, Lil-Pump-Ke-Mon, was used as a source of the reverse stripe (RS) trait for introgression into egg germplasm. Selections in an F_2 and one BC generation produced attractive egg inbreds with narrow green stripes and wide white RSs. Subsequent crosses provided genetic data on the interaction of *L-1, l-1^{BSt}, L-2* and *B* alleles in producing different phenotypes with reverse striping. Data support the hypothesis that the dominant *D* allele or possibly a dominant gene linked to *D* can act in a complementary fashion with *l-1^{BSt}* and either *l-2/l-2* or *L-2/l-2* alleles to produce reverse striping.

INTRODUCTION

Gourds are fruits, most common to the family Cucurbitaceae, with a hard or lignified rind or shell, used mostly for decorative purposes, but also as utensils,

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musical instruments and occasionally for food (Bailey 1937). This paper describes my breeding work with nest egg gourd [*Cucurbita pepo* L.ssp.ovifera (L.) D.S. Decker], a small (40 to 100 g), hard-shelled, white gourd about the size and shape of a chicken egg (Fig. 1). The egg gourd is smaller than its putative wild progenitor, *C. pepo* spp. *ovifera*, var. *texana* (Scheele) Decker (Decker 1988), and also lacks the extreme bitter principal associated with the wild species. The egg gourd, although attractive and amenable to decorative painting, has late maturity for northern temperate latitudes and has a large sprawling, vining habit that is relatively unproductive. I embarked on a project to breed egg gourd with two major objectives in mind: (1) introgress the '*Bu*' gene for compact growth habit into egg gourd, and concomitantly select for earlier maturity and more concentrated fruit set; and (2) develop egg gourds with a wide array of rind colors and patterns.

The bush ideotype

The bush phenotype in most cultigens of *C. pepo* is conferred by a single gene (*Bu*) which displays dominance or near dominance for shortening of internodes during early plant growth, but incomplete dominance in later stages of growth (Shifriss 1947). In addition to shorter internodes, bush plants are characterized by thicker stems, longer petioles, reduced tendril development, and often, earlier flowering (Loy 2004). Most of the modern hybrid cultivars of acorn squash and ornamental pumpkin (*C. pepo*) are heterozygous or homozygous for the *Bu* gene because of the amenability of such plants to higher density planting and cultivation.

In addition to the bush phenotype displaying short internodes and long petioles, a desirable trait affecting growth habit is multiple branching as contrasted to plants with a dominant main stem. Multiple branching promotes rapid and uniform leaf canopy development, and provides more nodes for formation of flower buds. In small-fruited cultigens such as egg gourd, high productivity depends upon high numbers of fruit per unit ground area, and thus, high numbers of staminate and pistillate flowers. Fresh weight fruit yields of 4.5 kg/m^2 are readily obtainable in large-fruited strains of ornamental pumpkin with relatively low flesh dry matter; therefore, in developing egg cultigens with average fruit size of 50 to 60 g, a goal of breeding cultigens capable of producing 50 to 100 fruits/m^2 at high planting densities appeared feasible.

Fruit pigmentation genes in *C. pepo*

There are several reported genetic traits for rind color and rind color patterns of fruit, and expression of such traits is complex because color and pattern changes can occur at different developmental stages of fruit (Shifriss 1949). Only those traits employed in the egg gourd breeding program will be summarized here. The complementary *L-1* and *L-2* alleles confer dark green rind color to fruits; whereas the recessive counterparts, *l-1* and *l-2* confer light green coloration (Paris and Nerson 1986). Also, in the presence of duplicate recessive genes, *mo-1* and *mo-2*, rind color changes from green to intense orange between 25 to 40 days after fruit set as a result of chlorophyll loss (Paris 1997). There are additional alleles at the *L-1* and *L-2* loci, but I will only mention two which are pertinent to the egg gourd breeding

project. In the presence of *L-2*, the allele *l-1*^{BSt}, recessive to *L-1* and dominant to *l-1*, confers striped fruit characterized by light blue-gray, fairly broad stripes over the ten primary vein tracts, and wider dark green strips in between (Paris 2000). However, the nature of the striping pattern elicited by *l-1*^{BSt} can be reversed in the presence of the allele *l-2*^{R}, such that dark green striping occurs over the vein tracts, and broad white coloration or white and mottled green coloration occurs between vein tracts (Paris 2009). *L-2* is incompletely dominant to *l-2*^{R}.

Another gene, *D*, confers dark green, nearly black pigmentation to stems and peduncles and can also affect fruit color (Paris and Nerson 1986), but is not expressed until 15 to 20 days after pollination. The *D* gene intensifies the green or orange coloration of fruit, and is epistatic to *l-1* and *l-2* alleles. The '*B*' gene (Shifriss 1981) confers precocious yellow coloration of fruits and sometimes peduncles at the time ovaries are differentiated prior to anthesis, but complementary interaction with the *L-2* allele is required for full expression (Paris 1988). As described in spoon gourds, the degree of expression of the *B* gene over the fruit surface, from completely yellow to yellow and green coloration (termed bicolor) is governed by at least two incompletely dominant modifying genes designated *Ep-1* and *Ep-2* (Shifriss and Paris 1981).

MATERIALS AND METHODS

Plant culture

During the summer months, June through October, gourds were grown either at the Woodman Horticulture Research Farm in Durham (NH,US) or at the Kingman Research Farm in Madbury (NH). Plants were grown on raised beds mulched with black polyethylene, and supplied with drip irrigation. Plants were either direct seeded or grown in 50-cell plug trays and then transplanted. Standard fertility and pesticide practices were used according to New England Vegetable Management Guides (published every year). In the greenhouse, during the months of January through May, plants were grown in 8.7 L plastic nursery pots in a soil-less mix (Pro-mix, Griffin Greenhouse Supply, Tewksbury, MA, US). Daytime temperatures were 24^{o}C (photoperiod 16 h) and nighttime temperatures were 18^{o}C. Phenotypes of gourds segregating for fruit color and pattern traits were evaluated at anthesis or shortly thereafter, at 18 to 25 days after pollination (DAP) and at maturity 45 to 55 DAP.

Plant material and pollinations

Self-pollinated seed of the original 'Nest Egg' gourd and bicolor spoon gourd was obtained from Hollar Seeds, Rocky Ford, Colorado (US). Seed of the cv. Lil-Pump-Ke-Mon, used for introducing the reverse strip phenotype, was obtained from Harris Seeds, Rochester, NY (US). All other germplasm used in the study was developed at the University of New Hampshire Experimental Research Farms. For pollination, staminate and pistillate flowers were closed the day before anthesis, using 10 cm 'Twist-ems' ties (Griffin Greenhouse Supply), and pollinations were performed before 9:00 h the following day. Immediately after pollination, pistillate flowers were re-closed with Twist-ems, tagged with the date, and if necessary,

covered with a poly mesh bag. Fruits were harvested 50 to 60 DAP, and the seeds were subsequently removed, cleaned, and dried at 30°C in a forced air dryer.

RESULTS AND DISCUSSION

Introgression of the bush growth habit into egg gourd

For transferring the *Bu* gene into an egg gourd background, I utilized NH123-12-4, a bush, orange-fruited pumpkin F_5 breeding line with multiple branching habit and fruit weighing 250 to 350 g. An F_2 population (Egg gourd x NH123-12-4)⊗ of 60 to 70 plants was grown at the UNH Horticulture Research Farm in Durham, NH (US) in 1996. Three white- and several yellow-fruited bush selections were made with mean fruit sizes ranging between 150 to 250 g. Fruit shape was oval to round to oblate, but none had a typical oval/ovate egg shape. The best white selection (G4-40), having a prolific fruiting habit and oblate fruit, was backcrossed to egg gourd. Two bush selections (G4-8 and G4-41) were crossed to spoon gourd, both for introducing the bush phenotype into spoon gourds, and for transferring the '*B*' allele for precocious yellow pigmentation from spoon into egg gourd. These crosses were self fertilized in the greenhouse in spring, 1997. A large (89 g) white egg selection (Egg x 4-40)BC_1⊗-2 ⊗ was obtained from the BC_1 population in the field, and a small dark orange egg carrying the '*B*' gene was obtained from one of the crosses to spoon gourd (Spoon x G4-8)⊗-2⊗. In addition, moderately small egg selections with light yellow and light orange pigmentation were made from F_2 populations generated in 1996. The white egg selection was backcrossed again to egg gourd.

Introgressing fruit pigmentation genes into bush egg gourd

The best egg gourd selections made in 1998 and 1999 were used to generate three complex crosses which were selfed in spring of 2000 to produce three populations (140 plants each) during the summer of 2000 for combining the multilateral bush phenotype with different color and striping patterns, conferred by different combinations of the *L-1, l-1^{bst}, L-2, l-2, B* and *b* alleles. In addition, white and colored flesh, conferred by the *Wf* and *wf* alleles (Paris 1995) appeared to be segregating in the above populations. Numerous selections were made of prolific egg gourd plants having a wide array of phenotypes: fruit with white/green normal stripes; solid orange/green bicolor; narrow white, broad yellow normal stripes; narrow yellow, broad orange normal stripes; and solid white. Two major breeding lineages were developed from selections from the above F_2 populations: (1) G4-2-4 selection, blue-gray/green broad normal stripe (BNS), segregating for narrow blue-gray/wide green striped fruit (*l-1^{BSt}/l-l^{BSt}, L-2/_Wf/_*); narrow yellow and wide dark green stripes (*l-1^{bst}/l-1^{bst}, L-2/_, wf/wf*); narrow white/wide gray-white striped fruit (*l-1^{bst}/l-1^{bst}, l-2/l-2,Wf/_*); and narrow light yellow/wide light yellow stripes (*l-1^{BSt}/ l-1^{BSt}, l-2/l-2, wf/wf*). Stripes on the latter two phenotypes have almost identical pigmentation and are barely discernable as ghost stripes, recognized by the raised ridges over the vein tracts associated with the *l-1^{BSt}/l-1^{BSt}* alleles; and (2) G3-3-4, homozygous for *L-1* and *L-2* alleles, but heterozygous for *B/b* and *Wf/wf*. The *B* gene in combination with Wf (white flesh) produces yellow or yellow bicolor

fruits, and in the presence of *wfwf, B_* produces orange or orange bicolor fruit. Two homozygous phenotypes were obtained from this line in 2001, plants having fruit with complete dark orange pigmentation (*B/B*) except for a dot of green at the blossom end of the fruit (G334-13 selection), and plants with solid dark green fruit (G334-22 selection). Hybrids produced from these two lines produced bicolor fruits with 1/3rd green at blossom end and 2/3^{rds} orange pigmentation (*L-1/L-1, L-2/L-2, B/_,wf/wf*) at peduncle end. The four striped phenotypes and two solid color phenotypes were selfed to homozygosity, and F_6 or F_7 seed was bulked for possible release to seed companies. The F_1 plants produced from crossing G424-25-3-11 to G344-13-3 produced green/yellow (*L-1/l-1^{BSt}, L-2/L-2, Wf/wf*) bicolor progeny exhibiting a similar bicolor pattern to crosses of G344-13-3 x G344-22-1, confirming dominance of the *L-1* allele to *l-1^{BSt}* and *Wf* to *wf*. An array of colors produced from a test cross of (G344-13-3 x G424-25-3-11) x G424-25-3-11, segregating for *B/b, L-1/l-1^{bst}*, and *Wf/wf* alleles is shown in Fig. 2A. Prolific fruit set on a bush gourd plant is illustrated in Fig. 2B.

Introgressing the reverse stripe trait into bush egg gourds

In summer, 2004, an ornamental pumpkin with reverse strips, 'Lil-Pump-Ke-Mon' was crossed to an inbred G424-16-2-4 (*l-1^{BSt}/l-1^{BSt}, l-2/l-2, Wf/Wf*), and the F_1 was selfed in the greenhouse in spring, 2005. An F_2 population of 270 plants was grown during the summer of 2005, and several reverse strip (RS) selections were made. As Paris (2009) noted, the reverse stripe is not normally expressed until about 11 to 15 days after pollination, but remains stable to gourd maturity. Selection #4, a 41g, oblate gourd showing precocious narrow yellow pigmentation (*B/_*) and wide white reverse striping similar to 'Lil-Pump-Ke-Mon' was backcrossed to G424-25-3-11 (blue-gray/green BNS). The BC_1 was selfed in spring of 2006, and two populations of 145 plants each were grown out in summer, 2006. Although several selections were made in 2005 and 2006 from the F_2 and BC populations, one BC_1 selection in particular, G2B-1, was especially productive and displayed prominent narrow, dark green stripes and broad white stripes (termed Type 1 RS). This line subsequently segregated approximately 3:1 for a uniform RS phenotype and the white/gray-white BNS phenotype (*l-1^{BSt}/l-1^{BSt}, l-2/l-2*). A white/gray-white BNS segregant (G12131-2W) from an F_6 population was selfed and bred true (Fig. 3C). Reverse stripe segregants in subsequent populations derived from a G121-9 selection were selfed for two more generations in the field and greenhouse to produce a line (G12194-3 F_7) homozygous for RS and near isogenic to G12131-2W (Fig. 3A, B). A summary of the principle gourd inbred lines developed between 2000 and 2011 is given in Table 1.

The model for reverse stripe elucidated by Paris (2009) suggested that the RS line, G1219-4, segregating for RS and white/gray-white BNS was likely heterozygous for the *l-2^R* allele conferring reverse stripe in the presence of the *l-1^{BSt}* allele. Fruits on plants heterozygous for L-2/*l-2^R* often exhibit only partially reverse striping (Paris 2009). However, when G121943-4 was crossed to either the green-fruited line G344-22-1 (*L-1/L-1, L-2/L-2*) or G424-25-3 with normal blue-gray/green stripes (*L-1^{BSt}/l-1^{BSt}, L-2/L-2*), all F_1 progeny of both crosses displayed complete

reverse striping (Fig. 3A, B). However, reverse striping in F_1 plants differed from Type 1 RS fruit from plants of G121943-4 In the case of G12194-3 x G424-25-3-11, fruit of F_1 plants had moderately wide stripes over vein tracts, similar to G12194-3, with broad, mottled white and green wide stripes between vein tracts (Type 2 RS, Fig. 3B). Fruits of F_1 plants of G12194-3 x G344-22 had very narrow green stripes over the vein tracts and mottled green and white wide stripes (Type 3 RS phenotype; Fig. 3A). Of more interest, when G12131-2W, near isogenic to 121943-4, but with white/gray-white BNS (*l-1*^{BSt}/*l-1*^{BSt}, *l-1/l-2*), was crossed to G344-22-1, the F_1 fruit (Fig. 3C) had the same Type 3 RS phenotype as those from G12194-3 x G344-22-1 (Fig. 3A). When the same white BNS line was crossed to G424-25-3-11, F_1 progeny displayed a BNS phenotype, but the stripes observed at 15 to 20 DAP were white/light green instead of light blue-gray/dark green as in G424-25-3-11. Fruits of F_1 plants of G424-25-3-11 x G344-22-1 displayed dark green fruit, and in a small F_2 population derived from that cross and grown out in 2007, plants bearing only dark green and BNS fruits were observed. Lastly, in two small testcross populations of RS F_1 plants (G424-25-3-11 x 12194) crossed to either G424-1-10-2 or G424-1-10-14 (BNS sister lines homozygous for *l-2*), backcross progeny segregated 20 RS, 10 white to blue-gray/green BNS, and 10 white/gray-white BNS, results most easily interpreted as G12194 being homozygous for *l-2* and possessing a dominant gene other than *l-2*^{R} conferring reverse striping. These results suggested several possibilities: 1. that another gene with full dominance may be responsible for the reverse stripe phenotype in egg gourd selections, 2. that in genotypes heterozygous for *L-2/l-2*, expression of the RS phenotype is intermediate in terms of pigmentation of the wide stripe; 3. when *L-2* is homozygous, RS is not expressed; and 4. in RS plants heterozygous for *L-1* in combination with *l-1*^{bst}, the dark green stripe over vein tracts is narrower and less distinct than when *l-1*^{BSt} is homozygous.

The possible nature of the genetics for reverse striping observed in egg gourd eluded us for some time, but some important clues turned up. Segregation data obtained during the summers of 2010 and 2011 from several F_2 and backcross populations involving G344-22-1, G12194-3, G12131-2W and G424-25-3-11 suggested that a completely dominant gene at a locus other than *l-2* was responsible for eliciting the reverse strip in G12194-3 in the presence of *l-1*^{BSt}/*l-1*^{BSt}. Finally, in the spring of 2012, it was observed that stems of the white BNS line (G12131-2W) were light green, and stems of Type 1 RS plants resulting from a cross of the white-fruited line G212-349-1 (*l-1/l-1, l-2/l-2, dd*) to the RS line 121943-4 (*l-1*^{BSt}/*l-1*^{BSt}, *l-2/l-2*) were dark green, indicating that F_1 RS plants were heterozygous for the D gene. A later planting was made in spring of 2012, revealing that plants of G424-25-3-11 have light stems (*dd*), and plants of G344-22-1 and G121943-4 have dark stems (DD). We also grew out a small population of 12 plants from the cross of the F_1 (G344-22-1 x 1213-1W) x G212-349-1. The segregation pattern was 8 plants with dark stems (*Dd*) and four plants with light stems (*dd*). The four light stem plants had the BNS phenotype, three with white/gray-white stripes and one with white/green stripes Of the eight plants with dark stems, six had reverse stripes and two were solid green. Lastly, in a cross of G1213-1W to a pumpkin line with the genotype l-1/l-1, l-2/l-2, D/D, fruit of all of the offspring exhibited Type 1 reverse

striping. These results, though preliminary, are consistent with the hypothesis that the D gene interacts in a complementary fashion with *l-1*^{BSt} and *l-2* alleles to produce reverse striping. In reverse striping putatively conditioned by the *D* gene, the *1-1*^{BSt} allele shows dominance over *L-1* in interacting with *D*, similar to reverse striping conferred by the *l-2*^{R} allele (Paris 2009).

CONCLUSIONS

Between the inception of the UNH gourd breeding program in 1996 and 2011, several inbred bush lines of egg gourd with an array of different rind colors and patterns have been developed. For identification of rind phenotypes in our gourd breeding work we have relied largely on the extensive inheritance studies with *C. pepo* reported during the past 25 years by Dr. H. Paris and colleagues. Moreover, the descriptions given in this paper do not do justice to the total array of variability in rind coloration and patterns. For example, the inbred G424-25-3-11 is described herein as having blue-gray/green stripes, but with short term storage, the narrow stripes turn white, giving a much more vivid striping pattern. Several different hues of yellow and orange pigmentations are exhibited in some of the egg gourd lines, presumably due to the interaction of the *B* or *b* alleles with *Wf*, wf, *D*, and *d*, and possibly with *Y* and *y* as well. Expression of the '*B*' gene is quite variable, resulting in different degrees of bicolor pigmentation, depending upon genetic background. Phenotyping plants of segregating populations is often extremely difficult, not only because of changes in pigmentation with time, but also because of the various gene interactions mentioned above. Lastly, we present preliminary evidence for existence of two genetic systems for producing reverse striping, one involving complementary action of *l-2*^{R} and *l-1*st alleles as reported by Paris (2009) and another involving the *D* gene together with *l-1*^{BSt}, in which reverse striping is occurs in the presence of homozygous *l-2 or L-2/l-2*.

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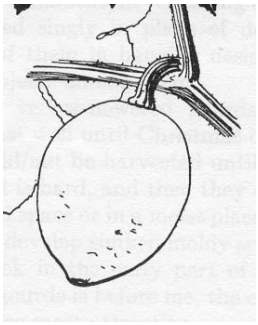

Fig. 1 Nest egg gourd described by Bailey (1937).

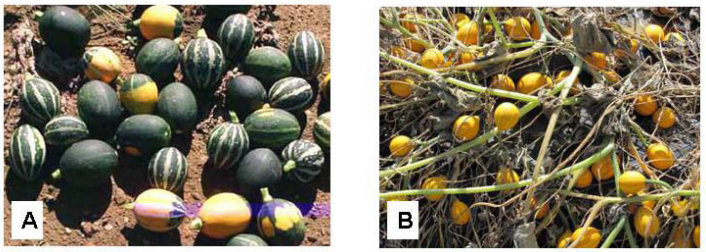

Fig.2. A. Egg gourd phenotypes produced from a three-way test cross involving breeding lines (G424-25-3-11 x G344-13-3) x G424-25-3-11. B. Prolific production of egg gourds on less than a square meter of ground area from a plant of the bush G37-24-2 breeding line.

Fig. 3. Fruit phenotypes for parents and F_1 progeny from three crosses. A. green x reverse stripe (Type 1); F_1 = green/mottled green-white, Type 3 RS; B. normal stripe x reverse stripe (Type 1); F_1 = wider dark green/mottled green white wide Type 2 RS; and C. green x white/gray-white BNS. F_1 = narrow green/ wide mottled green-white Type 3 RS.

Toward the development of molecular markers linked to the *fom-4* gene in melon

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Abstact

Melon Fusarium wilt (MFW), caused by *Fusarium oxysporum* f.sp. *melonis* (Fom), is one of the most destructive diseases of melon (*Cucumis melo* L.). The most effective control measure available is the use of resistant varieties. The unliked genes *Fom-1* and *fom-4* have been found to confer resistance to races 0 and 2 in 'Tortuga', a Spanish *cantalupensis* line. Identification of DNA markers tightly linked to genes conferring resistance to Fom is likely to have immediate application in MFW resistance breeding programs. In this work a bulk segregant analysis was used to search for randomly amplified polymorphic DNA (RAPD) markers linked to the *fom-4* gene, using segregating progenies derived from the cross between the 'Tortuga' line (carrying the genes *Fom-1* and *fom-4*), and the 'Piel de Sapo' line susceptible to Fom. The application of a 618-CAPS marker, tightly linked to *Fom-1*, together with artificial inoculations, allowed the identification of resistant F_3 plants carrying only the resistance allele *fom-4*. One hundred forty four decamer primers were screened to identify the RAPD marker OPN13 which amplified a fragment of approximatly 1.000 bp only in the 'Piel de Sapo' and the susceptible bulks. Further work is underway to determine the genetic distances between the marker and the *fom-4* locus, and to detect other markers that would be very useful in marker assisted selection to introduce this gene in commercial melon cultivars.

INTRODUCTION

Melon Fusarium wilt (MFW), caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *melonis* Snyder and Hans (Leach and Currence 1938) (Fom), causes yield losses in melon (*Cucumis melo* L.) crops worldwide (Zitter 1999). The development and deployment of resistant cultivars is generally considered to be the

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best approach to control MFW. Based on virulence to three differential cultivars of melon, Risser et al. (1976) established four physiological races of Fom, races 0, 1, 2, and 1.2. Two dominant resistance genes, *Fom-1* and *Fom-2*, control resistance to races 0 and 2 and 0 and 1, respectively. Fom isolates classified as race 1,2 are able to induce disease in melon lines carrying the above described resistance genes.

Recently, Oumouloud et al. (2010) reported a new recessive gene (*fom-4*) that confers resistance to races 0 and 2 in the 'Tortuga' melon line, which also carries *Fom-1*. Resistance under digenic control, involving two independent genes - one dominant and the other recessive - is not new in melon and was reported by Yuste-Lisbona et al. (2010) for resistance to *Podosphaera xanthii* races 1, 2 and 5 in line 'TGR-1551'. This kind of mechanism was also found in the snap bean cultivar 'Widusa' against *Colletotrichum lindemuthianum* race 38 (Ferreira et al. 2003).

The use of different resistance genes such as *Fom-1* and *fom-4* can provide higher levels of resistance to Fom races 0 and 2. 'Tortuga' therefore constitutes a new alternative source of resistance to these races. In addition, resistance controlled by more than one gene may increase its durability (Khetarpal et al. 1998). Thus, better protection against races 0 and 2 can be achieved by combining two resistant genes, such as *Fom-1*, and *fom-4* in melon varieties.

Standard procedures followed in breeding programs to introduce resistance genes into genotypes of interest include successive crosses, together with selection steps based on inoculations with the appropriate isolates, to finally fix and obtain the desired improved material. However, traditional artificial inoculations used to evaluate Fusarium wilt resistance in melon breeding programs is a time-consuming process. Moreover, occasionally, this problem is further complicated by lack of detection of susceptible plants because their identification is usually restricted to observations of the development of external symptoms, i.e. wilting, yellowing, plant death (Burger et al. 2003).

Thus, the availability of molecular markers tightly linked to a resistance gene would be extremely useful to avoid these drawbacks in melon breeding programs aimed to develop Fom resistant lines. In the last few years, several molecular markers linked to *Fom-1* (Brotman et al. 2005, Oumouloud et al. 2008; Tezuka et al. 2009, Tezuka et al. 2011) and to *Fom-2* (Wang et al. 2011; Oumouloud et al. 2012) have been developed. In this work, we report the localization of RAPD markers linked to the *fom-4* locus, which may be more useful for practical breeding purposes.

MATERIALS AND METHODS

Plant and fungal materials and inoculations

The melon line 'Tortuga' (Tor), carrying in homozygosis the resistance alleles *Fom-1* and *fom-4*, was used as the resistant parent, while the line 'Piel de

Sapo' (PS) was the susceptible parent. The F_1, and F_2 generations were developed from the cross Tor x PS. The analysis of 100 F_2 plants with a 618-CAPS marker, tightly linked to *Fom-1*, allowed the identification of 30 plants that did not carry the resistant allele *Fom-1*. These were selfed to obtain F_3 lines (Fig. 1).

In order to identify the F_2 homozygous resistant plants carrying only the resistant allele *fom-4*, the F_3 families, consisting of 20 plants each, were evaluated for resistance/susceptibility to Fom race 2; 20 homogeneous resistant F_3 families, coming from homozygous resistant F_2 plants, were chosen for the bulk analysis. Homogeneous susceptible families should come from susceptible F_2 plants and were used for building susceptible bulks. Segregating families were eliminated.

Plants were inoculated following the artificial inoculation method described by Oumouloud et al. (2010). The Fom isolate 'Fom 0124', belonging to race 2, was used for inoculum preparation, and the parental lines 'Tor' and 'PS' were used as resistant and susceptible controls, respectively.

DNA extraction, bulk preparation and molecular marker analysis

Genomic DNA was extracted from leaf tissue at the one-to two-leaf stages following the method described by Doyle and Doyle (1987) with minor modifications as described in Oumouloud et al. (2008).

Bulked segregant analysis (MichelMore et al. 1991) was used to identify RAPD markers closely linked to the *fom4* locus. Four "resistant" and four "susceptible" bulked DNA samples were prepared each with five homozygous resistant and five homozygous susceptible plants respectively from the F_3 population.

A total of 144 random sequence decamer oligonucleotide primers (Operon Technologies Inc. USA) were used to detect polymorphisms among the parents, 'Tor' and 'PS'. The selected primers were tested in the resistant and susceptible bulks. When a polymorphism was observed between the bulks, DNA from each plant included in the bulks was amplified separately with the polymorphic primers.

Amplifications of genomic DNA with the RAPD primers and electrophoresis were carried out under the same conditions as those described in Oumouloud et al. (2008). The 618-CAPS marker analysis was performed as described in Oumouloud et al. (2010).

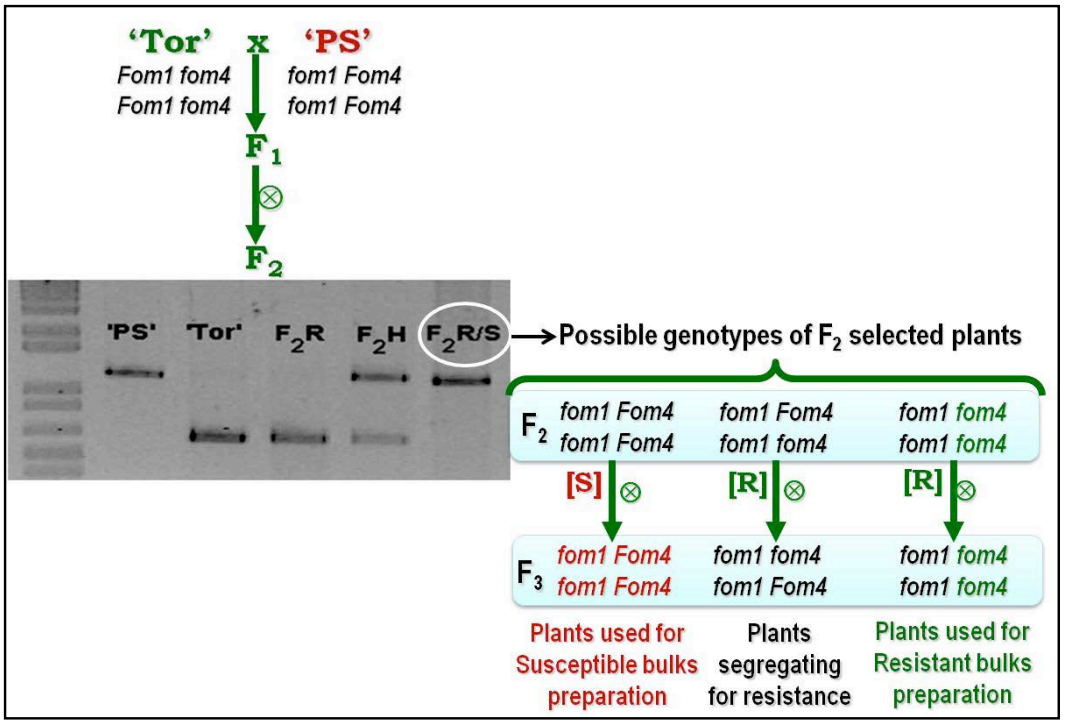

Fig. 1. Scheme of the processus followed for identification of melon resistant plants to *Fusarium oxysporum* f.sp *melonis* race 2 carrying only the *fom4* resistant allele.

RESULTS AND DISCUSSION

Identification of F_2 plants carrying only the *fom-4* resistant allele

The reactions of the melon differential host plants confirmed that the inoculations were carried out with Fom race 2. The resistant parent 'Tor' usually appeared free of MFW symptoms, whereas plants of the susceptible parent 'PS' showed typical systems of MFW and died within 3 weeks after inoculation.

We used a pair of specific primers (618-CAPS-F and 618-CAPS-R) to amplify the fragment of interest from 100 F_2 ('Tor' x 'PS') plants. Thereafter, *Tfi* I restriction enzyme digestion of PCR products showed patterns of 618- and 614-bp fragments in the resistant homozygous genotypes (*Fom-1–/Fom-1–*), three fragments (1232-, 618-and 614-bp) in the resistant heterozygous genotypes (*Fom-1–/fom-1–*) and a 1232-bp fragment in the susceptible genotypes (*fom-1Fom-4/fom-1–*), as well as in the resistant ones that do not carry the *Fom-1* gene suggesting that they are *fom-1fom-4/fom-1fom-4* (Fig. 1).

This analysis allowed the identification of 30 plants that did not carry the resistant allele *Fom-1*. These plants were selfed to obtain F_3 lines. Of twenty F_3 lines inoculated with Fom race 2, three were homogeneously resistant (*fom-1fom-4/fom-*

1fom-4), four homogeneously susceptible (*fom-1Fom-4*/ *fom-1Fom-4*) and thirteen segregated for resistance (*fom-1Fom-4*/ *fom-1fom-4*). The detection of F_3 resistant plants that do not carry the *Fom-1* gene confirms that resistance to Fom races 0 and 2 in the 'Tor' line is under digenic control, involving two independent genes, one dominant (*Fom-1*) and the other recessive (*fom-4*) (Oumouloud et al. 2010).

Detection of the RAPD marker linked to *fom-4*

From a total of 144 decamer-primers analyzed, 72 generated polymorphisms between the parent genotypes 'Tortuga' and 'Piel de Sapo'. On the average, each primer amplified six to seven fragments that ranged in size from 120 bp to 3 kbp. The polymorphisms observed were expected, because the resistant parent 'Tor' belongs to the var. *cantalupensis* whereas the susceptible one 'PS' is classified within the var. *inodurus*. All the 72 primers were screened for polymorphisms between the resistant and susceptible bulks. This analysis has resulted, until now, in the identification of the RAPD marker OPN13, which generated polymorphisms between the contrasted DNA bulks. This primer amplified a fragment of approximatly 1.000 bp only in the 'PS' parent and the susceptible bulks. BSA has proven to be a useful tool for identifying DNA markers linked to *Fom*-1 (Brotman et al. 2005, Oumouloud et al. 2008; Tezuka et al. 2009) and to *Fom*-2 (Wechter et al. 1995; Baudracco-Arnas and Pitrat 1996; Zheng et al. 1999; Zheng and Wolf 2000; Wang et al. 2000). In melon, Brotman et al. (2005) reported that this approach was more successful combined with RAPD as compared with its use with AFLP.

This marker will be analyzed on every individual of an F_2 population that is under development by crossing the F_3 resistant plants (*fom-1fom-4*/*fom-1fom-4*) and the susceptible parent 'PS'. This analysis will confirm the hypothesis that the OPN13 fragment is linked in repulsion to the *fom-4* resistance gene, and will allow us to determine the genetic distances between this marker and f*om-4*.

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Identification of the chromosomal region that controls naringenin chalcone accumulation in melon rind

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Abstract

Carotenoids and chlorophylls are the major melon fruit pigments. Recently we discovered the accumulation of naringenin chalcone, a yellow flavonoid, as the major pigment in the skin of 'canary yellow ' melon fruit as well as an additional pigment in other melon fruit types. Analysis of an F_2 population segregating for the accumulation of skin's naringenin chalcone indicated the existence of a single major gene that dominantly controls this trait. Analysis of candidate genes controlling similar trait in other crops failed to identify this gene. Thus, in an attempt to identify this gene, we sequenced RNA from the skins of developing fruit. Fruit skins from a pool of F_3 families having 'yellow canary' rinds, and accumulating naringenin chalcone, and a pool of F_3 families with white skin that do not accumulate detectable amounts of naringenin chalcone, at 10 DAP, 20 DAP and mature fruit were sampled and their RNA were extracted. We sequenced these RNA pools with Illumina/Solexa Genome Analyzer, assembled the resulting sequences into contigs, analyzed their expression patterns within and between the two pools at different fruit developmental stages and discovered SNPs and transcription patterns that differentiate between the pools. We localized the contigs and the SNPs on the publicly available cucumber and watermelon genomes. Most of the identified SNPs were localized to the same genomic region where contigs with significantly differential expression patterns between the two pools were co-localized. This indicates that the regulator gene for naringenin chalcone accumulation in melon rinds is localized in this small region. Our next steps include the identification and the cloning of this gene, finding what limits the accumulation of flavonoids to fruit rind only and finding ways to divert the accumulation of flavonoids to anthocyanins. This research could lead to the development of a complementary pigmentation system in melon fruit based on flavonoids.

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POSTERS

Study on *in vitro* culture of mini-watermelon

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Keyword: Mini-watermelon, tissue culture, clustered buds

Abstract

In vitro culture system of mini-watermelon was established in this study. This study demonstrated that the cotyledons paraxial end of aseptic seedlings served as the optimum explants from clustered buds of mini-watermelon induced by tissue culture. The optimal clustered buds-induced medium contained MS+6-BA 2 mg L^{-1} + NAA 0.1 mg L^{-1}, the proliferation rate of clustered buds was the highest in the medium of 1/2 MS+6-BA 2.0 mg L^{-1}; the adventitious buds of mini-watermelon differentiation formed the strong seedlings in the medium of 1/2 MS+KT 0.2 mg L^{-1} +IAA 0.01 mg L^{-1}, and the effect on rooting for seedlings of mini-watermelon was the best in the medium of 1/2 MS+IBA 0.3 mg L^{-1} by strengthening the seedlings.

INTRODUCTION

Watermelon (*Citrullus lanatus*) belongs to the gourd family of watermelon genera. Tissue culture of watermelon begins in 1970s and Andrus et al. (1971) firstly reported the establishment of asexual reproduction system of seedless watermelon from multiple shoot induced from hypocotyls. In China, Xu et al. (1979) successfully obtained the tube seedlings through multiple shoot induced from watermelon terminal bud explants. Then many researchers have made great progress in lots of studies such as explants selection, hormone proportion, plantlets domestication and prevention of vitrification (Pirinc at al. 2003; Helmle et al. 1992; Choi et al. 1994).

In this study, we take advantage of cotyledons as materials to investigate the influencing factors of tissue culture of mini watermelon in order to provide fundamental information of inducing tetraploid materials and breeding seedless watermelon.

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MATERIALS AND METHODS

Materials

H35, the female parent of mini-watermelon LongSheng No.1, which was bred by Heilongjiang Academy of Agricultural Sciences was used in this study.

Methods

Plump seed of mini-watermelon was chosen for the present study. The seeds were soaked with sterile water for 2 h, disinfected with 10% sodium hypochlorite solution for 10 min, washed with sterile water for 3 times, and inoculated on MS medium with 20 $g.L^{-1}$ of sucrose and 5 $g.L^{-1}$ of agar. Then the seeds were cultured at 28°C in the dark for germination. The seeds were transferred to culture room when radical was seen.

When cotyledons appeared before euphylla was seen, we choose the cotyledons with the same seedling ages and made a cross-cut. Both the adaxial explants and the abaxial explants were inoculated on shoot differentiation medium. Each treatment repeats 3 times (25 explants in 5 containers, i.e., 5 explants in one container).

Medium and culture conditions

Medium preparation

(1) Seed germination medium: MS

(2) Shoot induction medium: MS + 6-BA (0.5, 1, 2, 3 mg/L, respectively)+ NAA (0, 0.1, 0.3, 0.5 mg/L, respectively)

(3) Shoot reproduction medium: MS + 6-BA (1, 2, 3 mg/L, respectively)+ NAA (0, 0.1 mg/L, respectively)

(4) Seedling enhancement medium: 1/2 MS+ KT (0.1, 0.2, 0.3 mg/L, respectively) + IAA (0.01, 0.02 mg/L, respectively)

(5) Rooting medium: 1/2 MS+ NAA (0.1, 0.3 mg/L, respectively) or IBA (0.01, 0.02 mg/L, respectively)

MS was used as the basal medium, added with different hormone and kept pH value at 5.8. with 20 $g.L^{-1}$ of sucrose and 5 $g.L^{-1}$ of agar. The medium was disinfected at 121°C for 20 min and used after cooling. The temperature of culture room kept at 25±2 °C and the illumination was 12 light/dark at 3000 lx.

Inoculation and culture

The disinfected seeds were flatted on seed germination medium. The cotyledon without shoot tip was chosen for culturing and made a cross cut. After the cotyledon was transferred to shoot inoculation medium, we chose the strong and uniform clumps of multiple shoot clumps and cut into small pieces of 1 cm × 1 cm. Then the clumps were inoculated to shoot reproduction medium and the reproducible results were observed on 15th day after the inoculation. The strong and uniform shoots

were transferred to seedling enhancement medium and the shoots were transferred to rooting medium at 15 days later. When it grew 2-3 roots, the plantlet was moved to greenhouse for acclimatization.

RESULTS

Effect of the explants type on inducing multiple shoot

The results of Table 1 showed that there was significant difference in the ability of inducing multiple shoot between the adaxial explants and the abaxial explants. The inductivity of adaxial explants was no more than 2.6%, whereas that of abaxial explants was up to 100%. The present studies indicated that the adaxial explants have a better reproductive ability and higher inductivity. The multiple shoots were exclusively found at the joint of the cotyledons and hypocotyls, which was consistent with the reports of Zhang (2004).

Effect of hormone concentration on multiple shoot differentiation

The adaxial explants were cultured in mediums of different conditions. Light green shoots were generated from the joint of the cotyledons and hypocotyls at 10 days later, and the shoots turn green and formed cluster shoots by the 15^{th} day. We found that the more BA was used the more the multiple shoots were formed at the same concentration of NAA. However the formation of cluster shoots were restrained when the concentration of BA was kept more than 3 $mg.L^{-1}$. The best combination of the hormone was BA at 2.0 $mg.L^{-1}$ and NAA at 0.1 $mg.L^{-1}$, which resulted inducing number of multiple shoots of 100% and 6.3 per explants, respectively (Table 2).

Effect of different hormone combination on reproducing multiple shoot

It was reported that the induced multiple shoot usually suffered vitrification after reproduction for 2-3 times (Li et al. 2008). To diminish the vitrification, we chose 1/2 MS as the basal medium and managed to obtain an optimalizing combination of hormones. Some strong multiple shoots were selected to cut into small pieces of 1 cm×1 cm and transferred to medium with different hormone proportion. Reproduction was observed at 15 days later (Table 3).

When NAA was not used and concentration of BA was either at 2 $mg.L^{-1}$ and 3 $mg.L^{-1}$, the reproducibility of multiple shoot was relatively high but showed no significant difference. However, the multiple shoot grew better on the medium containing BA at 2 $mg.L^{-1}$. Thus we used medium with the concentration of BA at 2 $mg.L^{-1}$ and NAA at 0 $mg.L^{-1}$ as the shoot reproduction medium and kept the regeneration cycle at about 15 days.

Determination of seedlings enhancement medium

The survival rate of multiple shoot is very low if the shoot is used for rooting when it's very young due to its bad rooting ability. Therefore, we cut the shoot at length of 1 cm and transferred it to seedling enhancement medium to get vigor plantlets. Two weeks later, the seedlings grew vigor and vitrification was rarely observed at the medium concentration of KT at 0.2 $mg.L^{-1}$ and IAA at 0.01 $mg.L^{-1}$ (Table 4).

Note: Minimal medium is 1/2 MS.

Determination of rooting medium

The plantlets were transferred to 1/2 MS medium for inducing root when it is 1.5-2.5 cm high and with 3 leaves. Each container was inoculated with 5 plantlets, and roots can be seen at the stem base at 15 days later. The results showed that significant difference was not observed regarding to rooting ability when the medium was added with NAA or IBA at 0.3 $mg.L^{-1}$. However the plantlets grew vigorously when the concentration of IBA was at 0.3 $mg.L^{-1}$ (Table 5).

Plantlets transference and management

The suitable plantlets to transfer to matrixes were at 5 cm height with the roots of 1.5-2.0 cm length. Before transferring, the plantlets were moved to low sunlight at room temperature for acclimatization for 7 days and 2 days without the cover of the container. The matrixes used for acclimatization was vermiculite: turf: soil = 2:1:2. A survival rate of about 30% can be investigated at a month later.

The management of plantlets after transferring is very important. In particular the humidity should always be kept at about 70% and high humidity should be avoided. Only plantlets with roots longer than 1 cm can be transferred to matrixes.

DISCUSSION

In the present studies, we investigated the optimal medium used in several culture stages of the mini-watermelon; multiple shoot induction, reproduction, enhancement and rooting. We also established an ideal culture protocol in each stage and a better substrate composition for acclimatization.

Effect of disinfecting methods on seed germination

In the preliminary test we studied several disinfecting methods to seed germination. The seed coats are always peeled off before germination for the thick coats. Formal disinfecting materials such as corrosive sublimate and alcohol often diminish germination percentage of the bare seeds. Therefore we used sodium hypochlorite (10%) to disinfect the seeds for 10 min, and a better result and lower infection can be observed.

Effect of the explants type on inducing multiple shoot

We carried out the current study to disclose the influence of different explants type on inducing multiple shoot. Both adaxial explants and abaxial explants were cut off from the cotyledon which was obtained from sterile seedlings at 4-6 days after germination, and the results showed that the adaxial explants have a higher inductivity. In cabbage, Sun et al. (2006) suggested that the content of endogenous hormones were related to regeneration capacity. We suggested that the endogenous hormone distribution in different plant organs and tissues may results in the higher inductivity.

Rooting rate improvement by using seedling enhancement medium

Multiple shoot, induced from the adaxial explants of cotyledon, is always too weak to be cultured on rooting medium and transferring to field later because the weakness and relatively short internodes made it unsuitable to root. Thus we use a seedling enhancement medium to get the better seedlings with long internodes and strong roots.

Effect of hormones on multiple shoot differentiation

It was reported that combining use of BA and IAA was effective to differentiation of multiple shoot (Liu et al. 2009). In this study we achieved a better induction frequency by using BA and IAA, which is consistent with that of Compton and Gray (1994). When adding IAA to medium containing BA, callus became prosperous and the formation of shoot was restrained, which may be influenced by different genetic background of materials.

Methods to reduce vitrification

Plantlets of mini-watermelon showed severe vitrification in the course of multiple shoot induction and reproduction stages. In particular, the vitrification is always subjected to temperature and reproduction time, and the vitrification is likely to deteriorate when plantlets cultured above 25ºC or reproduction takes more than 15 days. We can decrease the vitrification by means of rising agar and ferric salt concentrations or reducing nitrate concentrations in the media, which are consistent with the results of Liu et al (2009).

Different genotype of mini-watermelon used as materials can lead to different inductivity and survival rate. We are planning to optimize the culture condition for those different genotypes in the future.

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Table 1. Effect of explant types on the cluster buds induction.

Condition	No. of explants	Concentration of hormone		No. of explants induced		Inductivity (%)	
		BA	NAA	adaxial explants	abaxial explants	adaxial explants	abaxial explants
Y1	75	1	0	61	0	81.3	0
Y2	75	1	0.1	66	0	88.0	0
Y3	75	1	0.3	70	0	93.3	0
Y4	75	1	0.5	75	1	100	1.3
Y5	75	2	0	75	2	100	2.6
Y6	75	2	0.1	75	0	100	0
Y7	75	2	0.3	69	0	92.0	0
Y8	75	2	0.5	60	0	80.0	0

Table 2. Effect of different hormone ratio on differentiation of multiple shoot clumps.

Condition	No. of explants	Concentration of hormone ($mg \cdot L^{-1}$)		Inductivity (%)	No. of adventitious buds per explant
		BA	NAA		
S1	75	0.5	0	69.3	4.4
S2	75	0.5	0.1	72.0	4.6
S3	75	0.5	0.3	77.3	4.9
S4	75	0.5	0.5	69.3	4.9
S5	75	1	0	81.3	4.7
S6	75	1	0.1	88.0	5.2
S7	75	1	0.3	93.3	5.5
S8	75	1	0.5	80.0	5.6
S9	75	2	0	100.0	5.8
S10	75	2	0.1	100.0	6.3
S11	75	2	0.3	92.0	6.1
S12	75	2	0.5	89.3	6.1
S13	75	3	0	94.7	5.7
S14	75	3	0.1	96.0	5.8
S15	75	3	0.3	86.7	4.9
S16	75	3	0.5	82.7	5.0

Table 3. Effect of different hormone ratio on proliferation of multiple shoot clumps.

Condition	No. of explants	Concentration of hormone ($mg \cdot L^{-1}$)		No. of adventitious buds per explant
		BA	NAA	
Z1	75	1	0	6.2
Z2	75	1	0.1	6.8
Z3	75	2	0	7.4
Z4	75	2	0.1	6.9
Z5	75	3	0	7.7
Z6	75	3	0.1	7.0

Table 4. Effect of different hormone ratio on shoot elongation.

Condition	No. of explants	Concentration of hormone ($mg \cdot L^{-1}$)		Shoot elongation (%)
		KT	IAA	
M1	15	0.1	0.01	67.7
M2	15	0.2	0.01	87.3
M3	15	0.2	0.02	70.6
M4	15	0.3	0.01	63.3

Table 5. Effect of hormones on *in vitro* rooting of shoot.

Condition	Concentration of hormones ($mg \cdot L^{-1}$)		No. of roots	Situation of root growth	Average height of seedlings (cm)
	NAA	IBA			
G1	0.1		3.7	weak and crisp	4.4
G2	0.3		3.6	medium and crisp	5.5
G3		0.1	3.3	medium	4.7
G4		0.3	4.0	vigor	5.3

Histological differences between watermelon grafted onto bottle gourd rootstock and self-rooted seedlings inoculated with *Fusarium oxysporum* f.sp. *niveum*

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Keywords: Anatomy, *Fusarium oxysporum* f.sp. *niveum*, grafting, tyloses, watermelon

Abstract

Effects of grafting on disease control and fruit quality of watermelon have been widely documented, while little work has been done on the histological response of the grafted plants to the pathogen. In this work, susceptible watermelon cultivar, 'Sumi No.1', was grafted onto fusarium wilt resistant bottle gourd (*Lagenaria siceraria*) 'Chaofeng', the grafted and self-rooted plants were inoculated with *Fusarium oxysporum* f.sp. *niveum* race 1 (FON 1) and the histological response of roots and hypocotyls was examined. In roots of self-rooted plants, infected xylem vessels were usually plugged with gums or tyloses, few occluded vessels were shed from the roots. Parenchyma cell surrounding the vascular cylinder were observed with cell wall thickened, cell disintegration and eventually formed cavities. In roots of grafted plants, only gums and tyloses in infected xylem vessels, together with the thickening of xylem parenchyma cell wall were observed. Moreover, we found that FON 1 caused a quick defense response in grafted plants compared with that in self-rooted plants, suggesting the earlier formation of barrier to fungal invasion. Hypocotyls of most self-rooted plants were colonized. Plenty of hyphae were seen in vascular tissues, the diseased vascular bundle finally became hollow and plants developed wilt symptoms, while most grafted plants' shoots did not colonized by pathogen and the plants became healthy. This work provides anatomical basis for fusarium wilt control in watermelon by grafting.

INTRODUCTION

Fusarium oxysporum f. sp. *niveum* (FON) is one of the most important soil-borne diseases of watermelon worldwide. The infection of FON could cause approximately 30-80% of yield losses, sometimes even worse (Martyn and Netzer 1991; Zhang et al. 2005; Guo 2008), which becomes the main obstacle for watermelon production.

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Watermelon [*Citrulls lanatus* (Thunb) Matsum and Naki]) is an important vegetable crop with a ~6% of total area in the world's vegetable production (http://faostat.fao.org/). Previously research on the interaction between watermelon and fusarium wilt mainly focused on the disease distribution, symptom expression and control measure (Wang et al. 2002; Chang et al. 2008; Wu et al. 2010), however, little is known about the histological response of the host plants to the FON infection, not even in the grafted watermelon. Understanding the interactions of watermelon plants to FON is of scientific and practical importance for disease control. This paper was undertaken to investigate the resistance to *F. o.* f.sp. *niveum* in roots of bottle gourd grafted and non-grafted watermelon growing in soil infested with the pathogen, and to determine whether there was any anatomical basis for the success of culture indexing as a control measure.

MATERIALS AND METHODS

Plant grafting & inoculation

The fusarium wilt-susceptible cultivar 'Sumi No. 1' and the fusarium wilt-resistant bottle gourd cultivar 'Chaofeng' were obtained from Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, China. The watermelon scions were grafted onto bottle gourd rootstocks using sprout insertion grafting method (Lee et al. 2010).

The FON race 1 (FON 1) suspension was grown in a 250 mL triangular flask with 150 mL liquid PDA medium at 25 ^{o}C on a rotary shaker at 150 rpm for 5-7 days. The concentration of conidia in the suspension culture was adjusted to 10^6 conidia per mL for plant inoculation with sterile distilled water.

For plant inoculation, roots were immersed in a freshly prepared spore suspension (10^6 conidia per mL) for 30 min, and then were replanted into the seedling medium. Control plants were similarly treated and immersed into sterile distilled water (Chang et al. 2008).

Microscopic examination

Roots were sampled at 8, 12 hour after inoculation (h.a.i.) and 1, 4, 5, 7, 9, 11, 15 days post inoculation (d.p.i.), five inoculated and five control grafted and non-grafted watermelon were removed from growth media, and then carefully washed to remove the soil. Samples were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2), and placed under vacuum to remove any remaining air. After dehydration with gradient ethanol and replacement with amyl acetate, the critical carbon dioxide point drying method was used to dry the sample. After sticking and coating using ion sputtering, samples were scanned by electron microscopy (XL30 ESEM, Philips, Holland).

RESULTS AND DISCUSSION

Histology of roots of susceptible 'Sumi No.1'

In this study, as the precise location of the infection process was not well observed, the infection process on the early stages was not discussed. At 4 d.p.i., a few pathogens entered the xylem vessel element of the vascular system (Fig. 1b), they continued to grow and eventually formed mass of mycelium (Fig. 1c). At the growing stage, the fungus was restricted to the xylem vessel, and once the fungus was established, they spread to adjacent tissues through the pit pairs (Fig. 1c). The phenomenon that the mycelium was trapped by the perforation plates of the vessel elements was not noted, which is disagreement with that described by Miao et al. (2004).

The first visible response of the histological change was the presence of gums in the xylem vessel at 12 h.a.i. (Fig. 1e), this substance appeared quickly after the pathogen entered the vascular system, thus is the rapid response of host plant to invasion of pathogen. Along with the further infection of mycelium, walls of parenchyma cells adjacent to colonized tissue and even surrounding the entire vascular cylinder were thickened at 5 d.p.i. (Fig. 1f), which functions as a barrier for fungus invasion.

By 7 d.p.i., tyloses were formed inside the surface of xylem vessels (Fig. 1g-j). Initially, several small tyloses were observed in vessel elements (Fig. 1g, h). It continued to grow and eventually occluded the vessel from which single (Fig. 1i) or multiple (Fig. 1j) tyloses were observed at 11 d.p.i.. These tyloses may function to prevent the spread of pathogen in the vascular tissue. Granular content was also observed in xylem parenchyma cells (Fig. 1h), suggesting the same function as tyloses. Previous studies on watermelon infected with FON (Miao et al. 2004) and cucumber (Chen et al. 2003; Ma et al. 2008) indicated the presence of tyloses formation in the vascular tissue. Mace et al. (1981) considered that formation of tyloses limited the expansion of mycelium and finally prevented its further invasion. The numerous tyloses in roots of watermelon appeared to be one of the major defense responses of watermelon to FON invasion, and further indicated its function in hosts' resistance.

The xylem parenchyma cells began to disintegrate at 7 d.p.i. (Fig. 1k) and resulted in cavity formation (Fig. 1l). When many vessels were occluded with gums and tyloses, the affected tissue was often employed a sloughing-off process. At 11 d.p.i.The affected tissue isolated from the vessel (Fig. 1l) and then was shed (Fig. 1m). Heavy degradation of the vascular tissues by the fungus resulted in hollow roots at 15 d.p.i. (Fig. 1n).

Shoots of the affected plants became diseased as that in roots. Shoots were also successfully colonized and mass of mycelium were localized at 9 d.p.i. (Fig. 1o). Heavy invasion triggered the formation of small cavities at 11 d.p.i. (Fig. 1p), which finally enlarged to encompass the whole infected vascular (Fig. 1q).

Histology of roots of watermelon grafted onto resistant bottle gourd

Colonization of the vascular system was scarce in roots of grafted watermelon, but sometimes occurred when these tissues were damaged. Histological changes of roots of grafted watermelon attacted by FON 1 were somewhat the same as that described for the susceptible 'Sumi No.1'. The colonization was limited to the xylem vessels. The first visible histological change was the presence of gums in the xylem vessel at 8 h.a.i. (Fig. 2a). By 12 h.a.i., tyloses were formed in the vascular (Fig. 2b) together with the wall-thicken of parenchyma cells surrounding the entire vascular cylinder (Fig. 2b). Some infected plant showed the combination of these described above (Fig. 2c). The extravascular tissue surrounding more root parts was never colonized, even when colonization of the vascular tissues was extensive. The only histological change in shoots of the infected plants was the presence of tylose in the vascular cylinder at 7 d.p.i. (Fig. 2d), while this process does not observed in shoots of self-rooted watermelon samples. Moreover, it is noted that FON 1 caused a quick defense response in grafted plants compared with that in self-rooted plants, suggesting the earlier formation of barrier to fungal invasion.

CONCLUSION

Self-rooted 'Sumi No.1' watermelon proved to be susceptible, and bottle gourd grafted watermelon be resistant to FON 1. Differences in the two materials responding to FON 1 were significant. In susceptible watermelon 'Sumi No.1', the histological changes included gums and tyloses in the xylem vessel elements, wall-thicken and disintegration of the xylem parenchyma cells, formation of cavities and shed of the infected vascular tissue. Whereas in grafted watermelon, the histological changes were characterized by gums and tyloses in the xylem vessel elements and wall-thicken of the xylem parenchyma cells. The main difference was the quick response of the grafted watermelon to FON invasion, though the histological changes only consisted of vascular plugging with gum and tyloses and cell wall-thicken, this action was enough to prevent the further invasion of mycelium.

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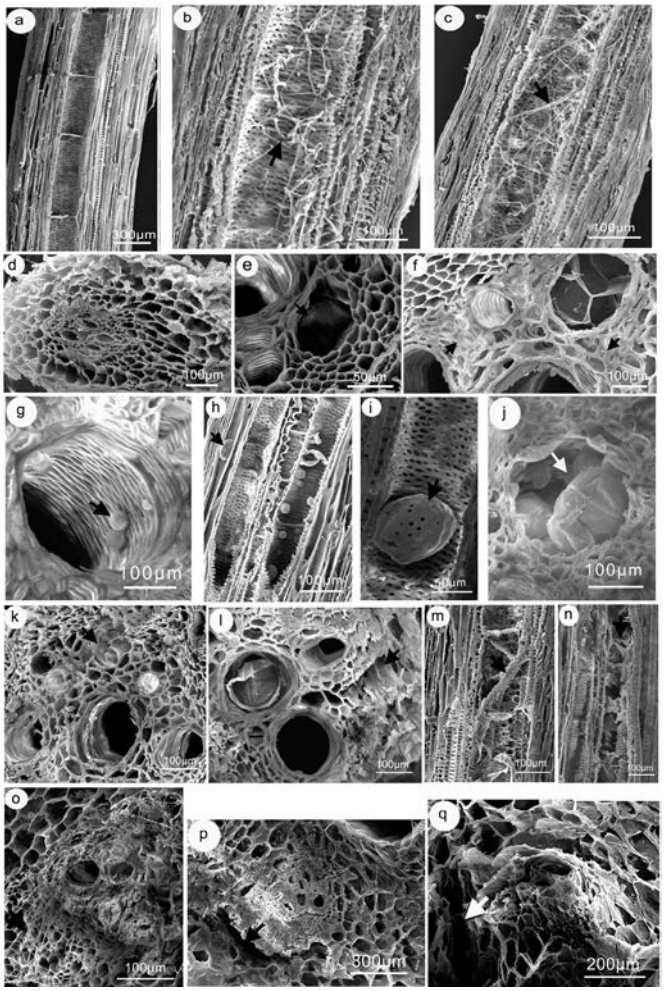

Fig. 1. Cross and longitudinal sections of roots and shoots of susceptible watermelon 'Sumi No. 1'. **a**: uninoculated root of watermelon (longitudinal section). **b**: mycelium localized in the xylem vessel elements through pit pair. **c**: mass mycelium established in xylem vessel elements at 4 d.p.i.. **d**: uninoculated root of watermelon (cross section). **e**: affected vessel occluded with gum at 12 h.a.i.. **f**: wall of parenchyma cells adjacent to colonized tissue and surrounding the vascular cylinder were thickened at 5 d.p.i.. Cross section (**g)** and longitudinal section (**h)** showing the initial formation of tyloses in affected vessel at 7 d.p.i.. **i**: a single enlarged tylosis in xylem vessel at 11 d.p.i.. **j**: multiple enlarged tyloses in xylem vessel. **k**: disintegration of the xylem parenchyma cells at 7 d.p.i.. **l**: presence of small cavity and shed of the affected vessel part. **m**: shed of the affected vessel part at 11 d.p.i.. **n**: heavy destruction of the xylem vessel elements at 15 d.p.i.. **o**: mycelium densely presence in vascular system at 11 d.p.i.. **p**: small cavity formation. **q**: enlarged cavity surrounding the affected vascular at 15 d.p.i..

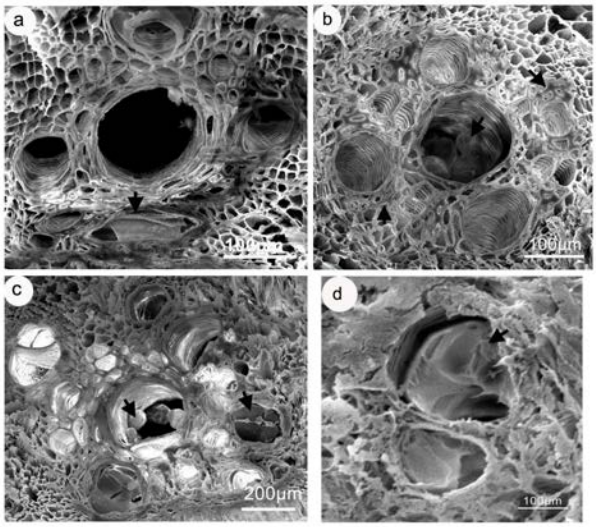

Fig. 2. Cross section of roots and shoots of resistant bottle gourd used as rootstock for watermelon. **a**: affected vessel occluded with gum at 8 h.a.i.. **b**: multiple tyloses in xylem vessel and the wall-thicken of parenchyma cells at 12 h.a.i.. **c**: defense response in combination with vascular gums, tyloses and wall-thicken of parenchyma cells at 2 d.p.i.. **d**: cross section of shoots showing a single enlarged tyloses which completely occluded the xylem vessel at 2 d.p.i.

Effects of drought on physiological and morphological features of wild and domestic Turkish watermelon genotypes

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Keywords: *C.citroides, C.colocynthis, C.lanatus, P.fistulosus*, drought, genetic resources

Abstract

This research was carried out between 2007 and 2008 to determine drought tolerance of domestic and wild watermelon genotypes in Sanliurfa (Turkey) condition. A total of 32 wild and domestic watermelon genotypes were used in this experiment. Morphological and physiological treatments such as leaf relative-water content, leaf temperature, chlorophyll contents, leaf water potential, leaf color, leaf area, number of stomata, width of stomata, length of stomata, plant height, number of nods on the plant, plant dry matter ratio, main stem diameter, turgority and final drought resistance tests were examined. Drip irrigation treatments included complete irrigation cut off, dry (I_0), full irrigation based on replenishment of soil water depleted from 0-90 cm profile (I_1) and 50 % full irrigation (I_2). At the end of the experiment, fifteen genotypes (Kar-24, Kar-25, Kar-27, Kar-59, Kar-86, Kar-114, Kar-143, Kar-147, Kar-163, Kar-185, Kar-197, Kar-203, Kar-215, Kar-218 and Kar-224) were found as tolerant and seventeen genotypes (Kar-26, Kar-35, Kar-37, Kar-39, Kar-98, Kar-99, Kar-117, Kar-140, Kar-154, Kar-177, Kar-184, Kar-212, Kar-234, Kar-243, Kar-330, Kar-332 and Kar-325) were found as intolerant.

INTRODUCTION

Drought comes from global. The climatic change causes drought in even mild climate conditions. Therefore resistance of genotypes to drought is our research's main subject. In spite of improvement in new watermelon cultivars, it is necessary studying on resistance to diseases and insects. Among C3 plants watermelon could be a very suitable model for drought studies. Southeastern Anatolia Region (GAP) has severe climatic conditions with high temperature (maximum temperature has been observed in August at 46.8 ^{o}C and a droughty climate (the lowest relative humidity was 33% in July) and still it has very extensive non irrigated area (Anonymous,

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2010). In this region local genotypes are used because of tolerance to drought. This project's goal is to evaluate all environments with high temperature.

MATERIALS AND METHODS

This research was conducted at GAP Agricultural Research Center of Turkish Ministry of Agriculture and Rural Affairs in Sanlıurfa-Turkey. This experiment was carried out on a clay textured soil (Vertic Calciorthid aridisol) during the 2007 and 2008 production season.

Thirty-two watermelon accessions were used in this study. The accessions were collected from different regions of Turkey and other countries (Sari et. al. 2007). Two of the genotypes are from the Egypt; one genotype was from Spain and wild watermelon as *Citrullus colocynthis*, *Citrullus lanatus* var. *citroides*, *Citrullus rehmii*, *Praecitrullus fistulosus* were used as controls (Table 1).

A control treatment was designated to receive 100% of soil water depletion from the top 90 cm soil profile which was measured by profile probe. Drip irrigation management treatments were (i) full irrigation based on replenishment of soil water depleted from 0-90 cm soil profile (S_1), (ii) 50% of full irrigation (S_2) and (iii) 0% irrigation (S_0). S_1 treatment was started from sowing to the harvest. S_1 and S_2 plots received 100% of soil water depletion replenishment till the initiations of irrigation regimes (true leaves). After formation of true leaves, S_2, water stress treatment, was initiated and continued till harvest. S_0, no irrigation (0% irrigation) treatment was started from sowing to the harvest.

Relative leaf-water content was measured between 13:00-15:00 hours by Formula [(Fresh Weight- Dry Weight) / (Turgor Weight- Dry Weight) × 100]. Leaf temperature was measured by infrared thermometer. Total leaf chlorophyll was measured by chlorophyll meter. A color meter was used for leaf color measurement. Leaf water potential (LWP) was measured two ways, as daily and seasonal. Leaf area measurements of all plant were measured by leaf area meter. Stomata diameter, stomata length and number of stomata were found on three leaves one time only according to Sari et al. (1999). Biomass was done from 2 cm of the root. Dry matter was measured by the formula [(Dry weight/Fresh weight/Fresh weight) × 100]. Number of nodes and main stem length was measured one time every month. 0-5 scale was used to determine plant turgor. 0.1-1 scale was used to carry out the last test. The experiment was designed and analyzed as a split plot.

RESULTS AND DISCUSSION

Physiological effects

The highest disparity leaf temperature (-40.8 ^{o}C) was found with the Kar-325 and at 0% level. The lowest disparity leaf temperature (-4.5 ^{o}C) was found with the Kar-197 with 50% level. The highest disparity of relative leaf-water content

(49.7%) was figured out in Kar-325 at 0% level, the lowest disparity of leaf relative water content (8.1%) was found in Kar-27 at 50% level. The highest disparity of chlorophyll content (69.3%) was found the Kar-37 0% level, the lowest disparity in chlorophyll content (9%) was found with Kar-215 at 50% level (Table 2). The highest daily leaf water potential disparity was found (-421%) with Kar-149 at 0 % level. The lowest daily leaf water potential disparity was found (-39.0%) with Kar-37 in 50% level. Seasonal leaf water potential changes the highest disparity data was calculated (311.0%) with Kar-149 at 0% level. The lowest disparity seasonal leaf water potential was found (-241.3%) with Kar-24 at 0 % level (Table 3).

Morphological effects

The highest disparity number of nodes was found (44.0%) with Kar-234 at 0% level. The lowest disparity number of nodes was figured out (1.3%) with Kar-203 at 50% level. The highest disparity ratio of biomass was calculated (10.9%) with Kar-24 at 50% level. The lowest disparity ratio of biomass was found (-221.7%) with Kar-114 genotype at 0% level. The highest disparity length was figure out (64.8%) with Kar-37 genotype at 0% level. The lowest disparity length was calculated (1.1%) with Kar-98 at 50% level. The highest disparity main stem diameter was found (49.4%) with Kar-26 at 0% level. The lowest disparity main stem diameter was figure out (-15.4%) with Kar-59-A genotype at 50% level (Table 4). The highest disparity leaf area was found (8.9%) with Kar-224 at 50% reduction of irrigation plot. The highest disparity number of stomata was found (-70.9%) with Kar-149 at 0% level. The lowest disparity number of stomata was found (-2.3) with Kar-330 at 50% level. The highest disparity in stomatal length was found (27.1%) with Kar-25 at 0% level. The lowest disparity in stomatal length was found (-12.2%) with Kar-59-A at 50% level. The highest weight of stomata was (29.0%) with Kar-39 at 0% level. The lowest weight of stomata was calculated (-13.6%) with Kar-215 at 50% level. The highest turgor was calculated at 0% level, the lowest turgority was found at the full irrigation (Table 5). The lowest L data was found (25) with Kar-330 at 0% level. The highest chrome data was 28.9 with Kar-330 at 0% level. The highest hue data was found (-61.6) with Kar-197 in the 0% level. The lowest hue data was calculated (-26.8) with Kar-212 at 0% level (Table 2-3). According to the last resistance test; Kar-24 and Kar-25 were found the most tolerant genotypes. The second most tolerant group consisted Kar-27, Kar-59-A, Kar-86-A, Kar-114, Kar-143, Kar-147, Kar-163, Kar-185, Kar-197, Kar-203, Kar-215, Kar-218 and Kar-224 genotypes. Kar-35 and Kar-39 fell into (0.5) a third group. Kar-37 and Kar-99 were found in a fourth group. Kar-26, Kar-98, Kar-117, Kar-154, Kar-177, Kar-184, Kar-212, Kar-234, Kar-243, Kar-330, Kar-332 and Kar-325 genotypes were found in the least tolerant group (Table 3). The results of this study showed a similarity with research by Lefi et al. (2004). The results of this experiment showed

a similarity with research and result of Gallardo et al. (2004) in terms of relative leaf water content. Leaf area was getting smaller relative to water stress level. Similar results were found by Liu and Stützel (2004) in terms of leaf area. The results of this experiment showed a similarity with research and the results of Kuo et al. (2004) in terms of number of stoma. In this experiment biomass increased during drought. This result was similar to the results of Liu and Stützel (2004).

CONCLUSIONS

At the end of the experiment; Kar-24, Kar-25, Kar-27, Kar-59, Kar-86, Kar-114, Kar-143, Kar-147, Kar-163, Kar-185, Kar-197, Kar-203, Kar-215, Kar-218 and Kar-224 were found as tolerant and seventeen genotypes; Kar-26, Kar-35, Kar-37, Kar-39, Kar-98, Kar-99, Kar-117, Kar-140, Kar-154, Kar-177, Kar-184, Kar-212, Kar-234, Kar-243, Kar-330, Kar-332 and Kar-325 were found as intolerant genotypes.

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Table 1. The watermelon genetic resources used in this study and their origins

Accession No (Tolerant)	Donor Identif. No / Name	Origin
Kar-24	No Name	Egypt
Kar-218	Kara karpuz	Elmacık-Canakkale-TR
Kar-25	No Name	Egypt
Kar-27	Surme	Diyarbakır-TR
Kar-197	No Name	Silivri-Istanbul-TR
Kar-59-A	TR 48544	Ege Agric.Inst.-TR
Kar-203	No Name	Usak –TR
Kar-224	No Name	Kızıltepe-Mardin-TR
Kar-86 A	TR 66064	Ege Agric.Inst.-TR
Kar-215	Akkarpuz	Aşagiokcular-Canakkale-TR
Kar-147	Medine Karpuzu	Bozova –Sanliurfa –TR
Kar-185	No Name	Akhisar-Manisa –TR
Kar-163	Gelin karpuzu	Kurtalan-Siirt-TR
Kar-114	No Name	Cizre-Sirnak-TR
Kar-143	No Name	Bozova-Sanliurfa-TR
Intolerant	**Donor Identif. No / Name**	**Origin**
Kar-212	No Name	Karapinar-Konya-TR
Kar-154	No Name	Batman –TR
Kar-117	No Name	Viranşehir –Sanliurfa-TR
Kar-149	Beyaz kislik karpuz	Erimli-Diyarbakır-TR
Kar-26	Pastéque à chair verte	Spain
Kar-184	No Name	Soma-Manisa-TR
Kar-177	No Name	Barbaros-Tekirdag-TR
Kar-243	Zerzuri	Hatay-TR
Kar-98	No Name	Birecik-Sanliurfa-TR
Accession No	**Donor Identif. No / Name**	**Origin**
Kar-234	PI 296 341	Seminis seed USA
Kar-325	PI 270564 04 SD (*C. lanatus var. citroides*)	South Africa (USDA, USA)
Kar-330	PI 632755 (*Citrullus colocynthis*)	France (USDA, USA)
Kar-332	PI 179660 01 SD *(Praecitrullus fistulosus)*	India Uttar Pradesh (USDA, USA)
Commercial Var.	**Donor Identif. No / Name**	**Origin**
Kar-39	Crimson Sweet	Commercial Variety
Kar-35	Sugar Baby	Old Commercial Variety
Kar-37	Halep Karasi	Old Commercial Variety
CT	Crimson Tide Fı	Common Commercial Hybrid

Table 2. Leaf temperature, relative leaf water content, Leaf color and chlorophyll contents of fully irrigated (control) watermelons and rate of reduction with 50 and 0 % irrigation level in watermelon genotypes

Genotypes	Leaf Color			Leaf temperature			R. leaf water content (%)			Chlorophyll contents (index)		
	0%											
	L	Chr.	Hue	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%
Kar-24	53	22.2	-41.5	29.8	-9.0	-22.2	76.8	15.0	26.2	458.5	23.8	51.3
Kar-25	53	22.8	-41.1	31.3	-4.6	-19.7	79.6	16.5	27.1	419.8	13.0	44.5
Kar-26	47	24.9	-28.8	29.6	-12.6	-28.0	79.8	13.0	45.9	357.3	-12.9	60.8
Kar-27	49	23.6	-47.4	30.3	-6.9	-17.3	79.8	8.1	28.8	399.3	21.7	42.4
Kar-35	42	25.4	-60.0	31.3	-7.1	-21.4	80.6	26.8	44.0	390.2	-7.9	39.8
Kar-37	36	25.4	59.0	30.1	-11.6	-25.4	78.9	11.8	43.9	447.8	-11.3	69.3
Kar-39	46	25.4	-58.2	29.6	-14.5	-29.1	79.2	12.3	37.4	389.5	-20.3	47.1
Kar-59	46	20.8	-51.1	30.7	-6.2	-23.0	78.3	10.0	23.7	458.1	23.6	47.3
Kar-86	51	20.3	-48.4	29.9	-5.4	-19.9	79.8	13.5	30.2	434.2	21.5	48.2
Kar-98	27	26.1	-29.4	29.1	-16.6	-39.0	77.4	11.7	38.1	486.5	-7.0	57.9
Kar-99	41	24.7	-56.4	29.6	-10.7	-26.6	79.3	16.5	37.2	394.8	-9.6	68.9
Kar-114	50	23.4	-51.4	29.9	-9.5	-23.5	80.8	12.9	27.9	478.0	21.1	52.1
Kar-117	28	26.4	-33.3	29.8	-12.0	-30.0	78.7	17.9	40.4	492.0	-5.2	66.7
Kar-143	48	22.5	-51.9	29.7	-10.4	-23.5	81.9	10.3	26.8	432.4	22.1	46.5
Kar-147	49	22.9	-51.4	30.4	-8.9	-20.6	76.3	12.2	30.0	476.7	21.7	53.5
Kar-149	34	22.5	-37.2	30.3	-14.8	-25.4	80.1	10.2	39.4	546.7	28.9	56.8
Kar-154	29	27.6	-30.3	30.5	-13.0	-25.0	82.2	21.0	46.6	387.7	-29.6	61.8
Kar-163	50	23.3	-51.6	30.6	-8.8	-23.2	80.5	10.9	26.8	381.5	-20.3	47.7
Kar-177	26	26.6	-28.0	33.5	-21.4	-21.4	79.0	10.6	38.0	564.3	33.0	64.8
Kar-184	26	26.8	-28.8	29.0	-18.7	-39.5	79.9	10.8	35.9	492.2	-6.8	56.8
Kar-185	44	25.0	-52.7	30.6	-8.1	-19.1	79.1	13.0	30.0	417.3	-16.5	43.5
Kar-197	48	24.3	-61.6	30.6	-4.5	-21.3	75.2	12.2	28.2	471.5	18.6	50.5
Kar-203	46	24.1	56.0	30.6	-5.4	-25.7	77.7	14.6	29.2	448.5	20.2	49.5
Kar-212	26	27.5	-26.8	29.1	-17.2	-31.1	78.8	14.8	42.6	476.3	22.0	62.1
Kar-215	45	23.5	-50.9	29.9	-7.7	-24.2	79.4	21.1	33.1	395.2	9.0	45.8
Kar-218	46	22.8	-60.9	30.6	-9.8	-21.1	76.5	9.8	28.3	496.5	28.6	54.7
Kar-224	46	25.3	-52.9	30.8	-6.6	-20.0	79.9	15.1	33.8	431.7	17.9	39.1
Kar-234	32	21.9	-35.5	29.1	-12.6	-31.2	77.8	14.8	37.5	493.2	27.6	64.2
Kar-243	28	27.0	-31.2	34.4	-14.6	-16.2	78.7	13.0	41.7	428.0	-26.4	68.2
Kar-330	25	28.9	-33.4	29.2	-17.7	-33.7	78.1	17.0	45.7	450.7	12.3	67.3
Kar-332	36	13.9	-42.7	27.7	-18.3	-40.1	76.6	12.1	43.1	362.7	-7.6	58.2
Kar-325	33	21.8	-37.0	28.3	-21.5	-40.8	79.0	17.1	49.7	436.8	-17.2	67.3

Table 3. Daily leaf water potential, seasonal leaf water potential, the last resistant test, Leaf color fully irrigated (control) watermelons and rate of reduction with 50 and 0 % irrigation level in watermelon genotypes

Genotypes	The Last Resistant Test	Leaf Color 50%			Daily Leaf Water Potential (%)			Seasonal Leaf Water Potential (%)			Leaf Color Control		
		L	Chr.	Hue	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%	L	Chr.	Hue
Kar-24	1	57	20	-41.7	-0.50	-66.0	-226.0	-0.80	-50.0	-241.3	64	18	-44.8
Kar-25	1	58	20	-43.0	-0.47	-48.9	-176.6	-0.90	-44.4	-233.3	64	18	-46.6
Kar-26	0.1	56	20	-42.8	-1.03	-62.1	-268.9	-3.27	-31.5	-108.0	61	18	-56.1
Kar-27	0.8	57	24	43.7	-0.50	-86.0	-240.0	-1.50	-60.0	-140.0	58	23	-43.8
Kar-35	0.5	53	19	-42.9	-0.67	-74.6	-183.6	-2.83	-13.1	-56.5	59	19	-48.6
Kar-37	0.3	54	19	-42.3	-0.77	-39.0	-133.8	-2.83	-30.7	-79.2	59	20	-52.5
Kar-39	0.5	56	24	-50.3	-0.97	-51.5	-113.4	-2.67	-31.1	-69.7	59	22	-48.7
Kar-59	0.8	56	25	-44.9	-0.63	-74.6	-169.8	-1.90	-35.3	-89.5	59	23	-45.2
Kar-86	0.8	56	25	-45.0	-0.43	-62.8	-295.3	-0.97	-103	-212.4	58	24	-44.9
Kar-98	0.1	53	23	-41.8	-0.67	-104.5	-347.8	-2.07	-65.7	-152.7	59	21	-51.8
Kar-99	0.3	55	23	-48.7	-0.73	-128.8	-397.3	-2.70	-30.7	-69.3	59	22	-49.4
Kar-114	0.8	56	24	-44.4	-0.73	-78.1	-156.2	-2.23	-40.4	-88.3	62	22	-45.2
Kar-117	0.1	50	21	-44.6	-0.70	-85.7	-404.3	-2.67	-42.3	-138.6	59	21	-49.8
Kar-143	0.8	54	23	-46.5	-0.80	-50.0	-158.8	-2.13	-45.5	-98.6	57	21	-45.8
Kar-147	0.8	56	23	-45.5	-0.40	-125.0	-342.5	-1.03	-97.1	-227.2	59	22	-45.4
Kar-149	0.1	55	20	-46.5	-0.60	-95.0	-421.7	2.83	241.3	311.0	63	21	-54.2
Kar-154	0.1	53	21	-47.1	-1.13	-65.5	-215.9	-2.47	-40.5	-127.9	59	20	-53.6
Kar-163	0.8	55	23	-44.8	0.77	229.9	333.8	2.27	217.6	301.3	60	22	-44.8
Kar-177	0.1	54	22	-42.3	-0.80	-146.3	-312.5	-3.00	-41.0	-119.0	60	20	-50.9
Kar-184	0.1	55	22	-42.2	-0.73	-74.0	-334.2	-3.13	-39.6	-111.8	60	20	-52.3
Kar-185	0.8	53	25	-48.8	-0.70	-100.0	-190.0	-1.20	-91.7	-194.2	57	22	-51.7
Kar-197	0.8	56	24	-46.7	-0.57	-70.2	-193.0	-1.30	-63.8	-179.2	62	23	-47.2
Kar-203	0.8	55	24	-46.9	-0.60	-111.7	-221.7	-1.30	-66.9	-159.2	59	22	-49.1
Kar-212	0.1	53	21	-43.5	-1.17	-99.1	-188.0	-2.17	-79.7	-191.7	58	20	-51.1
Kar-215	0.8	53	25	-45.2	-0.50	-140.0	-240.0	-1.60	-48.1	-137.5	57	23	-46.4
Kar-218	0.8	54	23	-48.3	-0.67	-59.7	-168.7	-1.30	-25.4	-161.5	58	19	-54.2
Kar-224	0.8	54	24	-47.5	-0.60	-111.7	-245.0	-1.47	-74.8	-160.5	58	23	-46.4
Kar-234	0.1	51	20	-47.1	-0.80	-178.8	-316.3	-3.33	-32.1	-94.3	59	18	-49.1
Kar-243	0.1	52	21	-47.5	-0.73	-91.8	-324.7	-3.03	-35.3	-110.2	61	20	-48.3
Kar-330	0.1	52	20	-45.0	-0.60	-171.7	-395.0	-3.10	-39.7	-124.8	64	16	-55.9
Kar-332	0.1	58	20	-47.7	-1.33	-80.5	-150.4	-3.00	-57.7	-131.0	65	17	-42.4
Kar-325	0.1	54	20	-49.7	-0.67	-168.7	-377.6	-3.33	-44.1	-106.3	61	18	-50.1

Table 4. Number of nodes, ratio of biomass, length of plant and main stem diameter fully irrigated (control) watermelons and rate of reduction with 50 and 0 % irrigation level in watermelon genotypes

Genotypes	Number of Nodes			Ratio of Biomass			Length of Plant			Main Stem Diameter (mm)		
	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%
Kar-24	33.3	-4.6	-15.2	23.7	10.9	-7.9	315.3	15.6	19.7	19.3	17.8	23.5
Kar-25	32.0	-5.4	-15.1	21.9	4.8	-12.5	307.0	8.2	19.7	19.2	16.0	20.8
Kar-26	50.0	28.1	33.2	16.6	-13.8	-32.6	331.5	11.1	26.7	24.9	52.1	49.4
Kar-27	38.2	16.0	6.9	15.8	-17.9	-87.2	226.2	12.3	22.8	16.5	21.0	11.6
Kar-35	41.8	8.3	-5.7	16.2	-1.7	-105.3	193.8	15.7	29.5	17.1	19.9	35.5
Kar-37	48.0	27.3	38.9	13.6	-36.4	-98.7	252.5	16.5	64.8	15.6	11.1	49.1
Kar-39	46.7	21.8	29.1	15.8	-23.2	-86.4	183.2	7.0	43.5	16.0	9.4	11.4
Kar-59	41.7	7.3	15.6	19.7	8.2	-75.7	286.2	15.7	34.9	12.7	-15.4	-12.0
Kar-86	34.2	13.7	21.4	11.9	-59.3	-229.5	246.7	15.4	45.7	15.0	6.8	41.8
Kar-98	44.0	28.6	32.3	13.7	-13.9	-110.9	225.3	1.1	51.5	19.2	27.8	30.2
Kar-99	43.8	33.6	38.9	17.0	-1.9	-46.6	214.8	7.8	47.7	21.0	35.3	31.6
Kar-114	41.5	10.8	6.2	13.2	-20.2	-251.7	242.3	11.9	25.2	17.3	13.7	30.7
Kar-117	42.0	28.1	38.5	18.9	-3.4	-54.1	201.5	1.3	39.3	17.3	14.8	22.0
Kar-143	44.7	15.7	13.9	15.5	-7.0	-183.5	287.5	18.5	34.0	20.7	18.5	37.4
Kar-147	31.7	7.7	11.6	13.2	-63.0	-165.0	234.8	29.3	54.4	16.3	11.1	30.3
Kar-149	45.8	19.4	25.4	15.9	-29.4	-68.0	217.2	16.2	31.9	18.6	21.3	31.5
Kar-154	39.7	26.0	17.5	18.5	10.1	-13.7	204.5	11.9	12.5	18.3	24.6	23.5
Kar-163	42.0	10.6	6.9	17.3	-4.3	-171.8	263.3	21.4	32.9	18.5	16.8	35.2
Kar-177	42.2	15.7	21.3	15.4	-17.3	-85.7	211.0	13.5	61.5	18.8	23.4	27.0
Kar-184	41.0	20.7	27.1	14.9	-16.2	-83.1	229.3	14.9	60.0	15.9	12.4	15.2
Kar-185	43.0	9.6	6.2	16.1	-9.1	-124.0	210.3	5.4	15.7	16.0	12.7	25.6
Kar-197	40.5	6.8	15.1	19.7	-15.0	-81.4	291.2	17.5	36.1	13.1	1.5	8.8
Kar-203	39.2	1.3	17.8	14.6	-29.4	-124.7	250.3	-5.0	35.3	12.3	8.7	20.0
Kar-212	38.8	22.9	14.0	16.1	-67.7	-139.7	242.5	-6.3	31.1	18.5	35.3	24.9
Kar-215	51.0	2.0	21.2	19.7	8.8	-46.0	252.5	5.3	31.8	15.4	23.3	21.1
Kar-218	44.0	14.3	22.9	17.4	-3.5	-76.8	285.2	22.4	34.7	12.0	13.0	-5.4
Kar-224	47.7	11.0	25.5	16.5	-13.8	-108.1	247.0	11.7	15.8	13.6	-13.0	-1.4
Kar-234	48.8	28.6	44.0	16.3	-36.1	-74.2	233.8	10.6	34.0	19.7	33.8	9.7
Kar-243	48.3	33.7	26.4	11.7	-74.6	-61.6	234.2	24.1	33.8	24.1	29.6	-5.0
Kar-330	32.7	20.2	32.9	7.7	-6.9	-11.1	190.0	15.4	24.5	23.4	43.0	47.7
Kar-332	47.3	29.5	35.3	16.2	-2.5	-34.5	295.7	27.3	54.3	24.3	36.2	53.8
Kar-325	50.0	29.9	34.0	18.8	-3.8	-25.1	372.0	6.5	36.9	9.7	12.1	25.9

Table 5. Number of stoma, length of stoma, weight of stoma, leaf area and turgorite fully irrigated (control) watermelons and rate of reduction with 50 and 0% irrigation level in genotypes

Genotypes	Number of Nodes			Ratio of Biomass			Length of Plant			Main Stem Diameter (mm)		
	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%	Control	Disparity at 50%	Disparity at 0%
Kar-24	33.3	-4.6	-15.2	23.7	10.9	-7.9	315.3	15.6	19.7	19.3	17.8	23.5
Kar-25	32.0	-5.4	-15.1	21.9	4.8	-12.5	307.0	8.2	19.7	19.2	16.0	20.8
Kar-26	50.0	28.1	33.2	16.6	-13.8	-32.6	331.5	11.1	26.7	24.9	52.1	49.4
Kar-27	38.2	16.0	6.9	15.8	-17.9	-87.2	226.2	12.3	22.8	16.5	21.0	11.6
Kar-35	41.8	8.3	-5.7	16.2	-1.7	-105.3	193.8	15.7	29.5	17.1	19.9	35.5
Kar-37	48.0	27.3	38.9	13.6	-36.4	-98.7	252.5	16.5	64.8	15.6	11.1	49.1
Kar-39	46.7	21.8	29.1	15.8	-23.2	-86.4	183.2	7.0	43.5	16.0	9.4	11.4
Kar-59	41.7	7.3	15.6	19.7	8.2	-75.7	286.2	15.7	34.9	12.7	-15.4	-12.0
Kar-86	34.2	13.7	21.4	11.9	-59.3	-229.5	246.7	15.4	45.7	15.0	6.8	41.8
Kar-98	44.0	28.6	32.3	13.7	-13.9	-110.9	225.3	1.1	51.5	19.2	27.8	30.2
Kar-99	43.8	33.6	38.9	17.0	-1.9	-46.6	214.8	7.8	47.7	21.0	35.3	31.6
Kar-114	41.5	10.8	6.2	13.2	-20.2	-251.7	242.3	11.9	25.2	17.3	13.7	30.7
Kar-117	42.0	28.1	38.5	18.9	-3.4	-54.1	201.5	1.3	39.3	17.3	14.8	22.0
Kar-143	44.7	15.7	13.9	15.5	-7.0	-183.5	287.5	18.5	34.0	20.7	18.5	37.4
Kar-147	31.7	7.7	11.6	13.2	-63.0	-165.0	234.8	29.3	54.4	16.3	11.1	30.3
Kar-149	45.8	19.4	25.4	15.9	-29.4	-68.0	217.2	16.2	31.9	18.6	21.3	31.5
Kar-154	39.7	26.0	17.5	18.5	10.1	-13.7	204.5	11.9	12.5	18.3	24.6	23.5
Kar-163	42.0	10.6	6.9	17.3	-4.3	-171.8	263.3	21.4	32.9	18.5	16.8	35.2
Kar-177	42.2	15.7	21.3	15.4	-17.3	-85.7	211.0	13.5	61.5	18.8	23.4	27.0
Kar-184	41.0	20.7	27.1	14.9	-16.2	-83.1	229.3	14.9	60.0	15.9	12.4	15.2
Kar-185	43.0	9.6	6.2	16.1	-9.1	-124.0	210.3	5.4	15.7	16.0	12.7	25.6
Kar-197	40.5	6.8	15.1	19.7	-15.0	-81.4	291.2	17.5	36.1	13.1	1.5	8.8
Kar-203	39.2	1.3	17.8	14.6	-29.4	-124.7	250.3	-5.0	35.3	12.3	8.7	20.0
Kar-212	38.8	22.9	14.0	16.1	-67.7	-139.7	242.5	-6.3	31.1	18.5	35.3	24.9
Kar-215	51.0	2.0	21.2	19.7	8.8	-46.0	252.5	5.3	31.8	15.4	23.3	21.1
Kar-218	44.0	14.3	22.9	17.4	-3.5	-76.8	285.2	22.4	34.7	12.0	13.0	-5.4
Kar-224	47.7	11.0	25.5	16.5	-13.8	-108.1	247.0	11.7	15.8	13.6	-13.0	-1.4
Kar-234	48.8	28.6	44.0	16.3	-36.1	-74.2	233.8	10.6	34.0	19.7	33.8	9.7
Kar-243	48.3	33.7	26.4	11.7	-74.6	-61.6	234.2	24.1	33.8	24.1	29.6	-5.0
Kar-330	32.7	20.2	32.9	7.7	-6.9	-11.1	190.0	15.4	24.5	23.4	43.0	47.7
Kar-332	47.3	29.5	35.3	16.2	-2.5	-34.5	295.7	27.3	54.3	24.3	36.2	53.8
Kar-325	50.0	29.9	34.0	18.8	-3.8	-25.1	372.0	6.5	36.9	9.7	12.1	25.9

Lycopene content and expression of phytoene synthase and lycopene β-cyclase genes in tetraploid watermelon

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Keywords: Tetraploid watermelon, different tissues, fruit development, gene expression, lycopene accumulation

Abstract

Watermelon (*Citrullus lanatus*) is one of the few crop that provide high lycopene to the human diet. In this paper, tetraploid watermelon was used to elucidate the molecular basis of lycopene accumulation and the gene expression levels of phytoene synthase (PSYA) and lycopene b-cyclase (LCYB). High performance liquid chromatography analysis was applied for the determination of lycopene content. Real-time polymerase chain reaction analysis indicated that the PSYA and LCYB genes were expressed in all the analyzed different organs of tetraploid watermelons with varied expression levels, particularly high in the flowers of watermelon. During fruit maturation, the expression levels of PSYA and LCYB increased during the mid-stages but decreased in the fully mature fruit. In addition, lycopene was accumulated as the flesh changed from white to red. Thus, PSYA and LCYB expression are correlated with lycopene accumulation during fruit maturation. Ripe fruit had higher lycopene concentrations than unripe fruit. Then we hope to provide a theoretical basis for optimization of high-lycopene watermelon breeding.

INTRODUCTION

Watermelon (*Citrullus lanatus*, family Cucurbitaceae) is a fruit characterized by a smooth external rind (green or yellow) and a juicy, sweet, red colored flesh. Nowadays, hundreds watermelon cultivars, both seeded and seedless are used commercially. The red color imparted to watermelon is due to the carotenoid pigment lycopene, an unsaturated hydrocarbon (11 conjugated and 2 non-conjugated double bonds) with an isoprenoid polyene chain structure made up of 40 carbons and 56 hydrogen atoms (Ronen et al. 1999). Because of the established beneficial effects of lycopene (free-radical scavenger) on human health, lycopene is often used as a food supplement as well as a natural food colorant (CondéNet, Inc. 2008). The

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plant matrix is an important factor for the digestion and absorption of lycopene from the gastrointestinal tract. For example, the consumption of watermelon juice has been shown to cause significant increase in lycopene concentration in human plasma (Alison et al. 2003). The health benefits resulting from a lycopene rich diet (Gerster 1997) include reduced incidence of cardiovascular diseases, lower risk for contracting some types of cancer (for example prostate cancer), improvement of bone mineral density and of sperm mobility, and better protection of skin exposed to UV radiation (Chen et al. 2001; Hadley et al. 2002, 2003; Andreassi et al. 2004; Stahl et al. 2001; Wattanapenpaiboon et al. 2003; Gupta and Kumar 2002).

Profiles and the concentrations of carotenoids of red-fleshed watermelons have been studied extensively (Collins and Perkins-Veazie 2006; Perkins-Veazie et al. 2001; Perkins-Veazie and Collins 2006a). The outcome of the study conducted on 50 watermelon cultivars (Perkins-Veazie et al. 2006b) showed that: (i) the total concentration of carotenoids in watermelon varies between 37 and 122 mg/kg and (ii) that lycopene is the most abundant (84–97%) carotenoid in watermelon. Lycopene is present in the fruit predominantly in the trans-form; the major isomers are 13-cis- and 5-cis-lycopene. The concentration of lycopene of different ploidy watermelons was determined (Wan and Liu 2011; Liu 2010; Wang and Dang 2009; Perkins-Veazie et al. 2001; Perkins-Veazie et al. 2006c). The results showed that lycopene content was increased in tetraploid watermelons compared with the diploid; seedless watermelon tended to have higher lycopene (>50.0ug. g-1fresh weight) than seeded types.

The carotenoid biosynthetic pathway has been studied extensively. In the first step of the carotenoid biosynthetic pathway (Fig. 1), phytoene synthase (PSY) catalyses the condensation of two geranylgeranyl diphosphates (GGDP) to produce a C40 phytoene. Then, two desaturases, phytoene desaturase (PDS) and z-carotene desaturase (ZDS), add four double bonds to form lycopene. Subsequently, the pathway divides into two branches. In one branch, an e- and a b-ring are added to lycopene to form a-carotene, which is used to synthesize lutein. In the other branch, two b-rings are added to lycopene to produce b-carotene, which is used to synthesize zeaxanthin, antheraxanthin and violaxanthin. The genes encoding almost all of the enzymes in the carotenoid biosynthetic pathway have been cloned and characterised from various plants (Castillo and Gomez 2005; Clotault et al. 2008; Guo et al. 2009; Kato et al. 2004; Ronen et al. 1999). In particular, PSY and PDS have important roles in controlling the flux in the carotenoid biosynthetic pathway. For example, during tomato fruit maturation, increased expression of PSY and PDS and decrease of LCYB are correlated with accumulation of lycopene (Bramley 2002; Cunningham 2002) and total carotenoid content (Fraser et al. 2002). In contrast, disruption of PDS in Arabidopsis decreased carotenoid biosynthesis (Qin et al. 2007).

In this study, the relationship between lycopene accumulation and the gene

expression of PSY and LCYB was investigated in different organs of and stages of fruit ripening in tetraploid watermelon. The goal of the present study was to investigate the role of transcriptional regulation of carotenoid related genes in carotenoid accumulation in watermelon.

MATERIALS AND METHODS

Plant material and fruit treatment

Autotetraploid watermelon seeds were obtained from the Research Group of seedless watermelon, Zhengzhou fruit research institute, CAAS and stored at 4°C. Watermelon seeds were germinated in a greenhouse, and then the seedlings were transferred to the experimental farm at Zhengzhou, Henan. The flowers (male flowers and female flowers) were capped the afternoon before flowers opened, self-pollinated, pollinated and then marked to identify fruit of a known developmental age. For the variety under this study, it usually takes 30 d for the development of watermelon fruit. Fruit were harvested 10 d, 15 d, 20 d, 25 d, 30 d respectively after pollination. Sampled flesh was separated from fruit rind and seeds, cut into small pieces, frozen in liquid nitrogen, and stored at -79 ^{o}C until requirement for total RNA extraction. The roots, stems, leaves, flowers, and fruit of autotetraploid watermelon were collected and stored at -79 ^{o}C.

Real-time polymerase chain reaction analysis of autotetraploid watermelon phytoene synthase and lycopene b-cyclase

Total RNAs from the organs of watermelon was extracted according to the method described by Li and Guo (2004), and then reverse transcribed to cDNA with the RevertAid^{TM} First Strand cDNA SynthesisKit (Fermentas). The Primer premier 5.0 program was used to design primers for quantitative real-time polymerase chain reaction (qRT-PCR) based on the watermelon PSY (GenBank Accession Number DQ494214) and LCYB (GenBank Accession Number EF014290.1) cDNA sequences (Table S2). The watermelon 18S rRNA gene (GenBank Accession Number AB490410), as a housekeeping gene, was used as an internal reference. Quantification was achieved by normalizing the number of target gene copies to a reference 18S rRNA gene by using the comparative Ct method. The ΔCt was calculated by subtracting the average Ct value of each tissue type from the average Ct values of 18S rRNA. The ΔΔCt was calculated by subtracting the ΔCt of each of the five fruit stages from the ΔCt of the fruit tissue. The formula $2^{-(\Delta\Delta Ct)}$ was used to calculate a relative fold change between the five fruit developing stages and different organs. Real-time PCR was performed on a Roche LightCycler 2.0 Real-Time PCR system with the SYBR Green q PCR Mix kit. Cycling conditions were 94°C for 1 min, followed by 40 cycles of 95°C for 15 sec, 57°C for 15 sec, and 72°C for 45 sec. Melting curves were performed at the end of each reaction run to detect

primer dimers. All experiments were performed in triplicate. The experimental conditions were similar as previously described (Tuan et al. 2010).

Extraction and high performance liquid chromatography analysis of lycopene from autotetraploid watermelon

Frozen watermelon tissue (3 g/sample) was thawed and pureed using an electric glass homogenizer (DY89-2, Ningbo Scientz Biotechnology Co., Ltd, China) to break cell walls. The sample was diluted by adding 5 ml absolute alcohol. The diluted sample was then added to 20 ml absolute alcohol, 30 ml methanol and 80 ml 2% dichloromethane + petroleum ether mixture to extract lycopene. Once extraction was sufficient to completely remove the color, the extracted solution was finally added to 100 ml.

For high performance liquid chromatography analysis (HPLC), the lycopene samples were separated on a waters HPLC system with a Wonda Sil C18 column (250× 4.6 mm, 5μm) and detected at 502 nm. Solvent consisted of methanol/ acetonitrile / dichloromethane water (20:75:5 v/v/v). The flow rate was maintained at 1 ml min^{-1}. Identification and peak assignment of lycopene were primarily based on comparison of their retention time and UV-visible spectrum data with that of standards (Howe and Tanumihardjo 2006) as well as spectral information from the literature (Table 1, Rodriguez-Amaya 2001) and quantified according to standard curves.

Statistical analysis

Each result shown in the figure or table was the mean of three replicated treatments. The significant differences between treatments were statistically evaluated by standard deviation.

RESULTS AND DISCUSSION

Dynamic changes of lycopene content during fruit development

In order to understand the relationship between carotenoid content and gene expression, HPLC was used for lycopene analysis in this study. HPLC results can be seen in figure 1. Lycopene, a red carotenoid, started to accumulate at the stage when the pulp began to turn red (15 days after pollination). The amount of lycopene in watermelon fruit was correlated with intensity of visualized red during fruit maturation from white to red. For example, lycopene was more abundant in stage 5 fruit than in less mature fruit. Lycopene was not detected in stage 1 or stage 2 when fruit flesh is white, at stage 3 when the fruit started to change color lycopene content was 5.05 mg/kg·FW, increased to 18.49 mg/kg·FW in stage 4 and 39.63 mg/kg·FW in stage 5 matching the increase of visualized red color (Fig. 1 and Fig. 3).

The red fruit of watermelon is rich in lycopene, which may reduce the risk of heart disease and cancer (Botella-Pav and Rodríguez 2006; Giovannucci

1999; Kritchevsky 1999). Previously, Liu (2011) and Wan (2010) showed by the UV-visible spectrophotometric method that lycopene accumulateS during the development of watermelon fruit. In this study we determined lycopene content by HPLC. We also extended the results by comparing lycopene content and expression of PSYA and LCYB in the carotenoid biosynthetic pathway during fruit maturation. As shown in Fig. 1 and Fig. 3, the expression of PSYA and LCYB was associated consistently with the accumulation of lycopene during fruit maturation in watermelon. In particular, lycopene was only found in stage 4 to 5 when the fruit flesh was red, along with a high level expression of PSYA and LCYB. The absence of lycopene in the early stages of fruit maturation was probably due to its conversion to downstream biosynthetic products, or due to the lycopene content being lower than the detectable minimum of the method.

This result suggests that PSYA and LCYB regulate the lycopene branch of carotenoid biosynthesis. In addition, the similar expression patterns of PSYA and LCYB during fruit maturation may be due to a common mechanism that regulates the transcription of carotenoid biosynthesis genes.

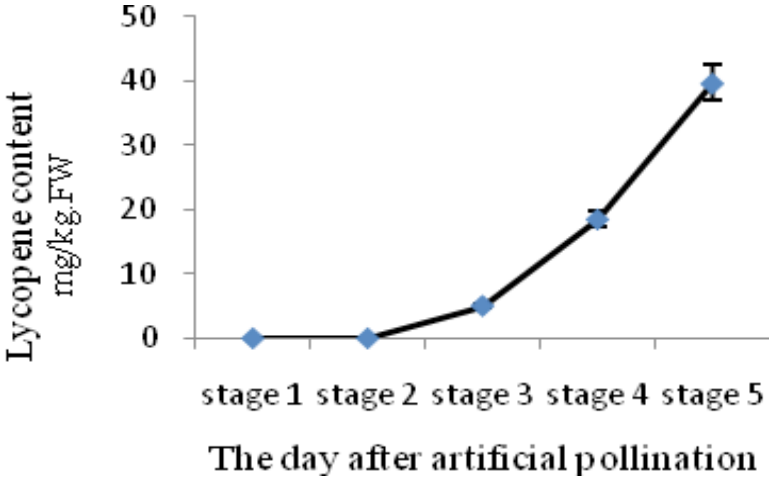

Fig. 1. Changes in lycopene content during fruit development.

Gene expression of phytoene synthase (PSYA) and lycopene b-cyclase (LCYB) in different organs of autotetraploid watermelon

The expression levels of PSYA and LCYB was higher in male flowers than in other organs except fruit (Fig. 1). Moreover, the relative expression (RQ) of PSYA in male flowers (111.43) was higher than that in female flowers (52.47). Also, the RQ of LCYB in male flowers (153.63) was higher than that in female flowers (24.99) (Fig.1). PSYA and LCYB were expressed at moderate levels in the stem, ovary, and leaves. The lowest expression levels were in root.

In plants, the abundance of carotenoids varied in different tissues. For example,

the flowers of most plants contain large amounts of carotenoid (Yamamizo et al. 2009). In this study, the expression levels of PSYA and LCYB were higher in the flowers, especially in male flowers. Tomato, has two isoforms of PSY; PSY1 that functions in the chromoplsts and PSY2 that fuctions in the chloroplast (Fraser et al. 1999). Additionally, lycopene b-cyclase was also found to have two different forms in tomato; one is LCYB and the other is chromoplast-specific lycopene b-cyclase (CYCB) (Ronen et al. 2000). In watermelon, only a single CYCB have been found (Bang et al. 2007) leaving the question how lycopene is accumulated in the fruit. Thus, further research is needed to elucidate the mechanism of carotenoid biosynthesis in the leaves and the fruit of watermelon.

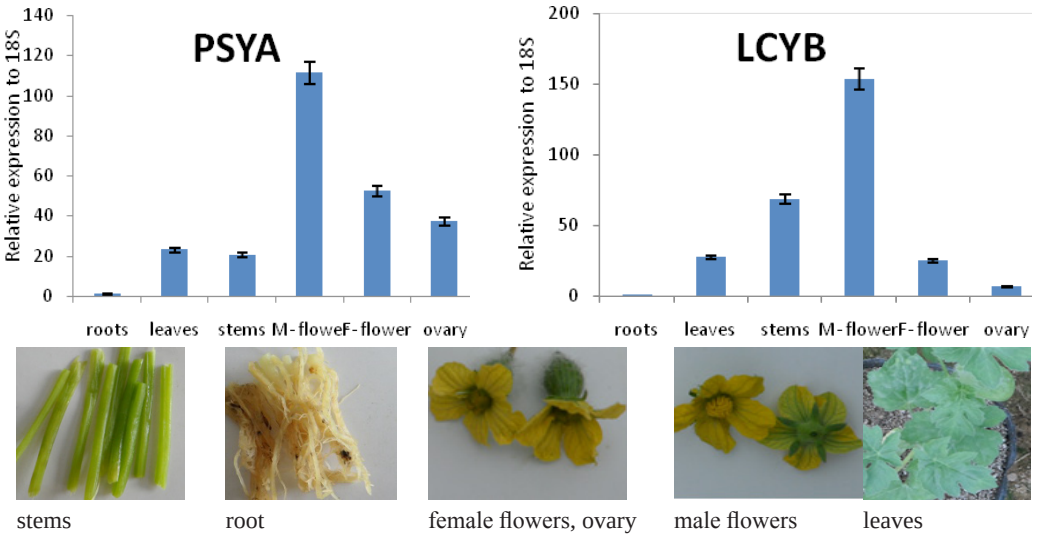

Fig. 2. Gene expression analysis of PSYA and LCYB in different organs in autotetraploid watermelon. Photograph of the six organs of autotetraploid watermelon. The height of each bar and the error bars show the mean and standard error, respectively, from three independent measurements.

Gene expression of PSY and LCYb during fruit maturation in autotetraploid watermelon

The expression levels of PSYA and LCYB during five stages of fruit maturation in watermelon (Fig. 3) were similar. Specifically, the expressions levels increased significantly at first, but then decreased when the fruit turned fully red. For example, the expression of PSYA increased from stage 1 (1.06) to stage 2 (RQ = 1.47) but decreased at stage3(1), and approximately 4 times from stage 4 (RQ = 14.58) to stage 5 (RQ = 3.89). Similarly, the expression of LCYB decreased about 45 times from stage 4 (RQ = 45.15) to stage 5 (RQ = 1).

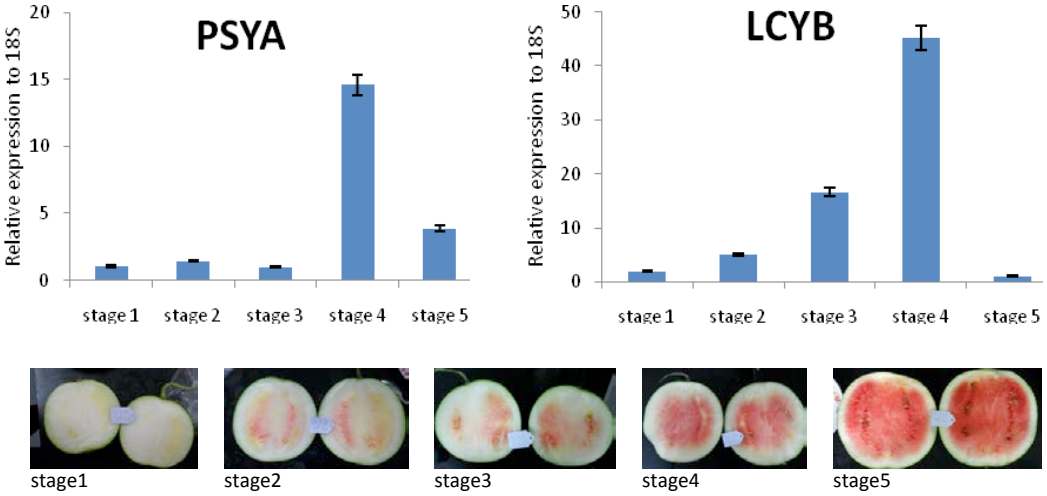

Fig. 3. Gene expression analysis of PSYA and LCYB during fruit maturation in autotetraploid watermelon.Photograph of the five developmental stages of autotetraploid watermelon fruit. Fruit stages were determined by days after pollination(stage1=10d; stage2=15d; stage3=20d; stage4=25d; stage5=30d;). The height of each bar represents the mean of three measurements and error bars indicate the standard error.

CONCLUSION

In this study, the relationship between lycopene content and the gene expression of PSYA and LCYB was investigated in different organs of and stages of fruit ripening in autotetraploid watermelon. PSYA and LCYB regulate the flux of the lycopene branch of carotenoid biosynthesis. In addition, the similar expression patterns of PSYA and LCYB during fruit maturation may be due to a common mechanism that regulates the transcription of carotenoid biosynthesis genes.

ACKNOWLEDGEMENTS

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Study on the changes of enzymes activity in the seed germination of watermelon

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Keywords: Enzyme activity, watermelon seeds, germination speed

Abstract

The studies compares peroxidase activity and the enzyme joint activity of Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in different position at germination of diploid and triploid watermelon seed. The results show that enzyme activities in different part of diploid watermelon seed are higher than in triploid watermelon seed, and enzyme activities of the seed coat is the lowest, enzyme activities of the endopleura is the highest in the three part of the triploid watermelon seeds. The peak enzyme activity of each part of the triploid seeds is later than the diploid. The enzyme joint activity of Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase had obvious change in 24h and 36h after germination respectively; the enzyme joint activity of Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase rose quikly in 0h-12h germination in the diploid's embryo , and peaked at 0.49μ·g-1·min-1 at 24h germination and began to reduced until it stabilized at about 0.2μ·g-1·min-1 at 36h germination. The enzyme joint activity of Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase rise very slowly in 0h-36h germination in the triploid's embryo, it stabilized at about 0.18μ·g-1·min-1 at 36h germination.

INTRODUCTION

The seed germination ability of diploid Watermelon is difference from triploid seed. Generally, the percentage of germination of diploid watermelon seed is 95% that of triploid watermelon seed is only about 40% enzyme activities of the seed is of great importance to the germination of seed.

There are many enzymes in dry seeds , including enzymes of the respiratory system, the protein synthesis system enzymes and hydrolytic enzymes, once they are hydrated, the activity immediately be restored. Roberts et al. thought that the citric acid cycle (EMP) into the phosphate sugar-free pathway (PPP) is the important conditions for seed germination, breaking seed dormancy When the PPP attain

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or exceed a certain level. The peroxidase enzymes have close ties with the PPP way, which enhancing the activity can promote the operation of the PPP pathway, finally result in breeking seed dormancy. Xuebao Long (1980) have reported that the activitie of the glucose 6 - phosphate dehydrogenase (6-P-GDH) and he glucose - 6 - phosphate dehydrogenase (G-6-PDH) is one of the key enzymes of the PPP pathway.

The studies compares peroxidase activity and the enzyme joint activity of Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in different position of diploid and triploid watermelon seed, which is of great significance to explore solutions to the triploid seed germination obstacle method.

MATERIALS AND METHODS

Plant materials

The test materials are seeds of HeiBao (diploid watermelon) and HeiNiu (homologous triploid watermelon) , provided by the Hunan Melons' Institute.

Processing methods

Seed germination as follows: Select the diploid and triploid full of seeds 100, soak seed 3h, repeatedly with tap water rinse 20min, rinse the seed surface of the sticky bar, into the training covered with moist gauze dish, then placed in 30 ° C incubator germination, in germination to maintain a moist gauze in a petri dish.

Seed germination rate statistics as follows:Germination standards, observe statistics culture time period seed germination to seed radicle breakthrough testa 0.5cm. The germination rate is calculated as follows:

$Gr = \Sigma Gt / T \times 100\%$

Gr is the germination rate, Gt is the number of germination of t days , T is the total number of tested seeds.

Determination of reference of Plant Physiology and Biochemistry Experimental Principle and Technology (Li and Shijie 2001), guaiacol method.

The crude enzyme extracting: were said to take the seed ministries located in a mortar, add 3 ml 0.2 mol / L PH 7.0 phosphate buffer, and home ice bath ground into homogenate and transferred to 5ml centrifuge tube, and then at 4 ºC, 4000r/min centrifuge 20min, the supernatant was the crude enzyme solution.

The reaction mixture: an accurate measure 50ml 0.2mol / L the PH7.0 phosphate buffer solution, adding guaiacyl phenol 28µl heated on a magnetic stirrer and stir until completely dissolved guaiacol, and after cooling, by adding 19µl 30%hydrogen peroxide, mixed to a uniform. Each determination of a configuration once the reaction mixture.

Enzyme assays: pipette accurately measure 3ml reaction mixture and the enzyme solution 20µl, 752 UV spectrophotometer to determine its absorbance in the 470nm wavelength, the every 1min record time absorbance values, recorded a

total of five times. Add 20μl of phosphate buffer as the control, repeat 3 times.

△ 470 per minute change of 0.01 for peroxidase activity units (μ). The following formula:

$$\text{Enzyme activity} = \frac{\text{V}470 \times Vt}{W \times Vs \times 0.01 \times t}$$

Formula, △ 470 for the absorbance values within the reaction time changes; Vt extract the total volume of enzyme solution (ml); W seed fresh weight (g); Vs for the determination of access to the enzyme solution of the volume (ml); t reaction time (min).

Glucose -6 - phosphate dehydrogenase and glucose 6 - phosphate dehydrogenase joint activity

Determination method reference Pu (1994) reported.

The crude enzyme extract: Weigh take various parts of the seed with 3ml 0.1 mol / L PH7.4 Tris-HCl grinding evenly transferred to the centrifuge tube, 4 ℃ 7000r/min centrifuge 30min, the supernatant as the enzyme crude extract.

The reaction solution: 0.1 mol / L, pH 7.4 and Tris-HCl (containing 5mmol / L $NADPNa_2$, 5mmol / L, D-glucose -6 - phosphate disodium salt and 5mmol / L of $MgCl_2 \cdot 6H_2O$)

Enzyme assay: accurate Pipette 1.35ml reaction mixture in the cuvette, then add 75μl of crude enzyme, quickly flip the cuvette, the enzyme solution and the reaction mixture was mixed with nucleic acid protein analyzer determination of absorbance in the 340nm wavelength value every 1min measured once recorded a total of five times the absorbance value. Control extraction buffer instead of enzyme solution with three replications.

For one unit of enzyme activity per gram fresh weight △ 340 changes in 0.1, is calculated as:

$$\text{Enzyme activity} = \frac{\text{V}340 \times Vt}{W \times Vs \times 0.01 \times t}$$

Formula, △ 340 changes in the value of the absorbance values within the reaction time Vt enzyme extract total volume (ml), W the sample fresh weight (g), Vs for the shift to take the enzyme sample volume (ml), t the reaction time (min).

Statistical analysis

Germination rate statistics:

It can be seen a small number of triploid watermelon seed germination at 24h germination from Table 1, the percentage of germination of it still only 34% until at 48h. Beginning of germination is at 12h, the percentage of germination of diploid watermelon seed peaking at 100% at 36h.

Table 1. The germination percentage of diploid & triploid watermelon seed.

Germination time	Germination rate （%）	
	HeiBao(diploid)	HeiNiu(homologous triploid)
0h	0	0
12h	39	0
24h	96	12
36h	100	20
48h	100	34

Germination of different seed parts of the peroxidase activity changes:

The peroxidase activity changes in different seed parts showed in Fig. 1. peroxidase activity of diploid and triploid watermelon seed endotesta was ascend in first and descend at last. peroxidase activity of diploid watermelon seed endotesta peaked at 10209.64μ • g^{-1} min^{-1} at 36h and declined after then. and that of triploid watermelon seed endotesta was ascend still at 48h.

Peroxidase activity in diploid embryo increased slowly in 0h-24h and peaked at 3901.11μ • g-1 min-1 at 36h and declined after then. Triploid embryo peroxidase activity was not obvious in 0h-24h,and increased rapidly in 24h-36h and peaked at 1109.21μ • g-1 min-1at 36h and declined after then.

Peroxidase activity in diploid and triploid exotesta changed obviously, and the activity was much lower than in endotesta and embryo. The peroxidase activity of triploid seed exotesta of was higher than that of diploid seed testa ,peaking at 475.2μ • g^{-1} min^{-1}.

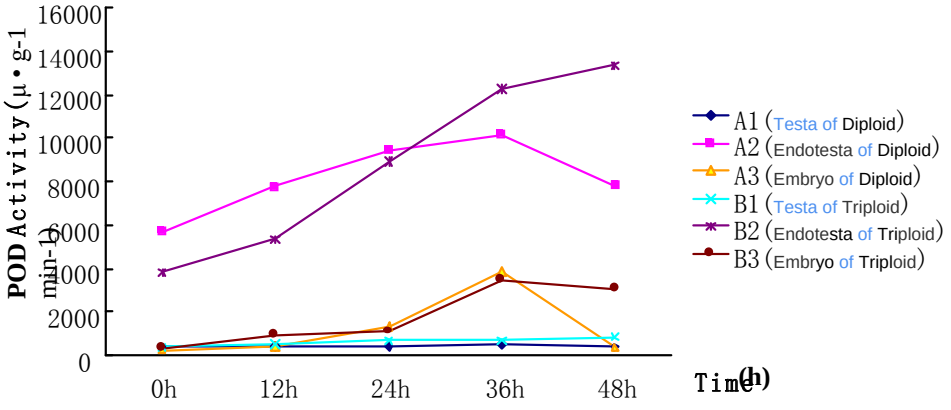

Fig. 1. The POD activity among different position of watermelon seed.

During the seed germination, peroxidase activity of the diploid and triploid seed exotesta, endoersta and embryo in the same germination time analysis of variance results shown in Table 2. After multiple comparison shows that the peroxidase activity of the diploid and triploid watermelon seed exotesta was not significant before germination 24h and was very significant during germation 36h and 48h. The peroxidase activity of diploid and triploid seed endotesta was very significant during the whole germination except 36h. The peroxidase activity of the diploid and triploid watermelon seed embyo was not significant an 0h and 36h and was very significant during other time..

Table 2. Results of variance analysis of POD activity among different position of watermelon seed.

Time	Testa		Endotesta		Embryo	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
0h	362.81Aa	359.00Aa	5676.52Aa	3847.40Bb	239.77Aa	280.65Aa
12h	580.07Aa	482.22Aa	7747.60Aa	5343.75Bb	404.03Bb	898.89Aa
24h	450.85Aa	636.68Aa	9463.88Aa	8907.89Aa	1358.79Aa	1109.21Ab
36h	463.04Ab	648.26Aa	10209.64Bb	13167.12Aa	3901.11Aa	3431.26Aa
48h	349.76Bb	824.96Aa	7789.12Bb	13355.65Aa	390.89Bb	2965.37Aa

Note: multiple using the LSD method, each treatment, different uppercase and lowercase letters, respectively, said the difference in the P <0.01 and P <0.05 level of significant with sex; with seed parts of the letters the same for the difference was not significant with the letters not the same as for the difference was significant with.

G-6-PDH and 6-P-GDH activity:

It can be seen from the figure 2 and table 3, the diploid G-6-PDH and 6-P-GDH activities of endotesta ,embryo and testa enhanced during the first of 24h, achieving the highest at the same time, respectively peaked at 1.44μg-1.min-1, 0.49μg-1min-1 and 0.2μg-1min-1, then slowly down and in a stable leavel. While the triploid seed have different changes in those indexes. It rised up at the initial stage in embryo, reaches the maximum after 36h germination,after that it stabilized at $0.18\mu g^{-1}min_{-1}$. However, a lower and steady enzyme activity apperead in testa in the first 24h , but an inverted V-shaped change emerged in the latter period. Trend in endotesta is complicated, showed a N-type trends and stabilized at $0.18\mu g^{-1}min^{-1}$ after 36h germination.

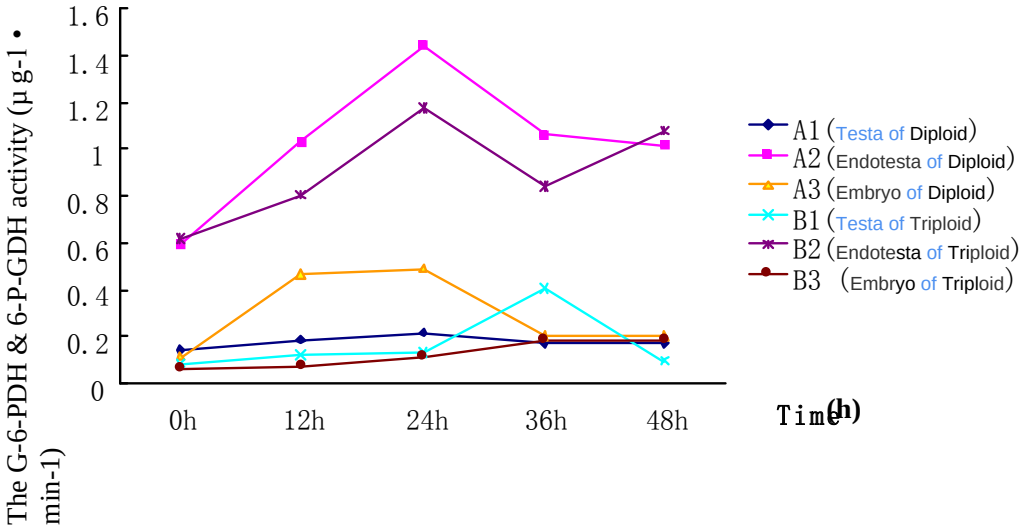

Fig. 2. The G-6-PDH & 6-P-GDH activity among different position of watermelon seed.

Table 3. Results of variance analysis of G-6-PDH & 6-P-GDH activity among different position of watermelon seed.

Time	Testa		Endotesta		Embryo	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
0h	0.14Aa	0.08Bb	0.59Aa	0.62Aa	0.11Aa	0.06Bb
12h	0.19Aa	0.12Ab	1.03Aa	0.81Ab	0.46Aa	0.07Bb
24h	0.22Aa	0.13Ab	1.44Aa	1.18Aa	0.49Aa	0.11Aa
36h	0.18Ab	0.40Aa	1.06Aa	0.84Ab	0.21Aa	0.18Aa
48h	0.17Aa	0.10Aa	1.02Aa	1.08Aa	0.20Aa	0.18Aa

Note: multiple using the LSD method, each treatment, different uppercase and lowercase letters, respectively, said the difference in the P <0.01 and P <0.05 level of significant with sex; with seed parts of the letters the same for the difference was not significant with the letters not the same as for the difference was significant with.

RESULTS AND DISCUSSION

The results indicate that diploid seed the POD , G-6-PDH and 6-D-GDH activity were higher than the triploid seed,and they all have lower enzyme activity at the seed coat had no significant changes within 48 h germination. According to the hypothesis of PPP ,the graph about the changes of enzyme activity in the seed can speculated that during seed germination the diploid respiratory are based on PPP pathway in the beginning while this happened after 36h in triploid .There are many factors limited the seed germination such as the endogenous inhibitor, the hormone

levels in the seed and the physiological and environmental conditions. Because a lot of differences existed in endotesta enzyme activity between the dioploid and triploid, we can found how energy substances changed in the seed germination through measuring the enzyme activity.

Further research work needs to be done to know the enzyme activity in embryo between the integrity of embryo and the decomposition of the storage substance. We also need to find out when POD activity increased, whether the tissue cells respiratory pathway experienced by the TCA / EMP pathway to a PPP means.

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Morphologic-molecular characterization and genetic relationships of different watermelon varieties

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Keywords: Watermelon, morphologic-molecular characterization, SRAP

Abstract

A total of 83 watermelon varieties registered by Ministry of Food, and Agriculture and Livestock of Turkey were used in this study. They were characterized both with 76 morphological traits according to UPOV and molecular SRAP markers. Genetic relationships were determined using NTSYS2.1 statistical software based correlation matrix and unweighted pair group method arithmetic average (UPGMA). The 76 morphological traits were used for principal coordinate analysis. The four main groups included 4, 3, 9 and 67 varieties, respectively. The 83 watermelon varieties were genotyped by 7 SRAP primer combinations. A total of 32 bands were produced and 11 of them were polymorphic. The watermelon cultivars used in this study showed a low level of polymorphism among commercial cultivars for SRAP markers. The results indicate the power of the morphological characters to discriminate among the watermelon varieties, and the usefulness in selecting the parental genotypes used in breeding programs.

INTRODUCTION

Vegetable production in Turkey is annually approximately 24 million tons from approximately an area of 1 million ha (TUIK 2010). About 35 % of the production comes from the species which belongs to the family Cucurbitaceae. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) is the most widely grown cucurbit in Turkey and is one of the most important cucurbit crop in Turkey. Turkey is the second most important watermelon producer country with 3.7 million tons on 150,000 ha area after China (FAOSTAT 2010). Almost 100% of its commercial production is conducted with F_1 hybrid cultivars. Hybrid watermelon

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varieties are the most important material for breeding new cultivars. Morphologic-molecular characterization and determination of relationships among watermelon varieties are important for breeding new cultivars.

The objectives of this study were characterize and determine genetic relationships among watermelon varieties in order to assist watermelon breeding program. The watermelon varieties registered by Ministry of Agriculture and Rural Affairs were morphologically evaluated according to UPOV, and molecularly characterized via SRAP markers.

MATERIALS AND METHODS

Plant Material

A total of 83 watermelon varieties registered by Ministry of Agriculture and Rural Affairs were used in this study **(**Table 1). Plants were grown in field of Verim Ziraat, Adana, Turkey.

Morphological Characterization

Seeds of the 83 accessions were sown in plastic seedling tray containing a mixture of peat and perlite (2:1 v/v) on March 20th, 2012 under plastic greenhouse conditions. Twenty plants of each accession were transplanted under plastic on April 24th, 2012 at 3–4 true leaf stage with 3 m x 0.5 m spacing for characterization.

Accessions were evaluated for a total of 56 phenotypic characters (*Seedling:* shape of cotyledon, size of cotyledon, intensity of green color of cotyledon, spots on cotyledon, depression of nerves of cotyledon, length of hypocotyls; *Plant:* growth habit, length of main stem, hermaphrodite flowers, number of nodes up to first node with female flowers; *Leaf:* length, width, ratio length/width, color, intensity of color, degree of lobing (beyond first flower), depth of incisions of margin of leaf of central third of plant, blistering, undulation of margin, flecking; *Flower:* Petiole length, size of petal of female flower (third to seventh flower), color of petal of female flower, shape of apex of petal of female flower, Ovary size, Ovary pubescence; *Fruit:* weight, shape of longitudinal section, ground color of skin, intensity of green color of skin, length of peduncle, size of insertion of peduncle, shape of basal part, depression of base, shape of apical part, depression at apex, size of pistil scar, grooves, stripes, intensity of green color of stripes, width of stripes, marbling, intensity of marbling, thickness of outer layer of pericarp, main color of flesh, intensity of main color of flesh, firmness of flesh, marbling of flesh, number of seeds; *Seed:* size, ground color of testa, secondary color of testa, type of distribution of secondary color of testa, area of secondary color in relation to that of ground color, patches at hilum, patches at margin) by using a modified UPOV (The International Union for the Protection of New Varieties of Plants) descriptor at cotyledon, flowering and mature fruit stages between March and July of 2012.

In addition to morphological characters, 20 quantitative traits were also measured for all accessions. These were: *Seedling:* Hypocotyl length (cm), Cotyledon width (cm), Cotyledon length (cm); *Leaf:* Leaf blade width (cm), Leaf blade length (cm), Petiole length (cm); *Flower:* Width of ovary (mm), Length of ovary (mm), Diameter of petal of male flower (mm); *Fruit:* Yield (kg/m^2), Fruit weight (g), Length of peduncle (mm), Diameter of peduncle (mm), Diameter of insertion of peduncle (mm), Diameter of pistil scar (mm), Fruit length (cm), Fruit diameter (cm), Firmness of flesh, Fruit Rind thickness (mm), Total soluble solids (%)). Mature fruits were harvested on July 28th in 2012 and yield (kg/plant) of each accession was recorded. At least three mature fruits from each accession were analyzed; Length of peduncle (mm), diameter of peduncle (mm), diameter of pistil scar (mm), fruit weight (g), fruit length (cm), fruit diameter (cm), rind thickness (mm) and total soluble solids (%) were recorded. The length measurements were determined by a meter, ruler or a digital compass, total soluble solids was analyzed by a hand held refractometer (Atago).

DNA extraction and SRAP analysis

For molecular analysis, genomic DNA was extracted from young leaves of 83 genotypes by the CTAB method as described by Doyle and Doyle (1990). DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc., Vinooski, USA), and 10 ng/mL DNA templates were made using TE (10 mM Tris– HCl, 0.1 mM EDTA, pH 8.0). A total 7 SRAP primers were used for all watermelon varieties (Table 2). PCR reaction components and PCR cycling parameters were performed as described by Uzun et al. (2009). PCR products were separated on 2% agarose gel in 1 x TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 3 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder were used for SRAP analysis.

Molecular analysis was carried out as follows: each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf 2000). A similarity matrix was constructed using SRAP data based on Dice (1945) coefficient. Then, the similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the cultivars studied. The genetic similarity matrix and ultrametric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test (Mantel 1967). The result of this test is a cophenetic correlation coefficient, *r*, that indicates how well dendrogram represents similarity data. The principal components analysis (PCA) of the original binary data matrix was also performed using NTSYS-pc version 2.1.

RESULTS AND DISCUSSION

The 76 phenotypic traits were used in clustering for PCA. The resulting cluster was presented in Fig. 1. The accessions were divided into four main clusters. Within these clusters, subclusters were identified including accessions from different regions. Correlation coefficients among pairs ranged from 0.03 to 0.65. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was $r = 0.75$, $P < 0.01$.The four main groups were detected at 0.03 of correlation coefficient. Group 1, 2, 3 and 4 include 4, 3, 9 and 67 varieties, respectively (Fig. 1). Solmaz and Sari (2009) reported that the collection comprising of traditional cultivars and local accessions collected from 15 different provinces of Southeastern Anatolia, Aegean, Marmara-Thrace, Middle Anatolia and Mediterranean regions of Turkey where local watermelon types are widely grown. They morphologically characterized 134 accessions according to UPOV descriptor list for 56 qualitative characters (6 seedling, 4 plant, 11 leaf, 5 flower, 23 fruit and 7 seed) in addition to 22 quantitative characters where morphologic relatedness were examined. The accessions studied showed large variation for most of the morphological traits. The accessions were divided into five different groups based on principle component analysis. Our results are consistent with that of Solmaz and Sari (2009).

Eighty three watermelon varieties were also evaluated by SRAP markers. A total of 32 bands were from 7 SRAP primer combinations used and 11 of which were polymorphic (33% of polymorphism). Number of bands per primer combinations was 4.6 whereas polymorphic bands per primer combinations were 1.6. Me6-Em7 had the highest number of polymorphic bands (3). The lowest number of polymorphic bands was obtained using Me6-Em8 primers. Highest polymorphism ratio was with Me9-Em11 primer combinations (66%) (Table 2). Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high (r=0.48, P< 0.01) suggesting that the cluster analysis strongly represents the similarity matrix. Correlation coefficients among pairs ranged from 0.84 to 0.98. The three main groups were detected at 0,95 of correlation coefficient. Group 1, 2 and 3 include 1, 81 and 1 varieties, respectively (Fig. 2).

The 76 phenotypic traits and SRAP markers form PCA were used in clustering. The resulting cluster was presented in Fig. 3 and Fig. 4. The accessions were divided into nine main clusters. Within this clusters, subclusters were identified including accessions from different regions. Correlation coefficients among pairs ranged from 0.03 to 1.00. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was $r = 0.95$, $P < 0.01$.The nine main groups were detected at 0.51 of correlation coefficient. Group 1, 2, 3, 4, 5, 6, 7, 8 and 9 included 6, 1, 23, 7, 3, 1, 3, 7 and 32 varieties, respectively (Fig. 3 and Fig. 4).

The watermelon cultivars used in this study showed a low level of polymorphism for SRAP. This is not promising for maintaining diversity

among cultivated genepool. Although, the SRAP markers were unsuccessfull in discriminating watermelon cultivars, they can be used for characterization and diversity analyses of watermelon genetic resources.

The results indicate the potential of the morphological and molecular techniques to discriminate among the watermelon varieties, very useful in selecting the parental genotypes used in hybrid breeding programs.

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Table 1. List of watermelon varieties used in this study.

Number	Variety	Number	Variety
1	Mart	43	Obenkar 55
2	Berta	44	Plantation Pride
3	Melani	45	RTTK 01
4	Triton	46	RTTK 04
5	Minos	47	RTTK 03
6	Global Star	48	RTTK 02
7	Hilton	49	Crimson Tide
8	Premium	50	Paradiso
9	Kıvılcım	51	Bora
10	Style	52	Speedy
11	Carmel	53	Belmore
12	Kamenta	54	Crimson Ruby
13	Trix Palomar	55	Nun 3094
14	Petite Perfection	56	Marmara 55
15	Green Globe	57	Crimsina
16	Pascal	58	Anchera
17	Hera	59	Karahan
18	Atlas	60	Bella
19	Data	61	Babba
20	Avira	62	Bozbey
21	501	63	Arashan
22	Erkan	64	Samkar
23	RTTK 06	65	Vole
24	Vole	66	Grenda
25	Odyssey	67	Crimstar
26	Shining Glory	68	Starfield
27	Cevval MK 13	69	Nort Point
28	Erdem 07	70	Ekvator
29	Othello	71	Gora
30	Dizayn MK14	72	Nun 502
31	Cargo	73	Daytona
32	Toraman	74	WDL 5036
33	Bonanzo	75	WDL 7028
34	Small Giant	76	Alkan
35	Şeker	77	Elize
36	Filiada	78	Perse
37	Meteor	79	İrem
38	Üstün	80	Galiks
39	Atalanta	81	Amphion
40	Bibo	82	Madelon
41	Ascrim	83	Karon
42	Vindex		

Table 2. List of SRAP primer pairs used to study the genetic relatedness of 83 watermelon varieties.

Name	No. of bands	No. of Polymorphic bands	Polymorphism (%)	Size (bp) (min–max)
Me9-Em11	3	2	66	180-700
Me6-Em7	7	3	42	250-1000
Me7-Em11	4	1	25	280-800
Me2-Em7	5	1	20	320-920
Me5-Em12	7	2	28	200-950
Me6-Em12	4	2	50	390-980
Me6-Em8	2	0	0	350-450
Total	32	11	-	-
Mean	4,6	1,6	33	

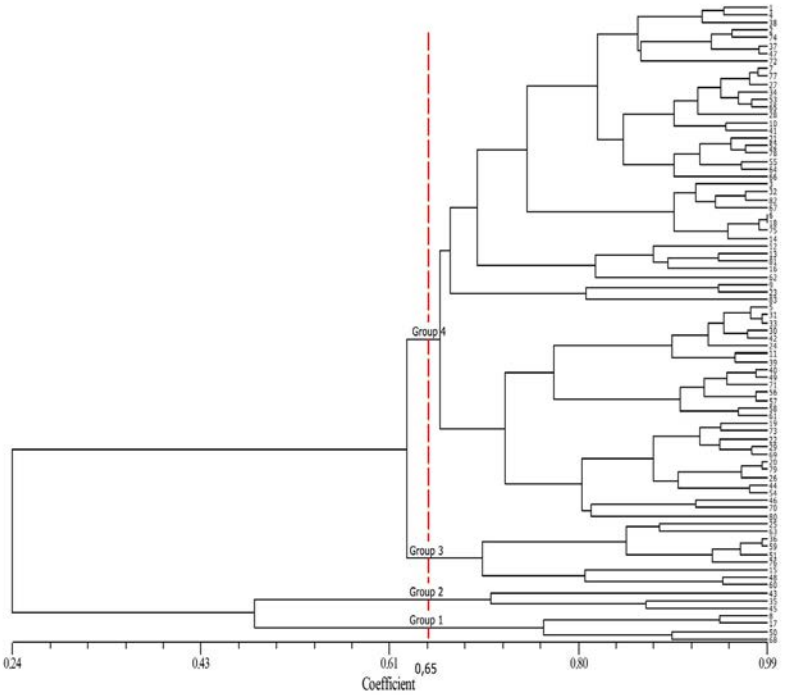

Fig. 1. Dendrogram of watermelon varieties obtained from cluster analysis of 76 phenotypic traits using average method.

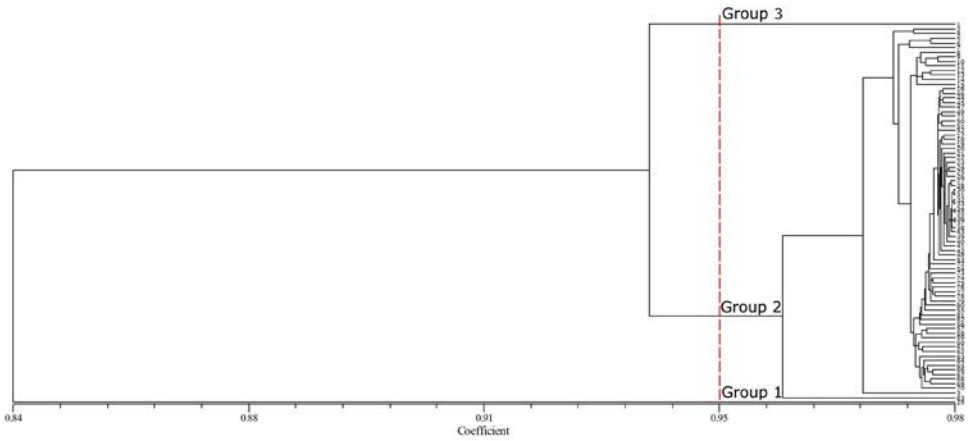

Fig. 2. Dendrogram of the 83 watermelon varieties using UPGMA method from SRAP markers.

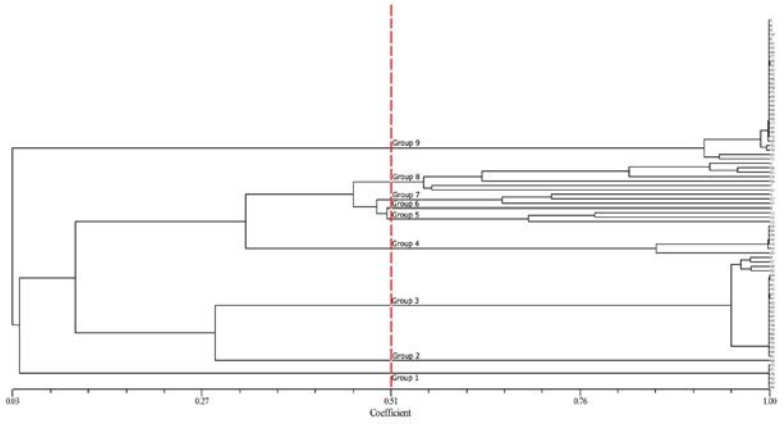

Fig. 3. Dendrogram of watermelon varieties obtained from cluster analysis of phenotypic traits and SRAP markersusing average method.

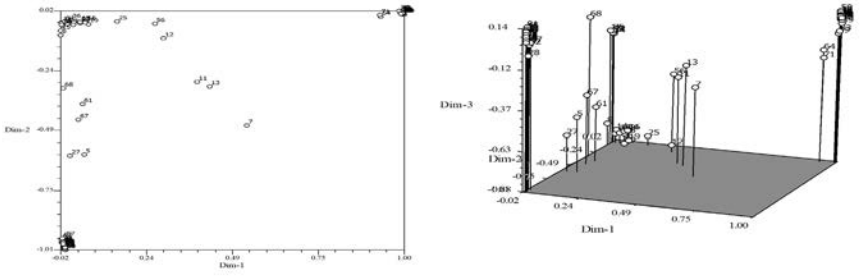

Fig. 4. Two-Three dimensional plot of watermelon varieties in the principal components analysis of phenotypic traits and SRAP markers.

Seed characteristics and seed-fruit correlation of Turkish watermelon germplasm

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Keywords: *Citrullus lanatus*, genetic resources, seed traits, fruit characteristics

Abstract

This study was carried out at the Department of Horticulture, Faculty of Agriculture, University of Cukurova. Totally, 327 genotypes were used which mostly collected from different regions of Turkey since 1993. Seed weight, seed length and seed width of 15 seeds per genotype were measured. The seed weight, length, and width ranged from 20 to 211 mg, 5.03 to 16.63 mm and 3.39 to 9.53 mm, respectively, among genotypes. Nine genotypes were selected based on seed size (3 small, 3 medium, 3 big) in order to evaluate correlation between seed and fruit characteristics. Correlation analysis was performed between measured seed parameters and fruit weight, length and diameter in the nine genotypes. Measured seed parameters were negatively correlated with fruit weight, length and diameter while seed weight was positively correlated with seed length.

INTRODUCTION

The genus *Citrullus* belongs to *Cucurbitaceae* family and contains 4 diploid (n=11) species that thrives in Africa, Asia and Mediterranean (Levi at al. 2001). Turkey is the second largest world watermelon producer (after China), with 3.7 million tones of watermelon produced on 150.000 ha area (FAO 2010). Altough Turkey is not the center of origin for watermelon it has valuable and extensive watermelon genetic resources because of the long cultivation history and the location of the country acting as a bridge between Asia and Europe. The successive conservation and the utilization of the plant genetic resources depends on the systematic identification and registration of the material in detail, examination of the genetic differences and determination of the useful traits which are desired in breeding. The detection of the polymorphism among the germplasm is necessary for the effective use of genetic resources (Che et al. 2003).

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The collection of watermelon genetic resources at Cukurova University had begun in 1993. Watermelon genetic resources were collected from most regions in Turkey and an extensive germplasm collection was formed with the addition of the reference materials and the different genotypes of other species which provided from different gene banks.

Wide phenotypic diversity in seed characteristics, including seed weight, size, shape and color exist among watermelon genotypes, which makes watermelon unique in this respect among Cucurbit species, including melon (Solmaz et al. 2008), *Cucurbita maxima* and *Cucurbita moschata* (Balkaya et al. 2006).

The objective of this study was to phenotype seeds of 327 genotypes belong to Turkish watermelon gene pool and determine if there is any association between certain seed and fruit characteristics.

MATERIALS AND METHODS

This study was carried out at the Department of Horticulture, Faculty of Agriculture, University of Cukurova. Totally 327 genotypes were used. Most of the genotypes used as plant material were collected from different regions of Turkey which have rich genetic resources of watermelon since 1993 and especially at the framework of an TUBITAK project (Sari et al. 2007). The other materials were consisted of 2 dihaploid lines (Sari et al. 1994), 44 genotypes from Turkish Gene Bank Aegean Agricultural Research Center (AARI), 19 genotypes of different wild species from United States Department of Agriculture (USDA), 7 genotypes from Northern Cyprus Turkish Republic (NCTR), 1 genotype from French National Institute for Agricultural Research (INRA), 1 genotype from Uzbekistan, 2 genotypes from Egypt and 12 open pollinated commercial varieties.

Watermelon genotypes were collected from Southeastern Anatolia (33.3 %), Middle Anatolia (16.8 %), Mediterranean (9.5 %), Aegean (8.9 %) and Marmara-Thrace (5.8 %) regions. *C. colocynthis* (accession number 318 and 322), *C. ecirhosus* (accession number 323), *C. lanatus* var. *citroides* (accession number 324 and 328) *C. rehmii* (accession number 329 and 330) and *Praecitrullus fistulosus* (accession number 331-335) were used as control to *C. lanatus*.

In this study, seeds were randomly sampled from each genotype and each seed was weighted with scale sensitive to 0.01 g for determination of seed weight. Seed width and length were measured with digital compass. Measurement was replicated three times and each replication contains 15 seeds. Seeds were classified as very light (one seed weight is lighter than 50 mg), light (one seed weight is 51-100 mg), medium (one seed weight is 101-150 mg), heavy (one seed weight is 151-200 mg) and very heavy (one seed is heavier than 201 mg) according to their weight. Seed were also classified as very short (shorter than 5.9 mm), short (between 6.0-8.9 mm), medium (between 9.0-11.9), long (between 12.0-14.9 mm) and very long

(longer than 15 mm) as regarded to seed length. Seed were classified as very narrow (smaller than 3.9 mm), narrow (between 4.0 -5.4 mm), medium (between 5.5-6.9 mm), wide (between 7.0-8.4 mm) and very wide (bigger than 8.5 mm) as regarded to seed width.

Nine accessions were chosen for correlation analysis between seed characteristics and fruit characteristics. Three genotypes from each seed size group (98, 101 and 107 for small; 105, 111 and 114 for medium; 171, 186 and 213 for big) were chosen. All chosen genotypes were belonging to *C .lanatus* var. *lanatus*. Correlation analysis were done between seed weight and fruit weight, seed length and fruit length, seed width and fruit diameter and also seed weight and seed length.

Seed were sown in trays containing mixture of peat:perlite 2:1 (v/v) for fruit measurements. Seedlings at the first true leaf stage were transplanted in open field with 3.0 m x 0.5 m spacing. Ten seedlings from each genotype were transplanted in each plot. Fruit weight, fruit length and fruit diameter were measured in three fruits from each genotype.

Data were analyzed in COSTAT statistical program. Correlation analysis was done in SPSS statistical program.

RESULTS AND DISCUSSION

Maximum, minimum and mean value of seed weight, length and width of 327 watermelon genotypes and statistical values were presented in Table 1. Significant variation was found as regard to seed weight, length and width. Seed weight was changed from 20 mg to 211 mg while seed length changed from 5.03 mm to 16.63 mm. Seed width showed variation from 3.39 mm to 9.53 mm.

As regarded to seed weight, 7.95 % of genotypes (26 genotypes) were lighter than 50 mg, 18.35 % of genotypes (60 genotypes) were between 51 mg and 100 mg, 48.93 % of genotypes (160 genotypes) were between 101 mg and 150 mg, 23.24 % of genotypes (76 genotypes) were between 151 mg and 200 mg and 1.53 % of genotypes (5 genotypes) were heavier than 200 mg. Wild genotypes, C. *colocynthis* (accession number 318-322) and *C. rehmii* (accession number 329-330) had small seeds, *C. ecirrhosus* (accession number 323) had small seeds. Genotypes of *C. lanatus* var. *citroides* (accession number 324-328) had medium size seeds. Genotypes belong to *Praecitrullus fistulosus* (331-335) had seeds weigh between 51 mg and 100 mg.

As regarded to seed length, significant variation was found between genotypes. Sixty six percent of accessions (216 genotypes) had long seeds, 20 % of accessions (67 genotypes) had medium seed length, 9% of accessions (31 genotypes) had short seeds, 3.7 % of accessions (12 genotypes) had very long seeds and only 1 genotype (*Citrullus rehmii*, PI 632755) had very short seeds.

Seed width was also measured and it showed great variation and changed

from 3.39 mm to 9.53 mm. Accessions separated into 4 different groups based on seed width. Majority of accessions took place in a group with wide seed width. Two hundred and eight genotypes had wide seed width, 52 genotypes had medium seed width, 42 genotypes had very wide seed width, 23 genotypes had narrow seed width and 2 genotypes had very narrow seed width. Correlation analysis was done between measured seed parameters and fruit weight, length and diameter. Significant negative correlation (r=-0.78) was found between seed weight and fruit weight. In our gene pool, genotypes with small seeds had bigger fruits (Fig. 1). Seed length was also significantly correlated (r=-0.64) with fruit length and there was a negative correlation between seed length and fruit length (Fig. 2). Significant negative correlation (r=-0.71) was found between seed width and fruit diameter (Fig. 3). In our gene pool, while seed dimensions increased, fruit dimensions decreased. Seed length was significantly correlated (r=0.94) with seed weight (Fig. 4).

As a result, it can be concluded that Turkish watermelon germplasm is consist of accessions mainly having big seeds. There was a significant correlation between seed weight, seed length and seed width with fruit weight, fruit length and fruit diameter. Measured seed parameters (weight, length and width) were negatively correlated with measured fruit parameters (weight, length and diameter). It was also found that seed length was significantly correlated with seed weight.

In this study we used diverse genetic material, including commercial watermelon varieties and wild species. Overall, the findings in this study concur with the results of Maggs-Kölling and Christiansen (2003) that showed watermelon genotypes with small fruits contained large seeds.

ACKNOWLEDGEMENTS

Researchers are grateful to Scientific and Technological Council (TUBITAK) and Scientific Research Projects, Unit of Cukurova University due to financial support to Project (TOGTAG 3370). In addition, researchers thank USDA-USA, INRA-France and AARI-Turkey for their kind assistance in seed supply and directorship of visited provinces and counties of Turkish Ministry of Food, Agriculture and Livestock.

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Table 1. Maximum, minimum and mean value of seed weight, length and width and some statistical values.

	Seed weight (mg)	Seed length (mm)	Seed width (mm)
Maximum	211	16.63	9.53
Minimum	20	5.03	3.39
Means	122	12.59	7.58
Degree of Freedom	326	326	326
Probability	0.000	0.000	0.000
F	53.4	2.96	1.63
Significance level (1%)	***	***	***

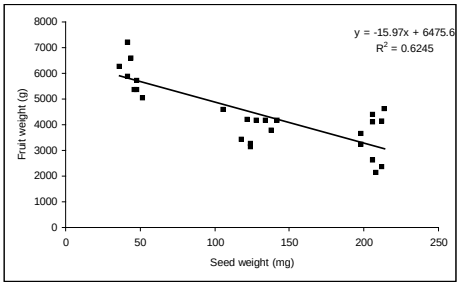

Fig. 1. Correlation between seed weight and fruit weight

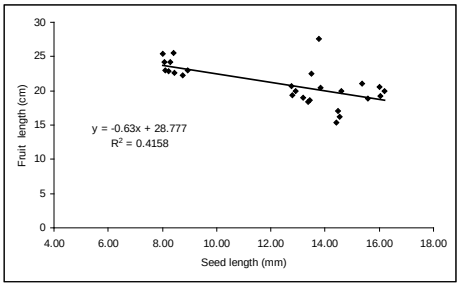

Fig. 2. Correlation between seed length and fruit length

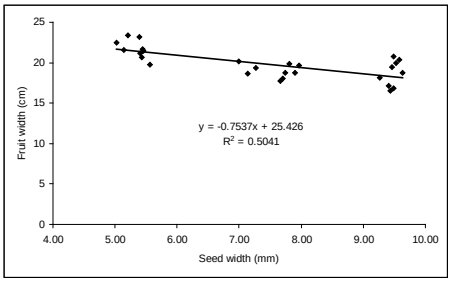

Fig. 3. Correlation between seed width and fruit diamater

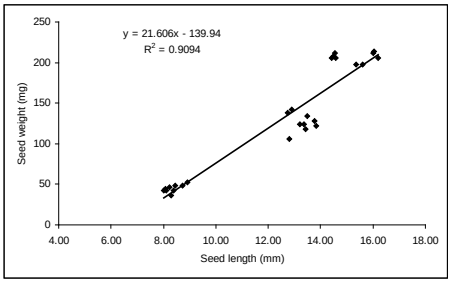

Fig. 4. Correlation between seed weight and seed length

The relationship of endogenous plant hormones to embryo abortion in fewer-seed watermelon

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Keywords: *Citrullus lanatus*, endogenous phytohormone, embryo development

Abstract

In order to explore the possible mechanism of embryo abortion in fewer-seed watermelon, three watermelon (*Citrullus lanatus*) genotypes, F_{011}, M_{05} and their hybrid F_1 generation were used to examine their endogenous hormonal change patterns. Indole-3-acetic acid (IAA), gibberellins (GA_3), abscisic acid (ABA) and isopentenyladenine (iPAs) in fruit of watermelon plants were measured by indirect enzyme linked immunosorbent assay (ELISA). Results showed that in comparison with its parents, F1 (the seedless genotype) had abnormal variation for the three hormones. IAA, GA3 and iPAs levels in F1 dropped 30%-70% during the globular stage whereas the content of ABA was twice that of F_{011} and M_{05}. Ratios of IAA/ABA, ABA/GA3, ABA/iPAs among the three were also different. It was proposed that the dynamic change patterns of endogenous hormones are closely related to embryonic development and the balance between them is important.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is one of the important commercial horticultural crops in the world and less seeds in fruit are considered as an important index to its good quality. Triploid breeding currently acts as the most prevalent approach to gain seedless watermelons, but it is still not the optimal method because of its low seed yield, low germination rate and low seedling emergence rate. So to innovate breeding materials may provide a more efficient way to obtain seedless and high-quality watermelons.

It has been reported that by means of irradiation, Wang et al. (1988), Cao et al. (1993) as well as Liu et al. (2006) have identified chromosome translocation seedless watermelons from a large mutation population. This had successfully made new seedless germplasm materials available. Embryo abortion caused seedless in tangerine (*Citrus reticulata)* (Yu et al. 1994; Lv et al. 1995). Other studies pointed out

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that seed development was regulated by plant hormones (Ji et al. 1992). And further evidence from research on *Litchi chinensis* (Chen 2000), *Citrus reticulata* (Huang et al. 1997) and *Ziziphus jujuba* (Qi 2002) indicated that abnormal endogenous hormone levels in fruit development may upset the physiological and biochemical metabolism of plants and this, in turn, finally would cause embryo abortion. In this paper, dynamic changes of endogenous hormones including iPAs, ABA, GA_3 and IAA were compared in fruit of F_{011}, M_{05} as well as their hybrid F_1 generation (hereafter referred to as F_1). The purpose was to reveal the internal mechanism of seedless characteristics in watermelon.

MATERIALS AND METHODS

Plant materials

Three watermelon genotypes F_{011}, M_{05} and F_1, were used to carry out the experiment in this study. Among the listed genotypes, F_{011} is an interesting natural mutation found in our experimental field which has a normal and stable seed yield level by selfing. But when it crossed with other watermelon strains as the female parent, all the hybrid F_1 generations produced abortive seeds. M_{05} is a normal diploid. And when it was crossed with F_{011}, F_1 plants had few seeds. Details are as follows: F_{011} had an average plump seeds of 482 per fruit, 356 in M_{05} but only 89 in F_1 (accounting for 18.5% of F_{011}). Another 31 in F_1 were black, shriveled seeds (accounting for 6.4% of F_{011}), but the amount of white shriveled seeds was 183 (accounting for 37.9% of F_{011}).

F_{011}, M_{05} and F_1 seeds were planted with the use of plastic film mulching in the open experimental field at Northwest F & A University, Yang ling, Shanxi, China. The three materials were randomly assigned to each plot with three replications and finally, 40 seedlings were kept in each plot. The plant spacing was 0.5m and row spacing was 1.7m.

When the plants entered flowering stage (about 60 days after sowing), we pollinated them artificially and recorded the date. Fruit setting on the same branches from plants with similar growth were gradually harvested on day 1 to 25 of the pollination, placed immediately into plastic bags, sealed and packed in ice, before returning them to the laboratory and storing at -20°C until use for endogenous hormones extraction. All fruit were divided into seven groups on the basis of the embryo and endosperm development status: (1) Fruit at pole nucleus mitotic stage (the first day of pollinating); (2) Fruit at zygotes mitotic stage (2 to 5 days after pollinating); (3) Fruit at early globular embryo stage (5 to 9 days after pollinating); (4) Fruit at late globular embryo stage (10 to 15 days after pollinating); (5) Fruit at heart-shaped embryo stage (16 to 20 days after pollinating); (6) Fruit at cotyledonary embryo stage (20 to 25 days after pollinating); (7) Fruit at mature embryo stage (25 days later after pollinating). If the fruit had been harvested posterior to the zygotes

period, then the embryos in it were isolated and discarded. Each group as a separate sample was used for assays of endogenous hormones.

Extraction and purification of endogenous hormones in fruits

Fruits were ground with cold 80% methanol at a rate of 5ml per gram fresh material. Then after a 20 min centrifugation at 10000 r/min (4 ºC), the supernatant was collected and passed through the C_{18} columns (C_{18} Sep-Park Cartridge). The efflux were collected, 1ml was taken out and dried with a stream of N_2 in a water bath (40ºC), finally, dissolved in 0.5 ml DBI buffer remaining 1-2 min for analysis by ELISA.

Assay of endogenous hormones

The procedures for endogenous hormones measurement were based on indirect ELISA described by Wu et al. (1988) as follows: coating, adding samples and the first antibodies, adding enzyme and the second antibody, adding substrate of the enzymatic reaction, stopping the reaction, measuring absorbency values. Each hormone of one sample was assayed in triplicate and the mean value used for data analysis.

The concentration of each hormone was estimated by a plotted standard log curve. Then its content was calculated based on the concentration.

RESULTS

Endogenous hormone levels in fruits at different stage

According to Fig.1, F_{011} and M_{05} had a similar endogenous hormone change pattern during the embryo development while F_1 showed a different one. More exactly, in F_{011} and $M_{05,}$ endogenous IAA, GA_3 and iPAs levels increased rapidly as soon as the flowers being fertilized, peaking at globular embryo stage and then decreasing gradually. Meanwhile ABA content stabilized at a comparatively low level. By contrast, though there was also a slight rise of endogenous IAA, GA_3 and iPAs levels in F_1, the mean levels were much lower than that in F_{011} and M_{05} whereas the mean ABA content was excessive, and its highest point was also found at the globular embryo stage. The facts indicate that it was the deficiency of IAA, GA_3, iPAs but excess of ABA in F_1 that resulted in embryo abortion. And the globular embryo stage was the key period for the regulation of hormones to embryo development (about 5 to 15 days after pollination).

In addition, the content of IAA in F_1 was 33% of that in M_{05} and iPAs was 55% of that in M_{05} at the zygote's mitotic stage; GA_3 level in F_1 was 30% that in F_{011} at heart-shaped embryo stage and 58% that in M_{05} at the early globular embryo stage. The highest ABA level in F_{011} was observed at the late globular embryo stage, 2.1 times as F_{011} and 1.9 times as M_{05}. This further implied that growth-promoting

hormone deficiency and ABA redundancy should be responsible for embryo abortion. And different embryo development stages had different dominating hormones. The putative sequence of dominating hormones acting on the embryo may be IAA, iPAs, ABA, GA_3.

Balance of endogenous hormones

The dynamic changes of ratios of the four hormones in the three watermelons were compared in this study (Fig. 2). The results showed that the ratio of IAA / ABA of F_{011} and M_{05} experienced a similar tendency during the period from pole nucleus mitotic stage to mature embryo stage. Both the two went up first and then dropped steadily with a single peak at zygote's mitotic stage and globular embryo stage, respectively. However, that of F_1 had a constant low level. In terms of ratios of IAA / GA_3, IAA / iPAs and GA_3 / iPAs, there were no significant differences among the three watermelons. But in the case of ABA / iPAs, ABA / GA_3, F_1 was of opposite trend to F_{011} and M_{05}. At first, both the ABA / iPAs, ABA / GA_3 of F_{011} and M_{05} declined, bottoming out at the globular embryo stage and then followed by an increase while those of F_1 increased first and the peak occurred at globular embryo stage. The facts suggest that in F_1, the disordered balance of hormones, especially the unusual ratios of IAA / ABA, ABA / iPAs, ABA / GA_3 caused its embryo abortion. Therefore, the balance of hormones plays an important role in the development of embryo.

DISCUSSION

Only 1 to 3 days are required for watermelon plants to complete double fertilization after pollination, and 2 to 5 days to start zygotic meiosis for the first time. When zygote goes into its division stage, its metabolism action is boosted. Our results, meanwhile, showed that the contents of endogenous hormones were increased rapidly at that time as well. This was also confirmed by Chen et al. (2002) in the study of cell embryogeny in Longan (*Dimocarpus longan*). There were no large differences in IAA and GA_{1+3} contents between the sterile and fertile plants in leechee (*Litchi chinensis)*. But CTK levels in sterile plants were dramatically lower than that in fertile plants. When the sterile plant suffered from embryo abortion, the content of IAA, GA_{1+3} and CTK decreased rapidly. Such abnormal decrease in growth-promoting substance had a bad effect on the division of embryonic cell and synthesis of biological macromolecules, this in turn, resulted in metabolic disorders as well as material and energy deficits. Thus, the final fate of the plant was sterile (Chen et al. 2000). Huang et al. (1997) also proved this changing pattern in the process of young fruit development of tangerine. In our study, results demonstrated that endogenous growth-promoting hormones in seedless watermelon (F_1) were insufficient, especially at the globular embryo stage. The deficit of IAA, GA_3 and

iPAs were as much as 30% to 70% while ABA, one of the growth inhibitors, greatly exceeded normal level resulting in dysontogenesis. On the other hand, the maximum of each hormone was observed at a different stage and the biggest gap of each hormone between F_1 and its parents was also different. This implies that endogenous hormones showed a particular successive function to embryo development, and the putative sequence was IAA, iPAs, ABA, GA_3.

Due to the potential complex interaction among different hormones, the balance of endogenous hormone levels can also provide further insight into the relationships of endogenous hormones to embryo abortion in seedless watermelon. Therefore, ratios of IAA /ABA, IAA / GA_3, IAA / iPAs, ABA / GA_3, ABA / iPAs and GA_3 / iPAs have been compared in the three watermelons. The data showed that in F_{011} and M_{05}, the maximum for ratio of IAA /ABA was found at the early stage of embryo development while in F_1, ratio of IAA /ABA did not change much within a low level. This suggests that growth-promoting substances were positively related to the differentiation and development of embryos. It can also be seen from the ratios of ABA / GA_3 and ABA / iPAs in F_1 that ABA content preponderated GA_3 content as well as iPAs content, especially at the heart-shaped embryo stage. Huang et al. (2003) claimed that the regulation of hormones to gene expression does not simply depend on an increase or decrease in the content of one specific hormone but the balance between them.

In conclusion, changes in endogenous hormone levels were closely associated with embyo abortion, which resulted in seedless watermelon. The cooperative interaction and balance of them was crucial to ensure normal embryos in watermelon. But the possible regulation mechanism of endogenous hormones to embryo abortion still requires further investigation.

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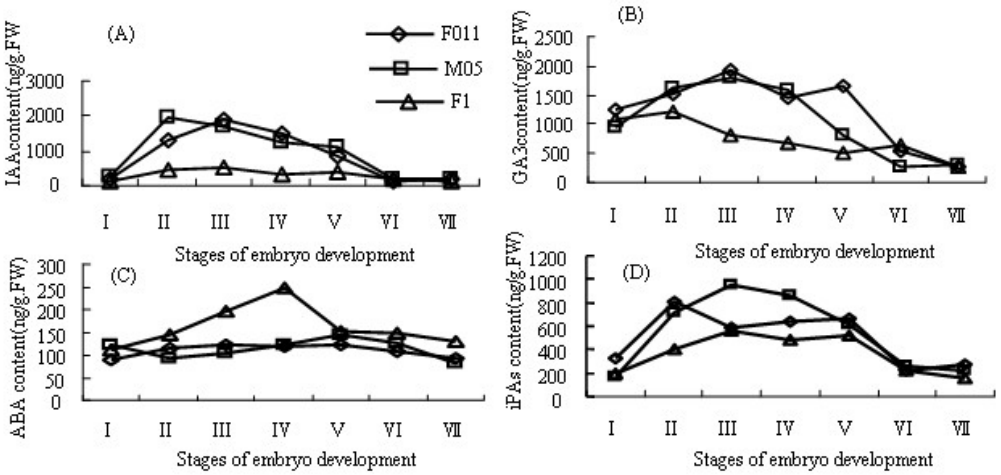

Fig.1. Changes of endogenous hormones in embryos of watermelon in different stages (I. pole nucleus mitotic stage; II. zygotes mitotic stage; III. early globular embryo stage; IV. heart-shaped embryo stage; V. cotyledonary embryo stage; VI. Mature embryo stage) . (A) IAA, (B) $GA_{3,}$ (C) ABA, (D) $iPA_{S.}$

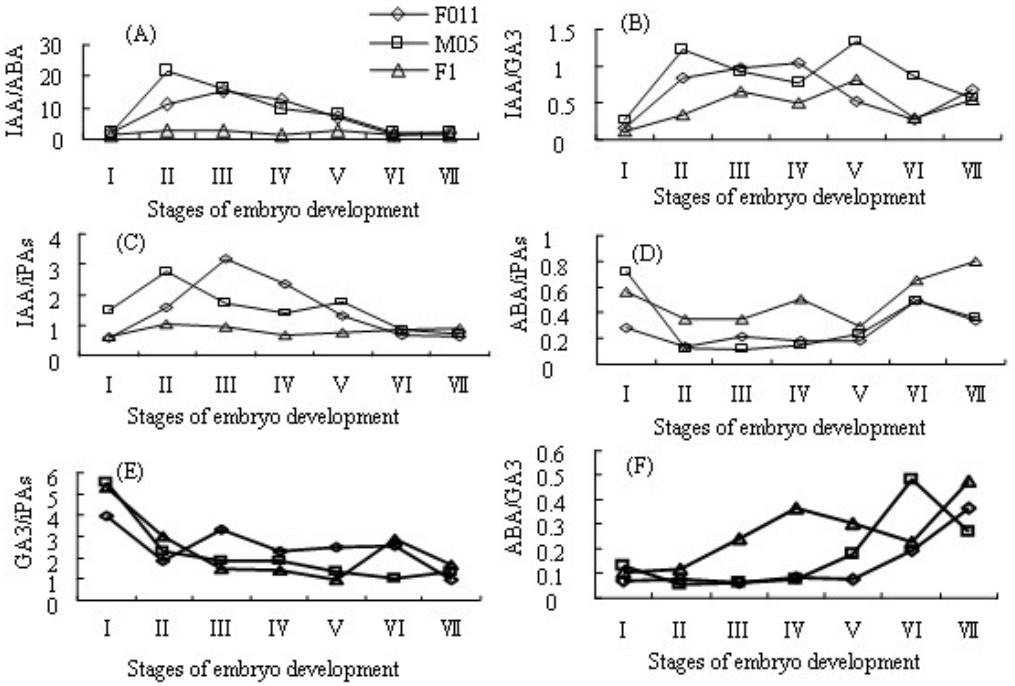

Fig.2. The ratio of endogenous hormones in embryos of watermelon in different stages (I. pole nucleus mitotic stage; II. zygotes mitotic stage; III. early globular embryo stage; IV. heart-shaped embryo stage; V. cotyledonary embryo stage; VI. Mature embryo stage). (A) IAA/ABA, (B) IAA/ GA_3, (C) IAA/ iPA_S, (D) ABA/ iPA_S, (E) GA_3/iPA_S, (F) ABA/GA_3.

Salt tolerance, seed size, cotyledon weight and the relationship of antioxidant enzyme activities in watermelon

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Keywords: watermelon, salt stress, the size of cotyledon, seed size, antioxidant

Abstract

In this study, four genotypes (Tunceli, Amasya, Malatya and Urfa), one standard type (Galactica) and one F1 hybrid varieties (Golden Crown) watermelon were used. Watermelon seedlings were grown in water culture using Hoagland nutrient solution in atmosphere-controlled climate chamber. When the plants had developed four to five true leaves, salt treatment was began, and 100 mM NaCl was gradually applied to medium. The purpose of this study was to investigate whether seed size and cotyledon weight may be used as a marker for salt-tolerant. As a result, genotypes with large seeds and cotyledons had higher enzyme activities than ones with smaller seed and cotyledon. Malatya ve Urfa with larger seed and codyledon leaf were less affected by salt damage, probably because they run better antioxidant enzymes. Galactica and Golden Crown F1 with smaller seed and cotyledon were more affected from slat damage, employing at a lower level of enzyme activities.

INTRODUCTION

In terms of salt tolerance, the differences between genotypes within plant species is a proven fact established by many studies. According to Ashraf (1994) and Yasar (2003), although there are differences in salt tolerance of genetic resources, a very limited number of genotypes show a real sense to salt tolerance. Therefore, assessment of the present genetic potential and selecting genotypes tolerant are important. However, that salt tolerance feature controlled by many genes is a conflict characters, and that parameters used in determination of tolerant genotypes vary in every species make difficult to establishment an appropriate selection method.

Several authors have reported that there are relationships between massiveness or shape of fruit and salt tolerance in tomato plants (Anastasio et al. 1987; Shannon et al. 1987; Caro et al. 1991), Indeed, it was determined that the cherry tomatoes having small fruit are more resistant to salt stress relative to tomatoes with normal-sized fruits. Pichai (1986) stated that the tomatoes with small fruit had more sucrose, invert sugar and acid content than those with normal-sized fruit, and reported that

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salt tolerance can be explained by the those characteristics.

When exposed to environmental stress such as extreme temperatures, drought, herbicide applications, nutrient disorders, plant produced reactive oxygen species and is subjected to oxidative damages. If antioxidants can not be activated adequate levels response to reactive oxygen species, the plant can not be protected enough against oxygen species and resulting in death. It was reported that plants containing high levels of antioxidant is more strength to oxidative damages. In the many previous study, it was documented that resistant genotypes had higher level enzyme activity than sensitive genotypes for protects against free oxygen radicals under salt stress (Dhindsa and Mathowe 1981; Wise and Naylor 1987; Cakmak and Marschner 1992; Polle et al. 1992; Gossett and et al. 1994a; Gosset et al. 1996).

The purpose of this study was to determine whether seed size and sotyledon weight may be used as a marker for salt tolerance and antioxidant enzyme activities in Watermelon.

MATERIALS AND METHODS

Before starting the study, twenty-two watermelon genotypes and cultivars are collected from various regions of Turkey. Among them, 'Malatya' and 'Urfa' with high cotyledon areas, cotyledon and seed weight, 'Tunceli' and 'Amasya' with medium value of cotyledon areas, cotyledon and seed weight, and Galactica and Golden Crown F1 with low cotyledon areas, cotyledon and seed weight were used in this study.

Table 1. Sort of status, and the place of supply of melon genotypes used in the study.

Genotypes	Type Status	Locality / Place of issue
Tunceli	Local Varie	Tunceli /Farmer
Amasya	Local Varie	Amasya I /Menemen
Malatya	Local Varie	Malatya/Farmer
Urfa	Local Varie	Urfa /Alata
Galactica	Standard Variety	Galactica/Alata
Golden Crown	Hybrid Variety	Golden Crown F1

Cultivation of Plants

Watermelon seed were sown in plastic containers 40x25x5 filled with pumice. After germination, seedlings were transferred into a climate chamber with temperature 26 C,70% humidity and 7000 lux light. After cotyledon leaves took shape of horizontal and the first true leaves occurred, the seedlings were placed into perforated plastic container filled with Hoagland nutrient solution. After the seedlings in Hoagland solution had 4-5 developed leaves, 100 mM NaCl salt stress was gradually applied into nutrient solution. Thereafter, control and salt treated

plants were grown in the climate chamber setting 25 °C temperature, 65% relative humidity. Leaf samples are taken 8 days after salt application for measuring and analysis.

Determination of Weights and Areas of cotyledon and seed weights

100 seeds were weighed and average of 1000 seed weight was calculated proportionally. Cotyledon area was determined one week after seed germination using planometer. Cotyledon weight was determined when it come horizontal position in randomly selected 10 seedling.

Description of the scale

The degree of morphological injury caused salt stress was defined by using following scale 8 days after the application of salt.

0: not affected
1: growth retardation, local yellowing and curling of leaves
2: yellowing and necrotic spots on 25% of the leaves
3: necrotic spots and fall of 25-50% of leaves
4: necrosis and fall of 50-75% of leaves
5: severe necrosis on 75-100% of leaves and completely death of plants

Enzymes extraction and assay

Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at -80 C for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium phosphate buffer pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15000 x g for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4 C.

Catalase activity was determined by monitoring the disappearance of H_2O_2 according to the method of Cakmak and Marschner (1992).

APX activity was determined by measuring ascorbate consumption by absorbance at 290 nm. One unit of APX was defined as the amount of enzyme required to consume 1 µmol ascorbate min^{-1} (Cakmak and Marschner 1992).

SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT.

All results reported were the means of three replicates. The each repetition had 10 plants Data were analyzed statistically and the means of each treatment were separated by Duncan's Multiple Range Test using SAS (1985) software.

RESULTS

Weight of seed and cotyledon, and scale values

Malatya and Urfa genotypes had higher cotyledon and seed weight, and cotyledon leaf area than Galactica and Crown F. Tunceli and Amasya were found at the medium level in terms of cotyledon and seed weight, and cotyledon leaf area (Table 2).

The degree of morphological injury determined by using scale (0-5) was more in the genotypes with larger cotyledon and seed than that in the genotypes with smaller cotyledon and seed (Table 2).

Table 2. Cotyledon leaf area, cotyledon weight, seed weight and scale point of watermelon genotypes exposed to salt stress.

Code	Cotyledon Leaf Area	Cotyledon Leaf Weight	Seed Weight	Scale
Tunceli	12,13 c	0,67 d	93,50 d	4,00 c
Amasya	19,20 b	0,85 bc	167,50 c	3,00 d
Malatya	23,40 a	1,11 a	168,30 b	1,50 e
Urfa	24,06 a	0,93 b	173,20 a	1,00 f
Galactica	7,73 d	0,28 e	52,90 e	5,00 b
Golden Crown	7,03 de	0,27 e	35,40 f	5,00 a

Difference between means with same letter in the same column is non significant at $P \leq 0.05$ level according to Duncan's Multiple Range Test

Antioxidant Enzyme Activities

In this study, CAT, APX and SOD enzyme activities in both the developed leaves and cotyledon leaves were examined. In both control and under salt stress, 'Malatya' had the highest CAT activities in its cotyledon leaves among genotypes used this study. Urfa had second highest CAT activities in its cotyledon leaves. The lowest CAT activity was observed in Golden Crown FI hybrid cultivars. When CAT activities in real leaves were evaluated, in control plant, the highest value was observed in Amasya genotypes. The other genotypes did not differ in CAT activity. Salt application increased significantly CAT activities in all genotypes. Among genotypes used in this study, Malatya and Urfa genotypes had the highest CAT activities in their real leaves under salt stress. Amasya had second highest CAT activity under salt stress. Galactica and Golden Crown F1 having the lowest value did not differ in CAT activity in real leaves (Table 3).

Table 3. Cotyledon and leaf catalase (CAT) enzyme activities (mol / min / mg FW) of watermelon plants under salt stress.

	CAT			
	Cotyledon		Leaf	
Code	Control	Salt	Control	salt
Tunceli	14,90 c A	4,11 c B	6,15 c B	27,22 c A
Amasya	11,90 cd A	3,63 c B	12,69 a B	64,71 b A
Malatya	42,73 a A	23,61 a B	5,53 c B	85,38 a A
Urfa	23,85 b A	12,34 b B	5,88 c B	82,18 a A
Galactica	8,16 d A	6,36 c B	5,19 c B	15,54 d A
Golden Crown	2,72 e B	2,72 c B	7,53 c B	18,41 d A

Difference between means with same small letter in the same column is non significant at $P \leq 0.05$ level

Difference between means with same capital letter in the same row is non significant at $P \leq 0.05$ level

While salt application increased significantly APX enzyme activities in developed leaves, it decreased APX activity in cotyledons in all genotypes. Malatya had the highest APX activity in nearly all case. Amasya and Urfa were other genotypes having high APX activities. Galctica and Golden Crown F1 had lower APX activities in all case relative to other genotype (Table 4).

Table 4. Ascorbate perocsidase (APX) enzyme activities (mol / min / mg FW) in cotyledon and developed leaf of watermelon plants under salt stress.

	APX			
	Cotyledon		Leaf	
Code	Control	Salt	Control	Salt
Tunceli	0,86d A	0,21c B	0,93b B	1,11c A
Amasya	1,02c A	0,24b B	0,91b B	2,49b A
Malatya	3,67a A	0,29a B	0,99a B	2,50b A
Urfa	2,40b A	0,23b B	0,69c B	2,70a A
Galactica	0,46f A	0,17d B	0,26e B	0,29e A
Golden Crown	0,67e A	0,10e B	0,59d B	0,76d A

Difference between means with same small letter in the same column is non significant at $P \leq 0.05$ level

Difference between means with same capital letter in the same row is non significant at $P \leq 0.05$ level

SOD activity in cotyledon was decreased by salt application, but increased in developed leaves of all genotypes except for Golden Crown F1. Malatya had the highest SOD activity among genotypes used in this study in all case. In the developed leaves, the second highest SOD activity was observed in Amasya under

salt stress. Galactica and Golden Crown F1 had lower SOD activity in both control and salt treatment relative to other genotypes (Table 5).

Table 5. Superocside Dismutase (SOD) (U/dak/mg F.W.) activity of watermelon plants under salt stress.

	SOD			
	Cotyledon		Leaf	
Code	Control	Salt	Control	Salt
Tunceli	141,3d A	124,0b B	115,0b B	175,0c A
Amasya	137,0e A	122,0c B	110,0c B	201,0b A
Malatya	204,0a A	129,0a B	141,0a B	247,7a A
Urfa	157,0b A	121,0d B	108,0c B	176,3c A
Galactica	133,0f A	90,3e B	86,0d B	99,3d A
Golden Crown	142,0c A	84,0f B	110,0c A	102,0d A

Difference between means with same small letter in the same column is non significant at $P \leq 0.05$ level

Difference between means with same capital letter in the same row is non significant at $P \leq 0.05$ level

DISCUSSION

The scale points based on morphological damage caused by salt application showed that Malatya and Urfa were less damaged by salt stress whereas Galactica and Golden Crown F1 were very sensitive. Cotyledon and seed weight of tolerant genotypes (Malatya and Urfa) were more than those of sensitive genotypes (Galactica and Golden Crown F1). These results were in accordance with results from pepper by Aktaş (2002) and watermelon by Yasar (2007) who also reported a relationship between salt tolerance and weight of cotyledon and seed.

When plant expose to any environmental stress, it enhances in extent of genetic ability the activities of some antioxidant enzymes such as SOD, CAT and APX. This situation was well documented in many studies with different species (Gossett et al. 1996; Harinasut et al. 2003; Yasar 2003; Yasar et al. 2006; Yasar et al. 2008a; Yasar et al. 2008b). CAT, APX and SOD activities in developed leaves were increased by 100 mM NaCl application in all varieties of watermelon used in this study. The increasing in these three enzymes activities was more in tolerant varieties (Malatya and Urfa) than sensitive varieties (Galactica and Golden Crown F1). This result is consistent with results in Setaria sp. by Sreenivasulu et al. (2000), in wheat by Karanlık (2001), in eggplant by Yaşar (2003), in melon by Yasar et al. (2006), in watermelon by Yasar et al. (2008a), in tomato by Shalata and Tal (1998), and in carrot by Lopez et al. (1996).

Tolerant varieties with high enzyme activity had larger seed and cotyledon than sensitive varieties with low enzyme activities. This result suggests that seed

and cotyledon size may be a genetic trait to determine salt tolerance level of plants. Probably, the varieties with larger seed and cotyledon may be better fed and run better their antioxidant enzymes due to have more carbohydrate, vitamins and nutrient elements.

In conclusion, the result of this study was showed that seed and cotyledon size can be used as preliminary criteria to determine salt tolerant level for watermelon varieties.

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Investigation on fruit quality of grafted watermelon

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Keywords: *Citrullus lanatus*, antioxidant activity, total phenolic compounds, sensory evaluation, soluble solid contents

Abstract

In this study, grafted and ungrafted watermelon cultivars were grown in Cukurova region to determine fruit pH, titratable acidity, soluble solids, antioxidant activity, total phenolic compounds and analysis of sensory evaluation. The highest soluble solids (11.32%) and titratable acidity 0.190 g/100g were in seedless cultivars. The highest antioxidant activity and total phenolic content was in Argentario+TP84, 80.21% and 137.40 (GAE) mg/kg fresh weight (fw) respectively. For sensory analysis, the most popular cultivars were grafted type.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is largely consumed as refreshing summer fruit throughout the Mediterranean basin. Watermelon provides dietary antioxidants such as carotenoids (lycopene and β-carotene), phenols, vitamins (A, B, C and E) and specific amino acids (citrulline and arginine) (Perkins-Veazie 2002; Perkins-Veazie et al. 2007). Carotenoids are thought to exert a protective role in reducing the risk of certain types of cancers, cardiovascular diseases and age-related degenerative pathologies (Rice-Evans et al. 1996; Giovannucci 1999; Rao 2006). The health benefits of eating watermelon, as well as its low caloric value, make it an ideal food (Tlili et al. 2011).

In recent years, the production of grafted watermelon fruit has increased rapidly. However, research is needed on the effect of grafting on fruit quality. Fusarium wilt is the most important factor limiting the production of watermelons in the world

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and higher resistance is needed. Four-year rotations are required to reduce Fusarium wilt incidence in fields used for watermelon production. Researchers (Yetişir et al. 2003; Koren and Edlstein 2004; Roberts et al. 2005; Yetişir et al. 2007) reported the benefits of grafting for watermelon production, including improved sugar content, lycopene and flesh texture.

Due to the complexity of the composition of foods, their antioxidant power depends on the synergistic effects and redox interactions between the different nutrient and ''non nutrient'' molecules, which together contribute to the possible health benefits. Therefore, recent attention has been given to the antioxidant activity of fruits and vegetables. A parameter that allows a real evaluation of the nutritional value of foods (Lenucci et al. 2006; Pellegrini et al. 2007).

In this study, four cultivars having different morphological characteristics were grafted onto rootstocks of *Lagenaria* spp., *C. maxima* x *C. moschata*. Ungrafted cultivars were used as controls. Grafted watermelon cultivars were grown in Cukurova region and studied for fruit traits such as pH, titratable acidity, brix, antioxidant activity, total phenolic contents and analysis of sensory evaluation.

MATERIALS AND METHODS

Four watermelon cultivars with different morphological characteristics (yellow fleshed, seedless, striped rind and black rind) grafted on rootstocks of a Argentario (*Lagenaria*) and Maximus (*C. maxima* x *C. moschata*). Ungrafted varieties were used as control plants. TP84 and 5299 is striped outer shell and red fleshed cultivars. Ant07 is slimline striped outer shell and yellow fleshed. Ç4 is seedless and black rind (Fig. 1). Cultivars were grown from March to July. Irrigation and fertilizer was through drip irrigation. Weed control was through tractor cultivation and hand pulling.

Watermelon harvest and sampling

Fruit harvest was made when the tendril at the fruiting node was brown and dry. Three independent samples of at least 3 injury-free watermelon fruits for each cultivar were hand-harvested randomly. Watermelon fruits were quickly delivered to the laboratory and immediately cut longitudinally from the stem-end to the blossom-end through 1 cm to the right and 1 cm to the left of the ground spot. Flesh samples were taken from the heart area (between locular and the fruit centre) of each watermelon. Soluble solids content (˚Brix) was measured by cutting a wedge of flesh from the heart area and squeezing the juice into a digital refractometer (Atago Pal-1) calibrated with a 10% sucrose solution. Surface pH was also measured on heart tissue of each replicate sample using WTW pH indicator. For titratable acidity, 10 ml of watermelon juice titratated 0.1 N NaOH solutions using a pH meter to be pH 8.1. The results in terms of fresh weight in grams of citric acid to be calculated per 100 g (Cemeroğlu 2007).

Total phenolic content determination

Phenolics were measured using 5 g fruit sample, with 5 ml of methanol solution (80%) added to a suitable rate of methanol solution (80%) with dilution be made with 4000 rpm, 4°C, centrifuged for 20 min. and collected in a 100 µL glass tube on the Folin-Ciocalteu solution of 100 µL and 3000 µL of distilled water for 10 min. Then the solution was added 100 mL of 20% Na_2CO_3 and waited 2 hours in the dark. The absorbance was read at 765 nm using a spectrophotometer. Results were expressed in mg gallic acid equivalent (GAE)/kg fresh weight (fw) (Abdulkasım et al. 2007).

Determination of antioxidant activity

The antioxidant activity of the cultivars was evaluated by DPPH free radical-scavenging method. Watermelon juice samples of 5 g of 5 mL of 80% methanol solution and the mixture was stirred sufficiently and then vortexed 4°C at 4000 rpm for 20 minutes in Heraeus Biofuge Primo R brand (Osterode, Germany) centrifuge. A 100 µL sample was centrifuged at 2460 µL of juice and 1.1-diphenyl-2-pikrilhidrazil (DPPH*. 80% methanol 0.025g/L) was added. A 100µ L of distilled water was used as the control sample. The absorbance of the samples was measured as the time to loss of 80% methanol for 0, 20, 30, 45, 60 minutes. A Perkin Elmer Lambda 25 UV/VIS (Massachusetts, USA. 2005) spectrophotometer set at 515 nm was measured and the measurement set at 5 minute data were used. Inhibition free radical DPPH in percent (I %) was calculated in following way:

$$I\ (\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) x100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound (Klimczak et al. 2007).

Sensory evaluation

Graphic scale of the samples using the method of sensory evaluation as a panelist by a group of 13 people of color, taste, odor, tissue characteristics and taking into account the overall impression is made.

Statistical analysis

Effects of cultivars on the nutritional properties of watermelon cultivars were assessed by analysis of variance (ANOVA). When a significant difference was detected means were compared using the least significant difference (LSD) test ($p<0.05$) at DUNCAN test. Statistical analysis were conducted using CoStat Statistics Software version 3.01 (CoHort Software, Monterey, CA).

RESULTS AND DISCUSSION

The amount antioxidant capacity, total phenolic, total, pH and titratable acidity in watermelon cultivars grown in the Alata Horticultural Research Station and harvested at ripening are reported in Table 1.

The highest soluble solids (11.32 %) and titratable acidity 0.190g/100g were in the seedless cultivar. Our results are in agreement with those of Davis and Perkins-Veazie (2005-2006) for soluble solids content in watermelon.

Watermelon cultivars were determined pH values 5.56 pH (Argentario+5299) between 4.977 (Maximus+Seedless) and titratable acidity 0.190g/100g (Seedless) between 0.096g /100g (TP 84). When data were averaged, pH and titratable acidity varied significantly between samples ($P < 0.05$) (Table 1).

The highest average total phenolic content was detected in Argentario+TP84 cvs (137.40 mg GAE/kg fw), Argentario+5299 (133.92 mg GAE/kg fw) and Maximus+5299 (127.98 mg GAE/kg fw) cvs whose values are not statistically different, showed the lowest average content of total phenolics. These results are consistent with those reported by Brat et al. (2006) and Tilli et al. (2011) who reported -116 mg GAE/kg fw and 92.3 mg GAE/kg fw-89.0 mg GAE/kg fw, respectively. Much higher values ranging between 870 and 910 mg GAE/kg fw were obtained in red-fleshed watermelon cvs by Perkins-Veazie et al. (2002). In our case, no statistically significant differences were found among cvs.

Many have studied correlations between bioactive compounds and antioxidant activities in numerous fruits and vegetables particularly tomatoes (Lenucci et al. 2006; Raffo et al. 2002; Giovanelli et al. 1999) The highest antioxidant activity have been identified Argentario+TP84 (80.21%).

The most popular type of sensory analysis of color assessment has been grafted and non grafted cultivars of 5299. Argentario+TP84 are the most popular cultivar for taste, aroma and texture. The highest scores of the overall impression received Argentario+TP84 (8.06). In sensory analysis Ant07 (grafted and non grafted cultivars) with yellow fleshed is received the lowest scores. In this case no statistically significant differences were found among sensory analysis cvs.

CONCLUSIONS

Argentario+TP84 has been determined highest value of antioxidant activity and total phenolic also kind of the most popular taste, tissue and the general impression. The antioxidant activity is Argentario rootstock grafted all cultivars of the red fleshed were higher than other cultivars. In terms the most popular cultivars of taste and aroma has been 5299. In terms sensory analysis, antioxidant activity and total phenolic content based on the above data higher values were grafted cultivars than non grafted cultivars.

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Table 1. Soluble solid, pH, titratable acidity, % antioxidant capacity, total phenolic contents in watermelon cultivars[1]

Cultivar	Soluble Solid Contents (%)	pH	Titratable acidity (g/100g)	Antioxidant activity (%)	Total Phenolic (mg/kg)
Maximus+Ant07	10.50 ^{b}	5.083^{g}	0.164 ^{c}	34.63 ^{de}	117.67 ^{cd}
Maximus+5299	9.43 ^{d}	5.300 ^{d}	0.124^{g}	30.30 ^{e}	127.98^{ac}
Maximus+Seedless	8.95 ^{e}	4.977 ^{h}	0.140 ^{e}	19.17^{f}	112.00 ^{cd}
Maximus+TP84	10.00 ^{c}	5.240 ^{e}	0.153 ^{d}	71.56^{ab}	73.61^{f}
Argentario+Ant07	9.00 ^{e}	5.167^{f}	0.143 ^{e}	38.87^{ce}	90.70 ^{e}
Argentario+5299	8.50^{f}	5.560^{a}	0.118 ^{h}	64.30 ^{b}	133.92^{ab}
Argentario+Seedless	10.00 ^{c}	5.140^{f}	0.153 ^{d}	74.83^{a}	92.03 ^{e}
Argentario+TP84	8.57^{f}	5.447 ^{c}	0.175 ^{b}	80.2 ^{a}	137.40^{a}
Ant07	8.50^{f}	5.503 ^{b}	0.132^{f}	73.52^{ab}	119.00^{bd}
5299	8.10^{g}	5.463 ^{c}	0.109^{i}	46.09 ^{c}	116.61 ^{cd}
Seedless	11.30 ^{a}	5.063^{g}	0.190^{a}	29.73 ^{e}	90.87 ^{e}
TP84	9.07 ^{e}	5.313 ^{d}	0.096 ^{j}	43.44 ^{cd}	108.03 ^{d}
LSD (5 %)	0.36	0.028	0.004	9.05	14.63

[1]Values are given as mean (n = 3), Letters indicate mean separation within each cultivar; values with different letters are significantly different at $P < 0.05$ by LSD test.

Table 2. Results of sensory analysis and evaluations.

Cultivars	Colour	Taste	Odor	Tissue	Overall Impression
Maximus+Ant07	5.66 ^{cd}	5.93 ^{ce}	4.58^{c}	5.54 ^{ce}	5.40 ^{cd}
Maximus+5299	7.79^{a}	7.50^{ac}	7.50^{a}	7.53 ^{ab}	7.55 ^{ab}
Maximus +Seedless	6.91^{ac}	5.70 ^{de}	5.40 ^{c}	6.30 ^{be}	5.83 ^{cd}
Maximus+TP84	7.03^{ac}	6.31 ^{be}	5.74^{bc}	6.61^{ae}	6.74^{ac}
Argentario+Ant07	7.43^{ab}	5.81 ^{de}	5.54 ^{c}	6.90 ^{ad}	6.21^{bd}
Argentario+5299	7.66 ^{ab}	8.23^{a}	7.59^{a}	8.0 ^{a}	8.00^{a}
Argentario +Seedless	4.58 ^{d}	4.94 ^{e}	4.81 ^{c}	5.53 ^{ce}	4.98 ^{d}
Argentario+TP84	7.55 ^{ab}	8.3 ^{a}	7.8 ^{a}	8.0 ^{a}	8.06 ^{a}
Ant 07	6.15 ^{ad}	5.65 ^{de}	5.41 ^{c}	5.20 ^{e}	5.48 ^{cd}
5299	7.71^{a}	7.65 ^{ab}	7.19 ^{ab}	7.80 ^{ab}	7.83^{a}
Seedless	6.00^{bd}	7.20 ^{ad}	7.13 ^{ab}	7.13^{ac}	6.84^{ac}
TP84	5.14 ^{d}	5.32 ^{e}	5.56 c	5.49 ^{de}	5.31 ^{cd}
LSD (5%)	1.45	1.51	1.45	1.41	1.34

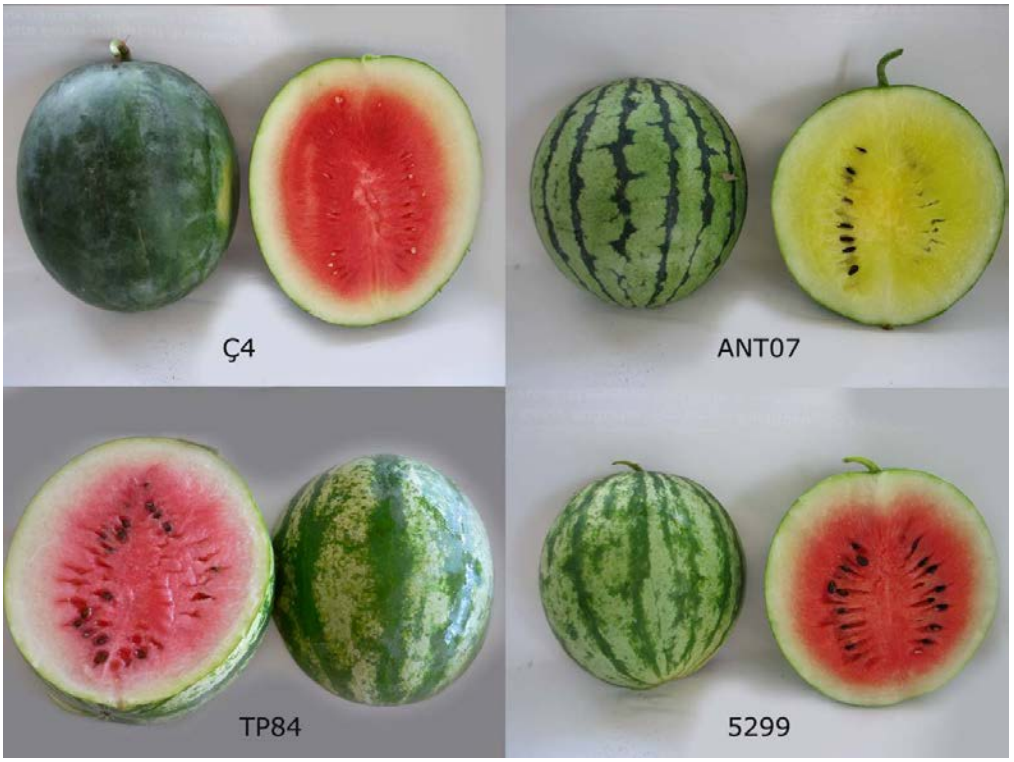

Fig.1. Fruit characteristics of the four cultivars studied.

Determining the agronomic characteristics of Turkish watermelon genotypes for developing breeding lines

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Keywords: *Citrullus lanatus* L., genetic resources, yield, quality

Abstract

Watermelon is an important vegetable in Turkey with approximately four million tonnes of annual production. Despite a wide range of morphological diversity in Turkish watermelons, mainly imported hybrid watermelon cultivars are used in production due to high yield and quality. The watermelon genebank in the Department of Horticulture in Çukurova University consists of 475 accessions collected from different regions of Turkey and provided from different genebanks of the world. These watermelon genetic resources were morphologically characterized, elite accessions that were chosen and self-pollinated for use in breeding studies. The aim of this study was to evaluate the horticultural characteristics of promising watermelon genotypes for two years. Length of main stem, diameter of main stem, number of branches per plant, number of nodes on main stem, yield, fruit weight, fruit length, fruit diameter, rind thickness and total soluble solids were measured in all genotypes. Data analysis indicated that the Turkish genotypes had high diversity for plant and fruit characteristics.

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. ve Nakai] is an important vegetable crop in Turkey. The production amount is 3 683 100 tonnes on 150 000 ha area (FAO, 2010).

Citrullus comprises 4 main species, however only *C. lanatus* var. *lanatus* is produced for marketing throughout the world. This species has many seeded and seedless (triploid) types. Fruits of *C. lanatus* var. *lanatus* are quite varied in size, shape and rind characters (Robinson and Decker-Walters 1997; Wehner 2008).

Turkey is not the center of origin for watermelon. Nevertheless Zhukovsky

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(1933) had reported the existence of wild types in Anatolia. It is known that Turkey is rich in watermelon genetic resources. Several local genotypes are still grown in some regions especially in Southeastern Anatolia. However, they are being replaced by higher yielding foreign cultivars (Solmaz and Sari 2009).

Çukurova University, Faculty of Agriculture, Department of Horticulture has a watermelon genetic resources collection which consists of 475 genotypes. Morphological characterization (Sari et al. 2007; Solmaz and Sari 2009) and molecular characterization (Solmaz et al. 2010) of these genotypes were done and seeds are regenerated and selfed for using in further breeding studies. Some of the main goals of watermelon breeding are fruit shape, earliness, high yield and fruit internal quality (Wehner 2008).

The objective of this study was to determine the agronomic chaacters of promising genotypes selected from the watermelon genetic resource collection of Çukurova University.

MATERIALS AND METHODS

This study was conducted at the research and application area of the Department of Horticulture, Faculty of Agriculture, University of Cukurova during 2011 and 2012. In 2011 14 (16 in 2012) promising genotypes chosen from the watermelon germplasm collection of Cukurova University and used as plant material.

Seedlings were planted on 1 April in 2011 and 18 April in 2012 under low plastic tunnels. Spacing was 2 m between rows and 0.5 m within rows. The experiment design was a randomized complete block with three replications and 15 plants per plot. Drip irrigation system was applied and plants were fertilized by $N:P_2O_5:K_2O$ according to 15:10:20 kg/ha pure ratio. Weeds were controlled by hand and fungucides and insecticides were applied against anthracnose and leaf mites. Low tunnels were opened on 18th May in 2011 and 16th May in 2012. Length of main stem (cm), diameter of main stem (mm), number of brances per plant, number of nodes on main stem were determined in 3 plants of each replicate in two years with a meter and digital compass (Mitutoyo CD-15D). Fruits were harvested on 4th July in 2011 and on 26th July in 2012. Total yield (kg/m^2), fruit weight (kg) fruit length (cm), fruit diameter (cm), rind thickness (mm) and total soluble solids (%) were measured in 3 fruits of each replicate for two years. Measurements were carried out with the help of a meter, ruler and a digital compass (Mitutoyo CD-15D). Total soluble solids were analyzed by a hand held electronic refractometer (ATAGO Pocket PAL-3l). Data were subjected to an analysis of variance by COSTAT statistical programme and means were compared by Tukey's test at 0.01 and 0.05 significance level.

RESULTS AND DISCUSSION

The mean values of length of main stem, diameter of main stem, number of branches per plant, and number of nodes on main stem were presented in Table 1. The analysis of variance indicated no differences in 2011 while significant differences existed among genotypes in 2012. Length of main stem ranged from 142.67-196.27cm in 2011 and 77.25-138.00 cm in 2012. Diameter of main stem ranged from 4.03 to 5.20 mm in 2011 and 2.00 to 4.67 mm in 2012. Number of branches is one of the characters that determines the plant vigor. In 2011 it was between 3.80-5.33 and 2.00-4.67 in 2012. Number of nodes on main stem ranged from 23.13 to 29.07 in 2011 and 15.75 to 25.67 in 2012.

Yield, fruit weight, fruit length, fruit diameter, rind thickness and total soluble solids were measured and mean values for two years are in Table 2. The analysis of variance indicated that significant differences existed between the genotypes for all fruit traits. The average yield ranged from 2.41 to 5.17 kg/m^2 in 2011 and from 1.48 to 5.28 kg/m^2 in 2012. High yield is one of the major goals for watermelon breeding. Watermelon fruits usually weigh 4 to 16 kg. However in Asia, even smaller fruit in the range of 1 to 4 kg are popular (Wehner 2008). The average fruit weight of the genotypes studied were varied from 2.17 to 5.43 kg in 2011 and 2.81 to 6.33 kg in 2012. Small size fruit, such as the mini watermelons are becoming more popular. Regarding fruit weight Turkish watermelon germplasm has good attributes for breeding small to medium size watermelons. The fruit shape of the genotpypes were round to cylindrical. The length of the fruit ranged from 15.58 to 26.75 cm in 2011 and 17.67 to 34.61 cm in 2012. Fruit diameter ranged between 13.60 and 20.97 in 2011 and 14.63 to 23.08 cm in 2012. Rind thickness is an important trait for resistance to damage during transport. It ranged from 11.64 to 22.05 mm in 2011 and from 10.45 to 25.38 mm in 2012. The analysis of variance revealed significant differences among genotypes regarding total soluble solids. In 2011 it ranged 5.63 %-9.63 % and 7.75 %-11.50 % in 2012.

High yield and good fruit quality are primary goals of watermelon breeders. Evaluating germplasm is one of the first steps in breeding. There are several reports on evaluating watermelon genotypes for developing new cultivars. Gusmini and Wehner (2005) measured fruit yield in 80 watermelon cultivars to identify high-yielding germplasm in North Carolina for use in breeding programs. The objective of their study was to measure yield in a diverse set of watermelon cultivars to identify. The researchers reported significant yield differences among the 80 cultivars across environments and high-yielding cultivars for use in breeding programs were identified.

de Queiroz et al. (2000) had evaluated some accessions of watermelon germplasm bank consisting 600 accessions for *Sphaerotheca fuliginea* and *Didymella bryoniae*. New triploid hybrids were developed and evaluated in field

experiments. They reported that some of the hybrids had high yield and high sugar content. Krasteva et al. (2000) evaluated the Bulgarian national watermelon collection obtained from different parts of the country and supplemented from various countries and commercial sources. The collection was evaluated for 30 characters. Main stem length ranged 1.6 to 2.8 m. Fruit weight, shape, length and diameter varied significantly among accessions. Fruit weight was 4.6-10 kg in local genotypes and 4-8 kg in foreign genotypes. Six watermelon lines were investigated in a full diallel for breeding purposes by Gvozdanovic-Varga et al. (2011). Genotypes were analyzed for 4 fruit traits as fruit weight, rind thickness, sugar content and fruit shape. The analysis of variance indicated highly significant differences. The average fruit weight of the genotypes ranged from 5.35 to 10.42 and rind thickness ranged from 0.9 to 2.1 cm. Maggs-Kölling and Christiansen (2003) evaluated local Namibian watermelon landraces and commercial cultivars. They reported that yield of watermelon landraces can be compared to commercial cultivars, although their fruit were significantly smaller.

The selected Turkish genotypes revealed high diversity in terms of measured plant and fruit characteristics. The next step is to plan breeding strategies for different markets and develop new hybrids using these genotypes.

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Table 1. Mean values of watermelon genotypes for measured plant characters.

Genotype	Length of main stem (cm)		Diameter of main stem (mm)		Number of branch per plant		Number of node on main stem	
	2011	2012	2011	2012	2011	2012	2011	2012
KAR 23	142.67	129.85 ab	4.03	6.32 ab	5.13	4.50 a	22.60	23.17 abc
KAR 58A	196.27	109.75 a-d	4.95	7.66 ab	4.33	3.25 ab	26.93	18.75 abc
KAR 84	187.73	97.55 bcd	4.93	6.91 ab	3.80	2.68 ab	28.07	17.17 bc
KAR 102	148.93	132.83 ab	5.22	6.66 ab	5.07	4.67 a	24.00	20.67 abc
KAR 116	147.00	110.17 a-d	5.20	6.84 ab	5.33	4.17 ab	23.13	21.84 abc
KAR 117	155.93	117.80 abc	4.31	6.80 ab	5.13	2.48 ab	23.93	22.89 abc
KAR 147	151.04	77.25 d	4.80	6.58 ab	4.38	2.00 b	23.62	15.75 c
KAR 154	172.67	113.25 a-d	4.79	8.02 a	4.40	2.75 ab	29.07	23.00 abc
KAR 175	166.78	133.59 ab	4.63	7.56 ab	4.91	2.50 ab	25.38	25.67 a
KAR 177	183.53	107.33 a-d	5.05	7.46 ab	4.27	2.78 ab	27.33	18.44 abc
KAR 180	-	106.84 a-d	-	8.08 a	-	3.33 ab	-	24.06 ab
KAR 181	161.87	108.50 a-d	4.53	6.72 ab	4.47	3.72 ab	25.33	22.06 abc
KAR 188	171.53	91.67 cd	4.40	5.43 b	4.47	2.34 ab	27.27	18.17 abc
KAR 189	157.33	115.50 abc	4.10	5.83 ab	4.33	2.00 b	25.20	22.50 abc
KAR 203	181.20	116.34 abc	4.66	6.70 ab	4.07	2.59 ab	25.47	20.06 abc
KAR 223	-	138.00 a	-	7.40 ab		4.33 a	-	23.50 abc
LSD 0.01		36.94						8.21
LSD 0.05	ns		ns	2.30	ns	2.29	ns	

Table 2. Mean values of watermelon genotypes for measured fruit characters.

Genotype	Yield (kg/m^2)		Fruit weight (kg)		Fruit length (cm)		Fruit diameter (cm)		Rind thickness (mm)		Total soluble solids (%)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
KAR 23	2.96 ab	4.79 ab	2.88 b	4.67 ab	26.75 a	34.61 a	13.60 b	16.50 ab	13.44 bc	20.22 abc	5.68 de	7.75 d
KAR 58A	2.41 b	5.28 a	2.88 b	4.06 ab	17.16 de	24.00 c-f	16.73 ab	20.13 ab	13.37 bc	19.81 abc	6.23 cde	10.59 abc
KAR 84	2.51 b	2.90 abc	2.98 b	2.89 b	17.58 de	18.05 ef	16.68 ab	17.50 ab	13.20 bc	17.00 abc	6.87 b-e	8.90 bcd
KAR 102	2.61 b	3.48 abc	2.98 b	4.94 ab	21.09 bcd	26.10 b-e	16.27 ab	19.01 ab	12.92 bc	16.14 abc	6.18 de	10.70 abc
KAR 116	2.77 b	4.75 ab	3.39 ab	5.15 ab	19.11 cde	24.79 c-f	17.36 ab	18.13 ab	14.13 bc	21.15 ab	6.58 b-e	9.86 a-d
KAR 117	2.30 b	3.71 abc	2.67 b	3.06 ab	16.82 de	29.76 abc	16.54 ab	14.63 b	16.53 b	17.00 abc	5.63 e	8.37 cd
KAR 147	3.70 ab	3.62 abc	4.29 ab	3.12 ab	20.27 cde	19.38 ef	20.97 a	16.73 ab	15.14 bc	11.83 bc	8.15 a-d	10.30 a-d
KAR 154	3.47 ab	4.66 ab	3.89 ab	6.33 a	18.98 cde	22.87 c-f	19.44 a	23.08 a	15.52 bc	25.38 a	5.79 de	11.01 ab
KAR 175	3.03 ab	5.17 a	2.71 b	4.06 ab	17.43 de	19.68 ef	17.07 ab	19.32 ab	12.85 bc	14.06 bc	6.70 b-e	9.54 a-d
KAR 177	5.17 a	2.38 bc	5.43 a	3.99 ab	25.99 ab	20.54 def	16.69 ab	17.56 ab	13.50 bc	12.36 bc	8.66 abc	10.38 abc
KAR 180	-	3.59 abc	-	4.37 ab	-	20.97 def	-	20.17 ab	-	18.65 abc	-	9.05 a-d
KAR 181	2.71 b	2.20 bc	3.20 b	3.50 ab	24.34 abc	27.97 a-d	16.06 ab	15.85 b	22.05 a	18.34 abc	9.63 a	11.50 a
KAR 188	3.39 ab	1.48 c	3.99 ab	2.81 b	20.92 b-e	17.67 f	19.07 ab	17.06 ab	15.11 bc	10.45 c	8.71 ab	10.99 ab
KAR 189	3.43 ab	1.56 c	3.46 ab	2.86 b	19.95 cde	18.86 ef	18.28 ab	16.78 ab	13.93 bc	12.67 bc	10.19 a	10.29 a-d
KAR 203	2.42 b	3.51 abc	2.17 b	3.66 ab	15.58 e	19.79 def	15.36 ab	19.04 ab	11.64 c	18.93 abc	5.41 e	8.54 bcd
KAR 223	-	1.63 c	-	2.74 b	-	34.24 ab	-	17.57 ab	-	19.50 abc	-	9.70 a-d
LSD 0.01	2.29	2.59	2.09	3.38	5.44	8.28	5.79	6.69	4.59	9.85	1.57	2.55
LSD 0.05												

Genetic diversity of watermelon and the contribution of community practices to its conservation on farm

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Keywords: Watermelon, practice, local cultivars, on farm, conservation

Abstract

Watermelon (*Citrullus lanatus*), belonging to the Cucurbitaceae family, is commonly grown in traditional agrosystems throughout the drought-prone areas in southern Africa. It is used as sweet watermelon accessions (*Citrullus lanatus* var. *lanatus*) containing almost as much variability as cow-melons (*Citrullus lanatus* var. *citroides*). In Zimbabwe, the wild-weed-landrace complex structured into three unique sets of alleles confirming the three major forms with limited admixtures. Evaluating the importance of a practice for the conservation of local cultivars illustrated that farmers grow different forms of watermelons based on their known value for cultivation and use. Most watermelon forms grown by many farmers on large areas have a high value for cultivation and use, mostly being used for human consumption and livelihood income. Many such forms of watermelon grown on large areas have a diversity of uses. The study also revealed that sustainability of a practice is closely related to the contribution of a practice to conservation of watermelon diversity. The rarity of local cultivars is also a reflection of the erosion of the indigenous knowledge systems concerning their value for cultivation and use. Practices that meet basic farmer livelihood strategies and conservation efforts should be promoted, and incorporated into national agricultural and biodiversity policies as a farmer incentive and to allow for germplasm exchange.

INTRODUCTION

Watermelon (*Citrullus lanatus*), belonging to the Cucurbitaceae family, is commonly grown in traditional agrosystems throughout drought-prone southern Africa as a staple food (edible seeds), a dessert food (edible flesh), and for animal feed. These traditional agrosystems constitute the marginal agricultural areas where modern crop cultivars and inputs are less available and less effective for resource-poor farmers. Such areas are a haven to farmers' continuing use of local cultivars or landraces, which due to crop adaptability contributes to stable food production and household income. Increased population, poverty, and land degradation have

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contributed to the loss of indigenous knowledge systems and the erosion of genetic resources in many crops impacting negatively on food security.

Traditionally, local cultivars embody substantial diversity, and continue to provide an essential component of sustainable crop production for many of Zimbabwean poor. This linkage between diversity and food security provides the rationale for enhancing the availability and use of local crop cultivars in the fragile ecosystems of arid and semi-arid areas of the country. The existence of watermelon diversity in Zimbabwe has been described in several studies (Mujaju et al. 2010; Mujaju and Nybom 2011; Mujaju et al. 2011; Mujaju et al. 2012).

The need to support and strengthen the conservation and use of farmers' cultivars is becoming more recognized as a valuable component in order to understand and enhance the mechanisms of *in situ* conservation on-farm. An approach that evaluates the importance of a practice for rare local cultivars and the contribution of a practice to conservation of local cultivars was used. The process aims to provide farmers as well as development agents and researchers with new insights into what keeps local cultivars on farm.

MATERIALS AND METHODS

Study sites & participants

One group was used in each of the two study sites. Sites were Matobo District representing the Ndebele farmers, and Zaka District representing the Shona farmers, with a minimum of 40 participating farmers. The two study sites were chosen based on original communities that have traditionally been made up of two different main cultures, growing local cultivars of watermelon. The groups were made up of farmers aged 45 to 60. The majority (80%) of the participating farmers were women. Most males reside in towns where they work. Similarly, it was difficult to get farmers below age 40, as most were working in towns or studying in schools.

Data collection and analysis

Data collection was done using the participatory extent and distribution analysis (also known as the four square analysis), which aims to identify the rare local cultivars, their traits, and possible practices for local cultivar conservation (Grum et al. 2008). A four-square analysis was used to represent the participatory extent and distribution of local cultivars of watermelon. Data on conservation practices and their relationship with cultivars was subjected to a principal component analysis (PCO) using NTSYS-pc version 2.1 (Rohlf 1993).

RESULTS AND DISCUSSION

The four-square analysis defines particular patterns and associations of watermelon cultivars. The four-square analysis for local cultivars (Fig. 1) showed that seed melon, and orange- and white-fleshed sweet watermelons were rare local cultivars, since they were grown by few households on small areas of land. The wild weedy melons (known as 'Kiriwani') were found in cultivation among many households but on small areas. This is mostly because farmers favour growing the red-fleshed sweet watermelons for income and human consumption as dessert, and the yellow- or orange- fleshed cooking melons for making porridge. The local cultivars that are grown by few farmers on small areas regardless of their positive traits of edible seeds and oil making (seed melons), and dessert (white- and orange-fleshed sweet watermelons) may result from the erosion of indigenous knowledge

systems.

The biplot of local cultivars and practices (Fig. 2) clearly elaborates that local cultivars are associated with their specific practices, which in many respects are their specific uses. The implication of the relationship between local cultivars and practices essentially is a reflection of the importance that different practices have for the maintenance of different local cultivars, which essentially means that no single practice will conserve large levels of germplasm diversity and that a range of practices is essential to maintaining that diversity.

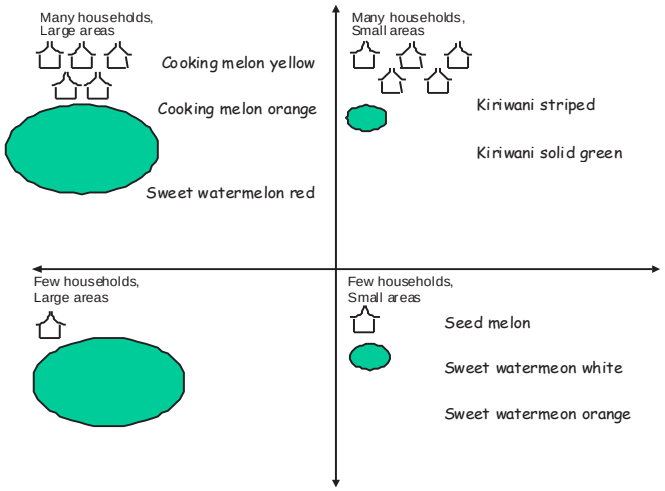

Fig. 1. Participatory extent and distribution analysis (four-square analysis)

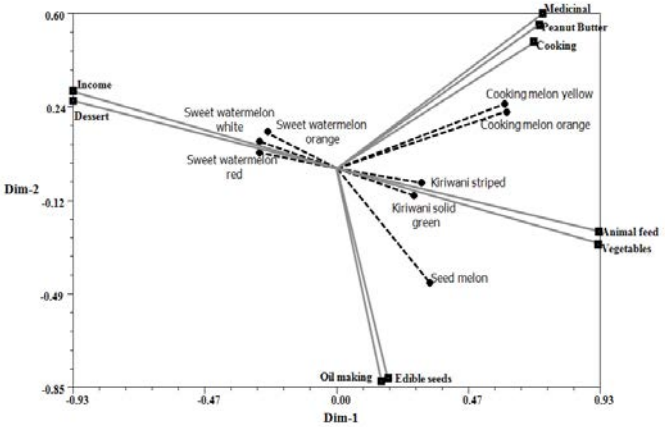

Fig. 2. Principal components analysis of the relationship between practices and local cultivars.

CONCLUSION

We concluded that for conservation of local cultivars to be realized by the communities, their practices relating to use or food security should be met. Germplasm conservation is driven by use. The many agricultural uses of cultivars correspond to the maintenance of high levels of diversity, and no single practice can accomplish that. Thus, communities should be encouraged to promote the use of

their best practices and, in the process, conserve genetic diversity of watermelons on farm. For policy makers it is critical to recognize the interplay between practices of farmers in support of their livelihood and the conservation of local cultivars. Agricultural policies should provide incentives for users to follow good practices and encourage conservation of local cultivars.

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a staple food (edible seeds), as a dessert food (edible flesh), and for animal feed. Traditional farming practices contribute to the maintenance of local cultivars, and increase diversity in those areas. There has been little work on investigating the relationships between wild and cultivated forms, and the contribution of farmer practices, to allow for better conservation strategies. Genetic diversity studies in Zimbabwe using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) exhibited considerable amounts of genetic diversity at all levels, with

Determination of peroxidase gene polymorphism in watermelon lines existing in Turkish genetic resources

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Keywords: Peroxidase gene polymorphism, stres related gene, watermelon

Abstract

Turkey is the second largest producer of watermelon in the world and a considerable number of local cultivars and ecotypes exist in the different regions of Turkey. Twelve peroxidase gene (POX) specific primers developed from the rice genome were used to detect peroxidase gene polymorphism (POGP) in 259 watermelon lines available in the Turkish genetic resources. Cluster analysis indicated that species based clustering and geographical origin of the genotypes were not significant in the clustering. Principle component analysis (PCA) revealed similar results and created three different clusters. The first three Eigen value was calculated and found to be 58.1, which explains the 58% of the total variation. Analysis of molecular variance (AMOVA) indicated 38.56 % variation among the groups, 7.39% variation between populations and within the groups and 54% variation within the population. The genetic polymorphism rate was found to be considerably high in the genotypes studied (98.2%). When both POGP and SRAP markers were considered, *Cophenetic Correlation Coefficient* obtained by Mantel's Matrix Correspondence Test was found to be r = 0.91, which indicates significant correlation between POGP- and SRAP-based similarity matrices. Considering the polymorphism level and cophenetic correlation between SRAP and POGP markers, POGP markers can be useful in evaluating genetic variation in watermelon genotypes.

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Citrulline can be used as a biochemical marker in watermelon screening studies for tolerance to drought and salinity

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Keywords: *Citrullus lanatus*, citrulline, resistance, salt stress, water stress

Abstract

Salinity and drought are two of the most important environmental factors that cause reduction in plant growth, development and productivity worldwide. In the present study, we have investigated the effect of citrulline on drought and salt tolerance in watermelon genotypes. Seeds were germinated in plastic pots containing vermiculite. Plants with three true leaves were subjected to drought and salt stress. Stress responses of the watermelon genotypes were evaluated in early plant development stages. Genotypes were classified according to their citrulline level. Watermelon genotypes with tolerance to salt, KAR-152, KAR-278, KAR-306 and KAR-338, were induced to accumulate citrulline. On the other hand, genotypes with drought tolerance KAR-98, KAR-234, KAR-278, KAR-300, KAR-306 and KAR-374 accumulated higher levels of citrulline than sensitive genotypes. The result of the study, evaluating citrulline as a biochemical marker for salt and drought tolerance of watermelon genotypes is discussed.

INTRODUCTION

Drought and salinity are the major environmental constraints to crop productivity. Plants have developed a variety of strategies and mechanisms in response to changes in their environments (Yoshimura et al. 2008). One of the biochemical changes occurring when plants are subjected to harmful stress conditions such as drought and salinity is the accumulation of reactive oxygen species (ROS) such as superoxide (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) (Xue and Liu 2008). Citrulline is a non-essential aminoacid that is reported to be an efficient hydroxyl radical scavenger and is a strong antioxidant (Rimando et al. 2005). Citrulline effectively protects DNA and metabolic enzymes from oxidative injuries (Akashi et al. 2001). Drought and salinity tolerant plants accumulate various organic osmolytes, especially organic compatible solutes, in response to osmotic stress (Rhodes et al. 2002).

Akashi et al. (2004) reported that one of the unique metabolic responses of

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wild watermelon under such stress conditions is the massive accumulation of a novel compatible solute, citrulline, which was found to be one of the most potent hydroxyl radical scavengers. However, this citrulline accumulation occurs at the later stages of the stress response, suggesting that this plant may possess other undiscovered mechanisms for protecting cellular components from oxidative damage.

Previous researchers have shown the variation of drought and salt tolerance in watermelon genotypes (Karipcin et al. 2008; Suyum et al. 2011). Yoshimura et al. (2008) reported that wild watermelon exhibits exceedingly high drought tolerance, although it carries out C3-type photosynthesis (Kawasaki et al. 2000; Miyake and Yokota 2000). Yokoto et al. (2002) indicated that the accumulation of compatible solutes and osmolytes can protect cells and cell components from stress damage, and that wild watermelon primarily accumulates citrulline, which may increase its tolerance to salt and drought stress. Kusvuran (2010) reported that citrulline levels in melon genotypes increased with drought and salt stress, and that this increase occured more in highly tolerant genotypes than in sensitive ones.

The aim of this study was to find out whether citrulline can be used as a biochemical marker of salt and drought stresses in a watermelon screening study.

MATERIALS AND METHODS

Sixty-five watermelon (*Citrullis lanatus* Thunb.) genotypes, most of them native genotypes originating from Turkey, were used as plant material (Table 2). Watermelon plants were grown under salinity, drought and control treatments for 24 days during the period of May 25 – June 17 in a Mediterranean climate. The experiment was carried out in a greenhouse with day and night temperatures of approximately 28-30^{o}C and 20-24^{o}C, respectively, and relative humidity in the range of 60 to70%. Seeds were directly sown into the vermiculite substrate in 2 liter plastic pots. For each genotype three independent plants in one pot were used as one replication and for salt, drought and control treatments fourreplicates were usedseparately. The plants were irrigated with a full strength nutrient solution at ppm concentrations of 177.2 N; 52.70 P; 240.44 K; 53.46 Mg; 120.30 Ca; 3.36 Fe; 0.85 Mn; 0.45 B; 0.50 Zn; 0.10 Cu; 0.05 Mo. The amount of nutrient solution applied in the treatments was determined based on the daily measured drainage fraction from the base of the pots (Dasgan et al. 2009). The range of the drainage fraction was kept at about 20% during the experimental period. The watermelon plants were grown without salt and drought treatments up to the third true leaf stage. From this point on a 50 mM increment of NaCl was added every day up to the final concentration of 200 mM. Similarly, drought stress was applied gradually for four days, and later the final drought rate was continued. When the plants were 16 days old, the young plants were subjected to salt and drought stresses and for 8 days. Con-

trol plants were grown under non-saline and non-drought conditions for the same period of time. Fully expanded watermelon leaves were sampled for measurement of citrulline from 24 days after sowing (DAS).

***Citrulline Determination*:** The solutions were prepared according to Knipp and Vasak (2000) and Dasgan et al. (2009).

Citrulline Extraction from Leaves: Five hundred milligrams of dry watermelon leaves were homogenized in 1.5 mL of EtOH (96%). Extracts were heated at 100°C until complete evaporation of EtOH. The residues were then dissolved in 1.5 mL of cold water and vigorously mixed. After centrifugation of the homogenates (10 min, 5000 g, 24°C) the supernatants (crude extracts) were removed and stored at -20°C until purification by an ion exchanger (Dasgan et al. 2009). Citrulline was measured according to Dasgan et al (2009).

RESULTS AND DISCUSSION

Drought and salinity increased citrulline accumulation in all of the watermelon genotypes compared with the control plants. However, the responses of the tolerant and sensitive genotypes differed significantly (Table 1). After eight days in 200 mM NaCI stress, the citrulline contents of tolerant genotypes KAR-126, KAR-152, KAR-173, KAR-278 and KAR-306 were 12.02 and 12.50 μmol gDW^{-1} (Table 1). However, under the same conditions, the salt sensitive watermelon genotypes KAR-92, KAR-281, KAR-285 and KAR318 contained 6.74 and 8.62 μmol gDW^{-1} (Table 1). Under salt stress, the citrulline amount actually doubled in the tolerant genotypes (Table 1).

The citrulline contents of the tolerant KAR-39, KAR- 268, KAR-278, KAR-282 and KAR-374, after ten days drought stress, reached 14.26 and 15.40 μmol gDW^{-1}, respectively (Table 1). Drought sensitive watermelon genotypes reached only 6.89 and 8.55 μmol gDW^{-1} (KAR-5, KAR-13, KAR-108, KAR-152, KAR-242, KAR-345). As can be seen in Table 1, citrulline accumulation in drought stressed watermelon occurred at a higher rate than in salt stressed watermelon (Table 1).

The citrulline contents of the watermelon genotypes increased under salt and drought stresses. However, the tolerant and moderately tolerant watermelon genotypes accumulated significantly more citrulline than the sensitive genotypes (Table 1). Greater responsiveness of the citrulline metabolism was indicated in the tolerant genotypes. The relationship between citrulline accumulation and tolerance remains to be ascertained, because citrulline overproduction might merely be one of the metabolic consequences of adaptation to high saline and drought conditions. Drought-tolerant wild watermelon accumulates high levels of citrulline under drought stress (Kawasaki et al. 2000). Akashi et al. (2001) reported that under drought conditions citrulline is a more effective scavenger of the hydroxyl

radical in wild watermelon than mannitol and proline. Citrulline accumulation in watermelon leaves is an effective defense against oxidative injuries during drought stress (Akashi et al. 2001). However, it should be noted that citrulline accumulation in desert watermelon plants growing in Botswana has been shown to be involved in osmotic adjustment, owing to its very high concentration, its compatibility, and its putative protective properties against oxidative stress through its high potency in scavenging hydroxyl radicals (Akashi et al. 2001). According to these authors, when wild watermelon was subjected to saline conditions, it overproduced gamma-aminobutyric acid, proline, and glutamine, not citrulline.

The result of the this study, screening watermelon genotypes in a large number of accessions or breeding lines for tolerance to salinity and drought during their young plant stage, is that the amount of citrulline accumulated in response to the given treatments may be considered a novel biochemical indicator of interest in early selection studies.

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Table 1. Citrulline in the watermelon genotypes grown under control, salinity and drought conditions. Ratio of salinity and dorught stressed plants in comparison to control plants without stress is presented.

Code no	Control (µmol gDW^{-1})	Salinity (µmol gDW^{-1})	Drought (µmol gDW^{-1})	Difference in salinity via control (%)	Difference in drought via control (%)
KAR 2	1.69 i-p	11.04 a-d	12.21 b-g	551.31	620.28
KAR 5	1.96 g-p	7.89 c-e	7.17 qr	303.72	267.01
KAR 13	2.61 a-h	11.05 a-d	6.89 r	323.37	163.76
KAR 23	2.09 f-p	10.65 a-d	11.39 b-l	409.14	444.83
KAR 27	3.27 ab	10.31 a-e	10.75 c-n	215.15	228.68
KAR 35	2.98 a-f	12.16 ab	10.67 d-o	308.80	258.97
KAR 37	1.70 i-p	12.19 ab	8.79 i-r	618.88	418.19
KAR 39	3.13 a-c	9.82 a-e	14.26 ab	213.73	356.18
KAR 59 A	2.58 a-h	11.32 a-d	10.23 d-p	338.98	296.55
KAR 77	3.01 a-i	10.50 a-e	8.74 i-r	249.09	190.50
KAR 92	1.42 op	6.74 e	9.17 g-r	373.60	544.59
KAR 98	2.92 a-f	9.80 a-e	11.94 b-i	235.43	305.40
KAR 104	1.53 m-p	9.24 a-e	10.71 d-n	504.91	601.49
KAR 108	2.72 a-g	8.99 a-e	7.40 p-r	230.33	171.87
KAR 126	2.56 a-h	12.13 ab	10.46 d-p	373.04	307.30
KAR 138	2.89 a-f	9.57 a-e	10.88 c-m	231.08	276.68
KAR 146	2.52 b-i	11.47 a-d	8.39 l-r	355.02	232.90
KAR 149	2.50 b-j	10.21 a-e	11.16 c-l	308.90	347.16
KAR 152	2.99 a-e	12.39 a	7.95 m-r	314.97	166.00
KAR 155	2.72 a-g	11.11 a-d	10.62 d-o	308.67	290.52
KAR 168	2.99 a-e	11.04 a-d	8.91 h-r	269.00	197.94
KAR 173	2.64 a-h	12.05 ab	9.11 g-r	357.50	245.86
KAR 174	1.98 g-p	9.60 a-e	10.86 c-m	384.46	448.09
KAR 177	3.40 a	10.25 a-e	10.78 c-n	201.63	217.44
KAR 203	2.79 a-g	9.26 a-e	7.67 n-r	231.62	174.72
KAR 212	3.27 ab	11.97 ab	10.22 d-p	265.70	212.43
KAR 234	1.44 n-p	9.21 a-e	11.23 c-l	541.54	682.73
KAR 242	2.70 a-g	9.94 a-e	7.44 p-r	268.24	175.67
KAR 246	1.62 k-p	10.03 a-e	9.81 e-r	518.50	504.86
KAR 254	2.00 g-h	8.99 a-e	11.24 c-l	349.50	462.59
KAR 260	2.83 a-g	10.10 a-e	8.20 l-r	257.38	190.30
KAR 268	2.27 c-o	10.56 a-e	13.36 a-d	365.19	489.46
KAR 278	1.78 h-p	12.02 ab	13.83 a-c	576.16	677.48
KAR 279	2.60 a-h	9.85 a-e	8.31 l-r	279.55	220.41
KAR 280	3.00 a-e	10.67 a-d	11.09 c-m	256.14	270.05
KAR 281	1.81 h-p	8.62 a-e	11.80 b-i	377.48	553.29
KAR 282	1.76 h-p	10.02 a-e	13.39 a-d	469.02	659.95
KAR 285	2.41 b-l	8.24 b-e	11.34 c-l	242.41	371.31
KAR 290	1.66 j-p	11.68 a-c	10.70 d-n	605.54	546.42
KAR 293	1.60 l-p	11.22 a-d	9.11 g-r	600.42	468.89
KAR 296	3.07 a-d	10.77 a-d	9.97 e-q	251.15	225.10
KAR 297	2.71 a-g	9.41 a-e	10.39 d-p	246.68	282.64
KAR 300	2.28 c-n	9.83 a-e	12.35 b-f	330.17	440.60
KAR 302	1.22 p	9.31 a-e	8.50 k-r	631.15	567.63
KAR 303	2.58 a-h	10.96 a-d	12.26 b-g	325.02	375.02
KAR 304	2.96 a-f	10.00 a-e	11.33 c-l	238.26	283.05
KAR 306	1.49 m-p	12.50 a	11.63 b-k	741.75	683.46
KAR 308	1.67 j-p	9.48 a-e	11.65 b-k	467.55	597.42
KAR 310	2.46 b-k	8.74 a-e	11.71 b-j	254.90	375.45
KAR 316	2.24 c-o	10.31 a-e	10.51 d-o	360.84	369.46
KAR 318	2.58 a-h	7.65 de	12.29 b-g	196.94	376.90
KAR 324	2.31 c-m	9.00 a-e	11.98 b-h	290.15	419.17
KAR 338	2.59 a-h	11.95 ab	9.34 f-r	361.54	260.71
KAR 341	2.55 a-h	9.14 a-e	10.75 c-n	258.45	321.57
KAR 344	1.97 g-p	8.95 a-e	8.62 j-r	354.03	337.09
KAR 345	2.47 b-k	9.63 a-e	8.22 l-r	290.93	233.56
KAR 348	2.24 c-o	10.18 a-e	8.43 l-r	355.68	277.19
KAR 349	2.59 a-h	9.08 a-e	7.60 o-r	250.20	193.31
KAR 350	2.18 d-o	9.32 a-e	10.90 c-m	328.09	400.87
KAR 351	1.82 h-p	10.06 a-e	8.55 j-r	453.26	370.43
KAR 353	2.43 b-l	9.95 a-e	13.20 a-d	309.37	443.26
KAR 355	2.92 a-f	10.18 a-e	8.23 l-r	248.60	182.14
KAR 374	1.60 l-p	8.96 a-e	15.40 a	460.77	864.18
CrimsonTide	1.66 j-p	11.48 a-d	13.37 a-d	590.90	704.88
Crisby	2.15 e-o	12.55 a	12.57 b-e	485.36	486.58
Mean	**2.35**	**7.71**	**10.43**	**357.95**	**374.31**
$LSD_{0.05}$	0.450	1.910	1.599		

Mega-environment identification for watermelon yield testing in the US

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Keywords: Watermelon, yield and yield components, mega-environment

Abstract

The concepts of target locations and mega-environments are important for identifying locations for use in multiple-environment cultivar trials. The objectives of our study were to study mega-environments, and identify test locations that were both discriminating among genotypes and representative of target regions for watermelon (*Citrullus lanatus* [Thumb.] Matsum & Nakai) fruit yield and yield components using GGE (genotype plus genotype x environment) biplot methodologies. Experiments were conducted in replicated, multiple-harvest trials for three years at eight locations. Two key locations, Quincy FL and Clinton NC were efficient representatives for the two mega-environments identified for marketable yield, fruit count, and % early fruit. College Station TX and Woodland CA represented one mega-environment, and Clinton NC represented a second for % cull fruit. The subdivision of major watermelon producing states for fruit size could not be justified. Identification of mega-environments for watermelon in the southern US has implications for future breeding and genotype evaluation in the US, including the use of specialized genotypes for the mega-environments identified to achieve optimum adaptation.

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INTRODUCTION

A mega-environment is a group of production areas that are similar in terms of genotype response, and that show a repeatable relative performance of crop genotypes across years (Yan and Rajcan 2002; Yan and Tinker 2005). Mega-environments are generally identified through the analysis of multiple-environment trial data of a diverse set of genotypes. The purpose of mega-environment analysis is to understand the genotype × location interaction patterns within a target region in order to explore the feasibility of dividing the target region into meaningful mega-environments. That permits the genotype × location interaction (which causes specific adaptation) to be exploited to maximize the response to selection within mega-environments, and increase the productivity of the target region (Yan et al. 2011).

Several methods have been used to analyze multiple-environment trial data including the genotype main effects plus genotype × environment interaction (GGE) model with a biplot display (Gauch 2008). A GGE biplot is constructed from the first two principal components (PC1 and PC2) and it graphically displays the genotype-by-environment data matrix, which allows visualization of the interrelationship among environments and genotypes, and their interactions (Yan and Kang 2003; Yan et al. 2000).

The research reported here was undertaken to analyze multiple-environment trial data for yield and yield components of watermelon (*Citrullus lanatus* [Thumb.] Matsum & Nakai). Our objectives was to use GGE biplot technique to (i) identify mega-environments for the main watermelon production areas of the US and (ii) identify locations having high discriminating ability and representativeness.

MATERIALS AND METHODS

Data analyzed in this study were obtained from set of watermelon yield trails conducted for 3 years (2009, 2010, and 2011). Each year, 40 genotypes were evaluated at 8 locations across the United States. The genotypes were chosen to represent key watermelon genotypes that were used in breeding purpose in the US in last century and the locations were chosen to represent the key watermelon production regions in the US (Wehner 2008). Locations were Kinston NC, Clinton NC, Charleston SC, Cordele GA, Quincy FL, College Station TX, Lane OK and Woodland CA. At each location, a randomized complete block design with four replications was used. Watermelon seedlings were transplanted at the two-true-leaf stage on raised beds (rows).

The 40 watermelon genotypes were evaluated for marketable yield (Mg ha^{-1}), marketable fruit number or count (thousand fruit ha^{-1}), % cull fruit (100 x cull fruit weight / total fruit weight), % early fruit (100 x first harvest of marketable yield / total harvest weight) and fruit size (kg $fruit^{-1}$). Data were not collected on % cull

fruit from Charleston SC in 2009, 2010, and 2011; and Quincy FL in 2009 and 2010. Data were analyzed using the SAS statistical package (SAS Institute, Cary, NC) and 'GGEBiplotGUI' package of R statistical software (R Development Core Team 2007).

RESULTS AND DISCUSSIONS

The combined analysis of variance (ANOVA) revealed highly significant environment, genotype, and genotype × environment interaction effects for all evaluated traits. The significant location x year interaction for all the traits evaluated in this study warranted separate ANOVA for each year (Fan et al. 2007). Within each year, location effect was significant for all the traits, except fruit size, evaluated in this study. The magnitude of the genotype x location interaction relative to genotype suggested the existence of different mega-environments (Yan et al. 2000).

The visualization of "Which won where?" pattern is important for searching for the existence of different mega-environments in a region. The two-dimensional GGE biplot of multiple-environment trial data was based on environment-standardized data and environment-focused singular value partition (Fig. 1). The straight line originating from the biplot origin and being perpendicular to the each side of the polygon divides the biplot into sectors. These sectors divide test locations into groups and indicate the existence of different mega-environments. The sectors were labeled 1 to 4 for all the traits evaluated in this study (Fig. 1).

The GGE biplot analysis showed that PC1 and PC2 together accounted for 60% to 95% of the total variation for watermelon fruit yield and yield components (Fig. 1). GGE biplot analysis identified two mega-environments for marketable yield, fruit count, % cull fruit and % early fruit; and it suggests that multiple-environment trials are essential and genotype recommendation must be based on both mean and stability (Yan and Kang 2003). On the contrary, for fruit size, all eight locations formed a single mega-environment (Fig. 1E). This indicate that one or a few test locations are sufficient to identify the best genotypes that can be recommended everywhere within the mega-environment.

The ideal test location should be both discriminating and representative. The small circle surrounded by concentric circles on the average environment axis, with an arrow pointing to it represented the ideal location (Fig. 2). According to Yan and Kang (2003) and Putto et al. (2008), the location that has high discriminating ability and high representativeness tends to easily differentiate the performance among genotypes and suggested that the selected genotypes have the desired adaptation in that location, respectively. For marketable yield, Cordele GA, Quincy FL and Kinston NC were closest to the ideal location, and therefore the most desirable of all eight locations (Fig. 2A). Among yield component traits, Kinston NC and Clinton NC; Texas followed by Woodland CA and Clinton NC; Quincy FL followed

by Kinston NC and College Station TX; and Quincy FL and Clinton NC were the most desirable locations for fruit count, % cull fruit, % early fruit and fruit size, respectively (Fig. 2B, C, D, and E, respectively).

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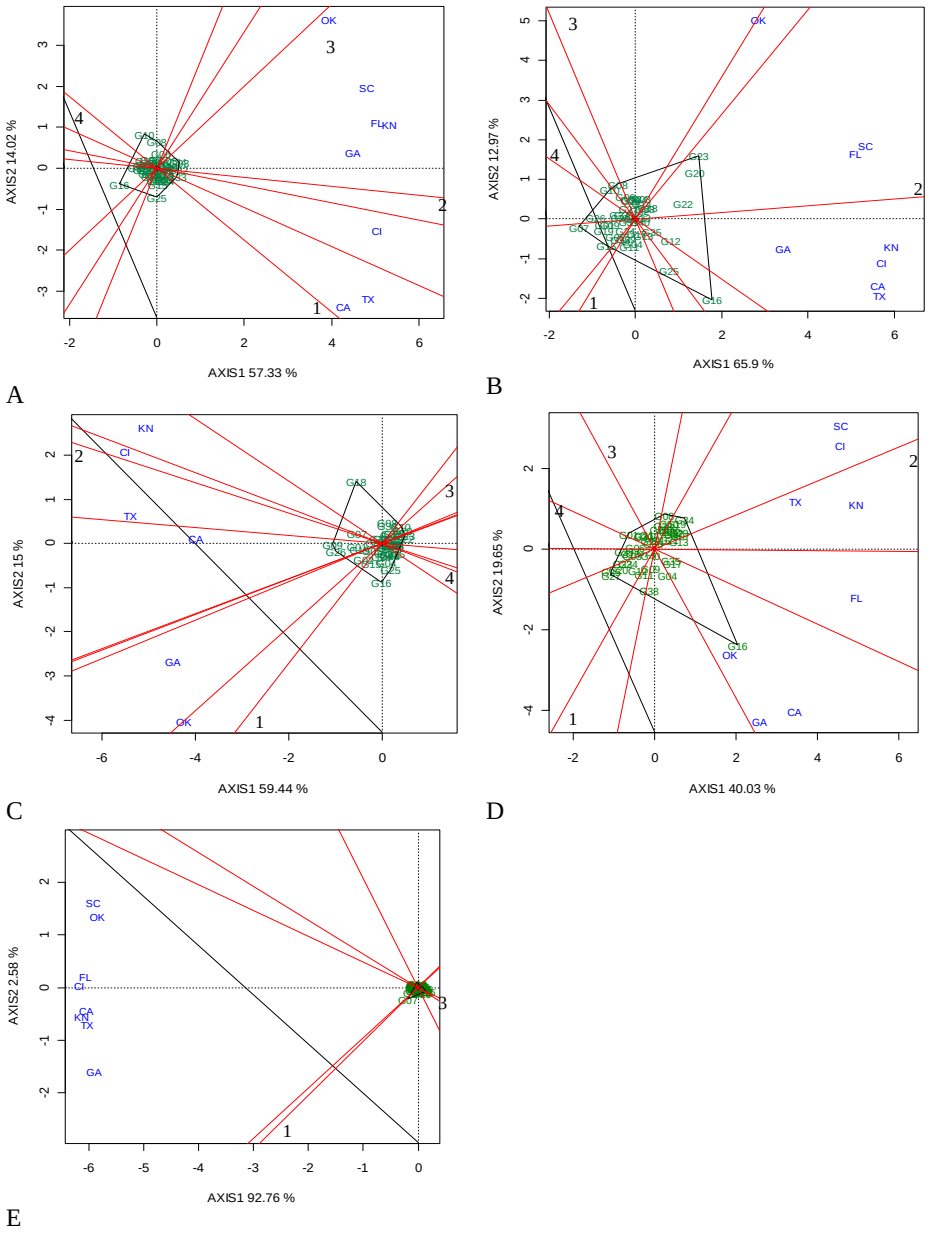

Fig. 1. Polygon view of the GGE biplot based on watermelon (A) fruit yield (Mg ha^{-1}), (B) count (thousand ha^{-1}), (C) % culls, (D) % early, and (E) size (kg fruit^{-1}) of 40 genotypes tested in 3 years and 8 locations.

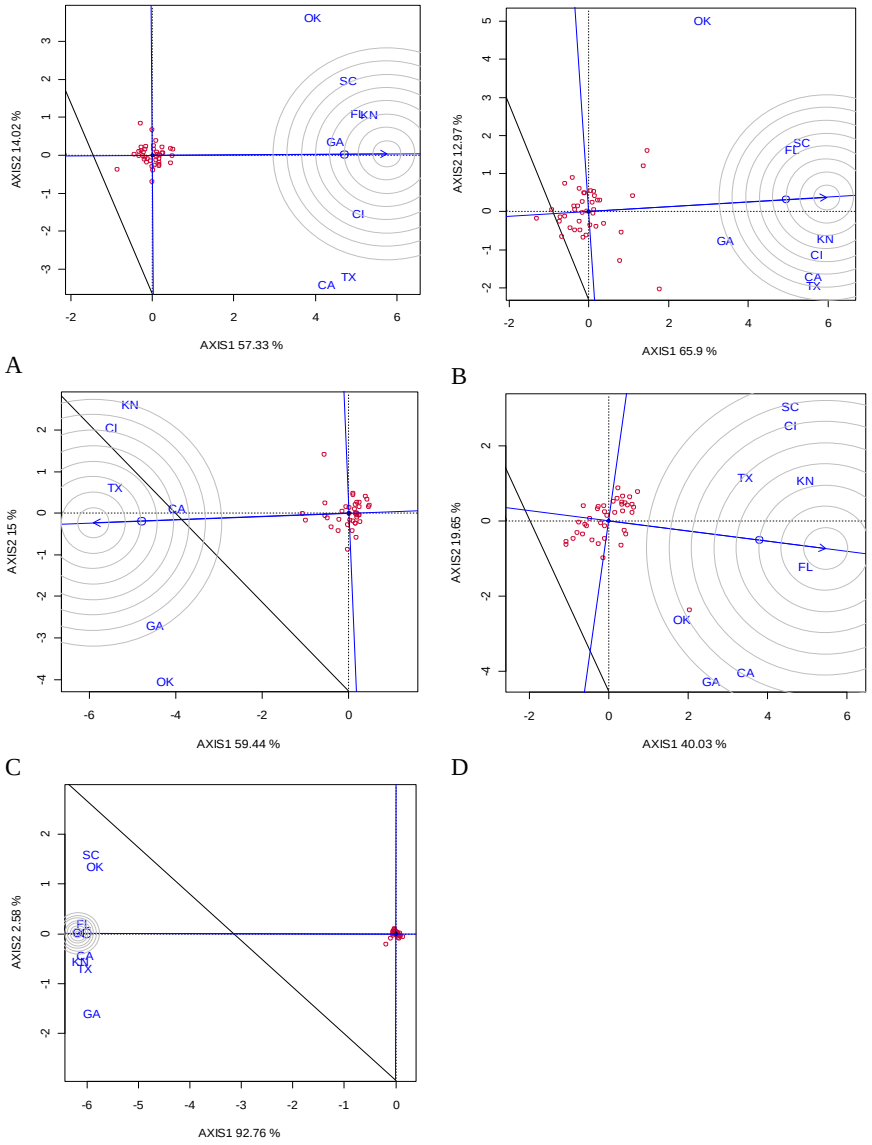

Fig. 2. Comparison of all locations with the ideal location for watermelon (A) fruit yield (Mg ha^{-1}), (B) count (thousand ha^{-1}), (C) % culls, (D) % early, and (E) size (kg fruit^{-1}) of 40 genotypes tested in 3 years and 8 locations. The ideal location was represented by the smallest circle, and was the most discriminating and yet representative of other test locations.

Analyses of population structure and genetic diversity among Turkish watermelons

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Keywords: *Citrullus lanatus*, watermelon, population structure, marker sampling, genetic diversity, UPGMA, PCA

Abstract

Watermelon is one of the most produced vegetable crop worldwide. Turkey is the second largest producer and considerable number of local cultivars and ecotypes arised during its cultivation in its different regions. Population structure, genetic diversity, and markers sampling of watermelon lines including many local cultivars were deduced in this study. Twenty-two sequence related amplified polymorphism (SRAP) primer combinations selected from 50 combinations were used to study 259 watermelon lines available in the Turkish genetic resources. Sub-structuring Bayesian analysis assigned the 259 watermelon lines into four subpopulations, and 208 out of 259 lines were admixed having at least 0.80 membership coefficient to minimum of one subpopulation and the rest were highly related. The 22 combinations yielded polymorphisms with genetic similarity ranging from 0.39 to 1.00. The UPGMA analysis indicated four distinct clusters among the watermelon lines in which the largest group contained 250 lines, while the other 9 lines were distributed in three distinct clusters. The principle component analysis indicated similar results. Overall, the results demonstrate that most of the Turkish watermelon lines are admixed.

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Genotypic variation in the response of watermelon genotypes to salinity and drought stresses

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Keywords: *Citrullus lanatus*, salt stress, water stress, screening, breeding, tolerance

Abstract

In this study, salinity and drought responses of 65 watermelon genotypes have been investigated in early plant growth stage. The plants were grown in vermiculite culture with 200 mM NaCl for salinity stress and irrigation was stopped gradually for terminal-drought stress. Control plants without stress have also been grown. Young plants (24 days old plant) were used for screening studies. Physiological measurements and analysis were conducted to identify tolerant versus susceptible watermelon genotypes; A scale evaluation of 0-5 for symptomatic appearance of the plants, shoot fresh and dry weights, root fresh and dry weights, leaf number per plant, leaf area, SPAD-Chlorophyll meter reading, membrane injury index, leaf relative water content, leaf water potential, leaf osmotic potential, stomata conductance, leaf temperature, Na, K, Ca, and Cl concentrations of the shoot and root samples. For each parameter, differences in salinity and drought stresses compared to control have been calculated. At the end of the study, large variations have been determined in watermelon genotypes for their salinity and drought responses. The sixty-five watermelon genotypes were classified as tolerant, mildly tolerant or susceptible.

INTRODUCTION

Limited water supply in the Mediterranean region is a major problem in irrigated agriculture. The progressive salinization of irrigated agricultural areas threatens the future of agricultural lands. Watermelons are major economic fruits in Turkey. It is grown in hot climate land, arid, and semi-arid regions in the country. Tolerant cultivars that are developed by breeding studies can be a useful solution for minimizing deleterious effects of water deficiency and salt stresses (Dasgan

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and Koc 2009). Drought and salt-tolerant accessions might exist in gene pools of various plant species (Dasgan et al. 2002). There are several studies related to local watermelons screenings for salt and drought tolerances. Karipçin et al. (2010) used wild and Turkish-domestic watermelons for drought tolerance in field experiment. Of these, fifteen genotypes were determined as tolerant and seventeen genotypes were susceptible. Zhang et al. (2011) reported that 820 accessions of USDA's *Citrullus* PIs and 246 watermelon breeding lines were evaluated for their drought tolerance at the seedling stage and the tested watermelon materials could be assigned into four groups, including tolerant, intermediate tolerant, moderately sensitive, and sensitive. The objective of this study is screening of Turkish watermelon germplasms for drought and salinity, and identify the most resistant genotypes for future breeding programs.

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MATERIAL AND METHODS

Sixty-five watermelon (*Citrullis lanatus* Thunb.) genotypes, most of them native genotypes originating from Turkey, were used as plant material (Table 2). Watermelon plants were grown salinity, drought and control treatments for 24 days during the period May 25 – June 17 under Mediterranean climate. The experiment was carried out in a greenhouse with day and night temperatures of approximately 28-30^{o}C and 20-24^{o}C, respectively, and relative humidity in the range of 60 to 70%. Seeds were directly sown into the vermiculite substrate in volume of 2 liters capacity plastic pots. For each genotype 3 independent plants in one pot were used as one replication and for salt, drought and control treatments 4 replicates used, separately. Plants were irrigated by the full strength nutrient solution in ppm concentrations of 177.2 N; 52.70 P; 240.44 K; 53.46 Mg; 120.30 Ca; 3.36 Fe; 0.85 Mn; 0.45 B; 0.50 Zn; 0.10 Cu; 0.05 Mo. The amount of nutrient solution applied in the treatments was determined based on daily measured drainage fraction from the base of the pots (Dasgan et al. 2009). Range of drainage fraction was kept about 20% during the experimental period. The watermelon plants were grown without salt and drought treatments up to 3 true leaf stage. Then, NaCl was added with 50 mM increments on every day until the final concentration of 200 mM. Similarly drought stress were performed gradually during 4 days, later on the terminal drought was continued. When the plants were 16 days old the young plants were subjected to the salt and drought stresses and continued during 8 days. Control plants were grown under non-saline and non-drought conditions for the same period of time. In order to screen tolerant and susceptible watermelon genotypes some physiological measurements and analysis were realized; 0-5 scale evaluation for the appearance of shoot damage, shoot fresh and dry weights, root fresh and dry weights, leaf number

per plant, leaf area, SPAD-Chlorophyll meter reading, membrane injury index, leaf relative water content, leaf water potential, leaf osmotic potential, stomata conductance, leaf temperature, Na, K, Ca, and Cl concentrations of the shoot and root samples in salt treatment.

RESULTS AND DISCUSSION

Mean values of screened physiological parameters of 65 watermelon genotypes in Table 1. In order to screen watermelons for salinity and drought stresses, among the 27 parameters the most relevant ones were shoot dry weight, root dry weight, plant total leaf area, leaf water potential, stomatal conductance, K, Ca, Na and Cl concentrations in shoot and root, K/Na, Ca/Na, membrane injury index and visual appearance of damages by scale evaluation.

According to the physiological data, watermelon germplasm was classified into 3 groups: tolerant, moderately tolerant, and susceptible (Table 2). For salinity response; 14 genotypes were tolerant, 34 genotypes were moderately tolerant, and 17 genotypes were suceptible. For the drought responses, 13 genotypes were tolerant, 43 genotypes were moderate tolerant and 7 genotypes were susceptible. Following the screening of watermelon genotypes in young plant stage, field experiment at fruit stage with the selected tolerant genotypes is underway. The screening for salinity and drought tolerances at early plant-growth stage may not produce tolerant adult plants (Dasgan and Koc 2009). On the other hand, performance of young plants has been considered highly predictive for the response of adult plants to the abiotic stesses (Qureshi 1990; Akhoundnejad 2011, Dasgan et al. 2012). The screening parameters at young plant stage in this study could contribute in reducing the number of potential genotypes for tests in later stages, including reproductive stage.

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Table 1. Physiological parameters of 65 watermelon genotypes were grown under saline and drought stresses during 8 days (mean of 65genotypes).

Gen İsmi	Control	Salinity	Drought	Differences in salinity compared to control (%)	Differences in drought compared to control (%)
Plant height (cm)	15.10	9.72	13.01	-35,63	-13.84
Number of leaf (leaf/plant)	4.46	2.58	3.46	-42.15	-22.42
Shoot fresh weight (g/plant)	10.89	3.12	5.24	-71.35	-51.88
Shoot dry weight (g/plant)	0.99	0.35	0.66	-64.65	-33.33
Root fresh weight (g/plant)	2.50	1.03	1.97	-58.80	-21.20
Root dry weight (g/plant)	0.17	0.07	0.18	-58.82	5.88
SPAD reading	51.67	57.00	59.36	10.32	14.88
Leaf area (cm^2/plant)	67.22	18.19	29.66	-72.94	-55.88
Relative water content (%)	86.25	66.44	74.17	-22.97	-14.00
Leaf water potential (-MPa)	4.38	8.48	8.46	93.61	93.15
Leaf temperature (^oC)	30.66	32.74	35.64	6.78	16.22
Stomatal conductance (mmol $m^{-2}s^{-1}$)	139.56	41.63	59.58	-70.17	-57.31
Osmotic potential (-MPa)	1.57	1.68	1.78	7.00	13.37
Potassium in shoot (%)	3.28	3.33	2.70	1.52	-17.61
Potassium in root (%)	0.43	0.96	1.49	123.25	246.51
Calcium in shoot (%)	3.214	2.489	2.614	-22.56	-18.67
Calcium in root (%)	1.04	0.87	1.24	-16.35	19.23
Sodium in shoot (%)*	0.87	2.41	-	177.01	-
Sodium in root (%)*	1.45	3.59	-	147.59	-
Chloride in shoot (%)*	1.39	8.07	-	480.58	-
Chloride in root (%)*	0.88	4.40	-	400.00	-
K/Na ratio in shoot*	4.06	1.43	-	-64.78	-
K/Na ratio in root*	0.28	0.28	-	0.00	-
Ca/Na ratio in shoot*	3.88	1.06	-	-72.68	-
Ca/Na ratio in root*	0.81	0.25	-	-81.56	-
Membrane injury index (%) **	-	20.35	3.96	-	-
Scale evaluation	-	2.49	3.31	-	-

* Salt treatment only, ** Control including for membrane injury index calculation.

Table 2. Salinity and drought responses of the watermelon genotypes have been classified as 3 levels: tolerant, mild tolerant and susceptible.

Salt stress responses of the watermelons			Drought stress responses of the watermelons		
Tolerant Genotypes	Mild- Tolerant Genotypes	Susceptible Genotypes	Tolerant Genotypes	Mild- Tolerant Genotypes	Susceptible Genotypes
KAR-13	KAR-2	KAR-27	KAR-98	KAR-2	KAR-5
KAR-23	KAR-5	KAR-39	KAR-104	KAR-13	KAR-108
KAR-98	KAR-35	KAR-92	KAR-149	KAR-23	KAR-152
KAR-104	KAR-37	KAR-168	KAR-234	KAR-27	KAR-280
KAR-146	KAR-59 A	KAR-174	KAR-278	KAR-35	KAR-324
KAR-152	KAR-77	KAR-234	KAR-282	KAR-37	KAR-345
KAR-278	KAR-108	KAR-254	KAR-293	KAR-39	KAR-351
KAR-279	KAR-126	KAR-281	KAR-300	KAR-59A	
KAR-302	KAR-138	KAR-282	KAR-304	KAR-77	
KAR-306	KAR-149	KAR-285	KAR-316	KAR-92	
KAR-308	KAR-155	KAR-293	KAR-344	KAR-146	
KAR-338	KAR-173	KAR-296	KAR-353	KAR-155	
KAR-341	KAR-177	KAR-297	Crisby	KAR-168	
Crisby	KAR-203	KAR-300		KAR-173	
	KAR-212	KAR-310		KAR-174	
	KAR-242	KAR-318		KAR-177	
	KAR-246	KAR-345		KAR-203	
	KAR-260			KAR-212	
	KAR-268			KAR-242	
	KAR-280			KAR-246	
	KAR-290			KAR-254	
	KAR-303			KAR-260	
	KAR-304			KAR-268	
	KAR-316			KAR-279	
	KAR-324			KAR-281	
	KAR-344			KAR-285	
	KAR-348			KAR-290	
	KAR-349			KAR-296	
	KAR-350			KAR-297	
	KAR-351			KAR-302	
	KAR-353			KAR-303	
	KAR-355			KAR-306	
	KAR-374			KAR-308	
	Crimson Tide			KAR-310	
				KAR-318	
				KAR-338	
				KAR-341	
				KAR-348	
				KAR-349	
				KAR-350	
				KAR-355	
				KAR-374	
				Crimson Tide	

Effect of boron supply under drought stress tolerance on watermelon

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Keywords: *Citrullus lanatus*, C. *colocynthis,* C. *lanatus* var. *citroides*, abiotic stress, SOD, CAT, GR

Abstract

In most parts of the world, drought has more adverse effects on agricultural production than any other combination of stress factors. Drought and micro-nutrient deficiency as well as toxicity, inhibit the important physiological and biochemical functions of plants and may result reduction in plant growth and ultimately the loss of productivity. Plants have developed some biochemical and molecular mechanisms to overcome both of those stresses. If plants are grown under conditions of adequate level of boron, they have healthy tolerance mechanism when they meet the abiotic stress conditions. This study was performed to determine the effect of boron supply under drought stress in controlled greenhouse conditions. Two different levels of drought (full irrigation, 50% of full irrigation), three different boron doses (0.5, 2.5 and 12.5 mg B kg^{-1}) with four different watermelon genotypes namely; tolerant to drought stress, 2012 (Citrullus *colocynthis*), 147 (C. *lanatus (*Thunb). matsum and Nakai) and sensitive to drought stress, 325 (C. *lanatus* var.*citroides*), and 98 (C. *lanatus* Thunb). All those watermelon genotypes were previously identified as resistances to drought conditions. In addition to lipid peroxidation levels of plants, some of the antioxidant enzyme activities (SOD, CAT and GR) were also determined.

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Genetic relationships among various Sihke melon landraces

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Keywords: Characterization, melon, phenological, Sihke

Abstract

Landraces and older varieties face with extinction in modern agriculture. Therefore, the preservation of diversity becomes vital for future breeding efforts because landraces and older varieties might contain genes that current varieties do not. The present study aimed to determine genetic relationships among various Sihke melon landraces for phenotypic traits. The main materials of the study were 15 Sihke melon genotypes collected from various parts at Van province in Turkey. Moreover, 2 foreign standard melon genotypes (Sembol F_1 and Sempati F_1) and 13 local melon genotypes that previously have not been characterized were included into the phenotypic characterization. Total 63 measurements or observations were used to define the genetic similarity among the melon genotypes by dendrograms or two- and three-dimensional scalings obtained from Euclidian distance matrix. Based on the molecular Euclidean distance matrix, Sihke melon landraces were different from the others and tended to be grouped together. Among the studied genotypes, the most distinct genotype was Genotype 30 called Semame. The remaining genotypes were divided into three main groups.

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Investigations on inhibitory effects of some essential oils against *Fusarium oxysporum* f. sp. *melonis* races on melon

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Keywords: Melon, *F. oxysporum* f. sp. *melonis* races (0, 1, 2, 1-2), essential oils

Abstract

Fusarium wilt is a severe disease causing economic losses in melon production fields throughout the world. However, conventional pesticides are not sufficient to control of the pathogen. In addition, their frequently unconscious use result in environmental pollution beside their ascending phytotoxic effects. Recently health and environmental issues regarding the use of pesticides highlight the necessity of alternative methods to control of these pathogens. Essentail oils are considered to be an alternative method since they have no or less hazardous effects on environment and human health.

In this study, three essential oils of cumin (*Cuminum cyminum*), thyme (*Thymus vulgaris*) and cinnamon (*Cinnamomun zeylanicum*) were tested against *F. oxysporum f. sp. melonis'* races (0, 1, 2, 1-2) in *in vitro* condition. Three concentrations of different essential oils (cumin, thyme and cinnamon) showed significantly strong inhibition in the mycelial growth of four races. These results suggest that these essential oils seem to be an alternative method controling Fusarium wilt in problematic areas though we need to establish convenient formulation of the practical use. Use of essential oils seem possible to be efficient fungicides in order to control of the soil borne pathogens, in enviromentally friendly manner, once the right formulation and application method are established.

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A new developed test method against to the melon Powdery Mildew (*Podosphaera xanthii*) in *in vivo* conditions

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Keywords: Melon, disease, powdery mildew, *in vivo* and pot tests

Abstract

In the world, one of the biggest problems of melon production is powdery mildew disease (Robinson and Decker-Walters 1997). Powdery mildew is usually the most common disease of cucurbits and easily identifiable. Plants are infected with the disease agent in hot and dry weather (Robinson and Decker-Walters 1997). Besides controlling of the disease with the chemicals, to use disease-resistant varieties are seen as the best solution (Hosoya 1999).

Identifying of melon genotypes reactions to the disease is important. A test method is needed to optimize in our conditions for the development of resistant varieties. In the world, a leaf disc method used for the powdery mildew disease in melon (Pitrat et al. 1991). We used this method in our laboratory. But leaf discs were not consistently achieving the desired results due to the contaminations.

Therefore, we used greenhouse compartments in Vegetable Department of Batı Akdeniz Agricultural Research Institute, to realized pot tests in vivo. Melon seedlings which are 3-4 true leaves are sprayed with a liquid spore suspension containing 5×10^6 spores/ml of powdery mildew. Tested plants are incubated under the average temperature 26, 9°C and 60,8 % of humidity. The first symptoms of sensitive plants were seen on the leaves at 9 days, Assessments can be done on the leaves, at the end of 15 days.

In vivo test results are evaluated according to the 1-4 scale of Yuste-Lisbona et al. (2010). In vivo tests which developed in our conditions were more quickly, less labor and less cost then the leaf disc method. The results are clearly seen. In conclusion, in vivo pot tests against to powdery mildew of melon can be easily applied to the resistant disease breeding studies.

INTRODUCTION

Turkey is one of the important melon producing countries in the world. In the world melon production, our country has come in second place after China (FAO 2010). One of the biggest problems in melon production is powdery mildew

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disease of cultivated areas (Robinson and Decker-Walters 1997). Powdery mildew is usually the most common disease of cucurbit and easily identifiable. Disease symptoms are white powder on the leaf, on the petiole and rarely seen in the fruit (Zitter et al. 1996). Disease agent infected the plants in hot and dry weather conditions. In advanced stages, causes the death of the leaves, reduces yield and quality (Robinson and Decker-Walters 1997). Two different species of disease [*Podosphaera xanthii* (*Sphaerotheca fuliginea* Schlech previously ex Fr. Poll.) and *Golovinomyces cichoracearum* (formerly *Erysiphe cichoracearum* DC ex Merate)] have been identified. In Europe, the first *P. xanthii* was demonstrated and then both types of diseases spread was reported later (Cohen et al. 1993; Epinat et al. 1993; Krístková et al. 2004; McCreight et al. 1987; Sowell 1982). The main common disease of melon powdery mildew in Japan is *P. xanthii* (Hosoya et al. 1999, 2000; McCreight 2006). There are many physiological races of powdery mildew agents (Zhihao Xu 1999). To date, in melon production, by *Podosphaera xanthii* are known 5 races (0, 1, 2, 3 and 5), in *Golovinomyces cichoracearum* two races (0 and 1) have been identified (Bardin et al. 1999), however many other races are known (Lebeda et al. 2008, 2011). In Turkey, Ünlü et al. (2010) was conducted a study in Antalya-Kocayatak region. In this study they collected samples of powdery mildew in melon greenhouses and looked the micelle structures in the laboratory. After then they used differential cultivars (Rochet, PMR 45, PMR 6 and Edisto 47) to determine the race of the diseases. At the end they determined the causal agent is *Podosphaera xanthii* race 5 in Antalya-Kocayatak region of Turkey.

MATERIALS AND METHODS

A hundred pure lines were used in test studies. Also, 1 susceptible standard variety (Ananas, BATEM) and 1 resistant genotype (PMR 6) were used. *Podosphaera xanthii*, race 5 isolate were used for inoculation of melon seedlings.

Tests were made in 2 different ways. The first method**;** leaf disc method (Pitrat et al. 1991) is used for the determination of powdery mildew resistance. This method is done in the laboratories of the West Mediterranean Agricultural Research Institute of Plant Protection Department (Fig. 1).

Fig. 1. Leaf disc method.

Leaf discs of (1 cm in diameter) melon seedlings were taken from the third or fourth leaves of melon. Leaf discs placed in the Petri dishes which containing water agar (Cohen 1993). Powdery mildew spores were blown with a pipette to the leaf discs. Petri dishes incubated under these conditions (16 h light and 8 h dark at 24°C) in an incubator. After 15 days Petri dishes were observed (Fig. 1).

The second method; This study is done in the West Mediterranean Agricultural Research Institute of Vegetable Department. 3-4 true leaves of melon seedlings sprayed by the spores (5×10^6 spores/ml) of powdery mildew suspensions (Fig. 2). Tested plants were put in a greenhouse compartment containing 60.8% humidity and 26.9˚C average temperature.

Fig. 2. Spraying of powdery mildew

Symptoms are observed at the end of 15 days according to the Yuste-Lisbona et al. (2010)' s 1-4 scale.

1-No sporulation , **2-**Weak sporulation, **3-**Medium sporulation , **4-**Intensive sporulation

According to these scale; 1 and 2 is resistant, 3 and 4 susceptible varieties (Fig. 3).

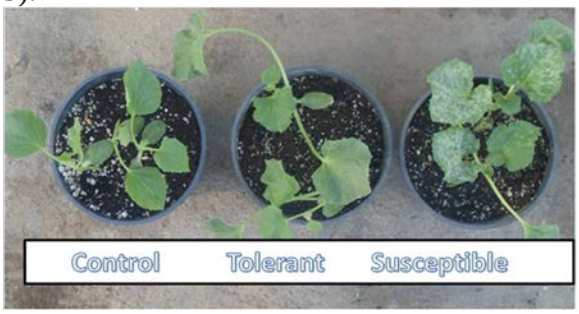

Fig. 3. Resistant and susceptible varieties

RESULTS AND DISCUSSIONS

In this study, the determination of the reaction of genotypes against to the powdery mildew, two different methods were used. These methods are leaf disc method and pot test. Pot test method has been more successful than the leaf disk method according to this study. Leaf disk method applied under laboratory

conditions, but too much contamination has occurred in Petri dishes and powdery mildew micelles are not clearly seen on leaf discs (Fig. 1). Pot tests under greenhouse conditions are done in a shorter time, less labor performed and the results are clearly showed. In addition, pot test is a practical method against to the determination of powdery mildew so it can be easily used in melon breeding studies.

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Effects of grafting and soil solarization on melon production in greenhouse

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Keywords: Grafting, soil solarization, melon, rootstocks

Abstract

Effects of grafting and soil solarization on Fusarium wilt, flowering dates, early and total yields, brix, weight and number of melon fruits were investigated in a greenhouse of Bati Akdeniz Agricultural Research Institute located in Antalya in 2008 spring season. Trials were set in the split plot design with 3 replications. The main plots were solarization in which the grafting plots were placed. In this study, Çıtırex F1 cultivar was grafted on Ferro F1, Nun 9075, RS 841and RZ 6415 rootstocks. Non-grafted Çıtırex F1 plants and non-solarized plots were used as controls. Non-grafted Çıtırex F1 plants grown in the solarized plots provided the highest number fruits as well as early and total yields. Number of *Fusarium* wilt infected plants was higher in the non-solarized plots than the solarized plots. On the other hand, Çıtırex F1 plants grafted on RZ 6415 rootstock were the most heavily affected by the Fusarium wilt among plants grafted on the other rootstocks. Higher brix values were obtained from the non-grafted Çıtırex F1 plants than the plants grafted on different rootstocks. In conclusion; grafting was not a good option to grow melon on different rootstocks; however, solarization always provided better quality and higher yields in melon production regardless of the rootstocks used.

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the important crop grown in open fields and protected areas in all over the world with 25 million tons of production. According to 2010 statistics (FAO, 2012), 1.6 million tons of melon was produced in Turkey. Melon is affected by many different pests. One of the great problems of melon is the decrease in fruit yield and quality caused by soil diseases, mainly *Fusarium oxysporum* f. sp. *melonis*; however, other pathogens such as *Verticillium dahliae*, *Rhizoctonia solani* and *Meloidogyne incognita*, also result in important plant damages and reduction in yield and quality of melon (Miguel 2005).

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Grafting is a developing integrated management strategy to deal with biotic and abiotic stresses in solanaceous and cucurbitaceous crops. It has been employed with great success to control a wide spectrum of soil-borne diseases and nemotodes for cucurbits and other vegetables (Rivard et al 2010; Gisbert et al. 2011; Yilmaz et al. 2011). In addition, grafting increased yield, fruit quality, growth promotion, extended production periods and crop longevity, more efficient fertilizer use, tolerances to soil salinity, low temperature and flooding, and reduced the number of plants required per hectare (Colla et al. 2010; Louws et al. 2010; Maršić and Jakše 2010). However, presently the major concern with respect to grafting is the enhanced risk of spreading diseases throughout grafting handling procedure and reduction in yield and fruit quality even plant death in some species such as melon due to graft incompatibility between scion and rootstocks (Edelstein et al. 2004; Louws et al. 2010).

Combining chemical and non-chemical alternatives such as grafting, solarization and use of resistant varieties as a component of an IPM program provides the best performance to control soilborne problems (Davis et al. 2008; Rivard et al. 2010; Yilmaz et al. 2011). In this regard, root knot nematodes and soilborne diseases were efficiently controlled by preplant soil fumigation with specific nematicides (1,3 D) combined with grafting (Bogoescu et al. 2010). Moreover combining soil solarization with grafting significantly increased cucumber yield and reduced soilborne pathogens and nematodes. In recent years, this technology has been commonly accepted as an environmentally friendly and cost-effective application by the growers in Turkey (Yilmaz et al. 2011).

The purposes of this study were to determine the combining effects of soil solarization applied with grafting on first flowering date, early and total yields, total fruit weigth, Content of soluble solids and fusarium wilt on roots.

MATERIALS AND METHODS

Material

Characteristics of rootstocks used are RS 841 commercial hybrids of *Cucurbita maxima x Cucurbita moschata*; Ferro F1, commercial hybrids of *Cucurbita maxima x Cucurbita moschata* resistant to *Fusarium oxysporum* and *verticillium sp.*; Nun 9075 (Nunhems Co) commercial hybrids of *Cucurbita maxima x Cucurbita moschata* and Rz 6415 (64-15 RZ) is an experimental rootstock variety of the Lagenaria type (*Lageneria siceraria*) for the grafting of watermelons.

The Çıtırex F1 (Clause Tezier, Turkey) is a hybrid that belongs to Galia type. It is recommended for open field culture grown in the spring. It is a strong plant with resistant to cold stress, powdery mildew and Fusarium (F0,1,2) in Turkey. It is in medium size and very good color. Fruit weight changes from 1.8 to 2 kg.

A virtually blue impermeable film (VIF) with 350 micron thick and which

reduces the methyl bromide emissions up to 96 percent was used in this application of solarization.

Method

Çıtırex F_1 (*Cucumis melo*) plants were grafted with four cultivars rootstocks Ferro F1, Nun 9075, Rs 841 and Rz 6415. Çıtırex F_1 Cultivar was used as scions. Both rootstock and variety seedlings were kinds of gifts supplied by Antalya Tarim Inc, Antalya-Turkey. Plants were grafted following the procedure described by Lee (1994). Plants were grown under controlled conditions, with a relative humidity of 60-70%, temperature of 18-25^{0}C, a photoperiod of 16 h. The soil in greenhouse was a sandy clay loam (54% sand, 31% silt, 15% clay) with pH 8.0, EC 0.57 dS m^{-1} and organic matter 2.8%. Soil temperatures in the greenhouse in 10 cm depth were measured by HOBO (temperature data logger, Henna H141JH) for solarized and non-solarized plots in every hour intervals from March 2008 to July 2008.

The trial was set in regular glasshouse in Aksu department of the Bati Akdeniz Agricultural Research Institute (BATEM), in Turkey. The experiment was set up completely randomized plots in split plot design with 3 replications and each plots contained 20 plants.

The glasshouse was separated two sections. Solarization was initiated on July 20, 2007 at first. Soil solarization plastic remained on the ground of this section until February. The second division of the plot in the glasshouse was the control section. Seedlings were done on 22 Feb. 2008 in each plot with 0.4 x 1.20 m (2000 plants /da) distances. Without tilling again the VIF plastic was cut just enough to plant the melon seedlings.

Observations taken from trial

First flowering date was recorded when 50% of plants were flowered in plots. The first two cumulative yields for per plot were considered as early fruit yields. Total yield was compilation of 5 harvests from May 15 until July 5. Number of fruits was the total number of fruits harvested in each plot. Hazards of *Fusarium oxysporum* f. sp. *melonis*, on plants were evaluated as symptom-free and infected plants. The measurements of the content of soluble solids were done with refractometer and sensory panel test were conducted from fruits in each plot. In the sensory panel test**,** 8 people were asked to give marks to the samples after the harvest of the fruit in each grafted and non grafted plot. The evaluation marking was made as below: 1-2 points for bitter fruit, 3-4 for not sweet fruit; 5-6 for less sweet fruit; 7-8 mark for sweet fruit; 9-10 points for very sweet fruit. Data for all measurements were subjected to analysis of variance in JMP 5.01 statistical program (SAS Institute Inc., Campus Drive, Cary, NC 27513). When the overall F test was significant ($P \leq 0.05$), means were compared by LSD test.

RESULTS AND DISCUSSION

Combined with solarization application and grafted and non-grafted Çıtırex F1, the first date of flowering, yield per plant, early yield, total yield, disease of *Fusarium oxysporum* f. sp. *melonis* has different effects. All these parameters were significantly different between the applications.

Soil temperatures

Average monthly soil temperatures for solarized and non-solarized soils were given in Fig. 1. Average soil temperature differences between solarized and non-solarized plots were 4.9 ºC, 4.1 ºC, 2.6 ºC, -0.6 ºC, and 0.5 ºC in March, April, May, June and Jule respectively. According to results of this trial, soil solarizations induced plant growth. Yılmaz et al. (2011) showed that 4 and 5-Month Soil Solarization and grafting induced plant growth of cucumber. Krug and Theiel (1985) reported that increase in soil temperature significantly promoted the growth of cucumber.

First flowering time

Effect of grafting and solarization on first flowering time was statistically significant ($P \leq 0.0001$). Early flowering time of the plants grown in solarization plot was in 10 days and late flowering time of the grafted plants grown in control plots was in 13 days (Table 1). The earliest flowering time was 8,3 day in nongrafted Çıtırex F1, the lowest flowering time was 15,3 day with Çıtırex F1 grafted on Nun 9075 rootstock.

Early yields

Statistical analysis showed that both solarization and grafting resulted in significant early yield increases ($P \leq 0.01$). The highest early yield was 6242 $kg.da^{-1}$ with nongrafted Çırıtex F1 in the solarization section, while the lowest early yield 571 $kg.da^{-1}$ of RZ 6415+ Çıtırex F1 was found in the control (Table 1). According to Yilmaz et al. (2011) reported that the early fruit yield was almost doubling in 4 and 5-month period Soil Solarization plots compared to the early yield of cucumber in the control plot. El-Nemr (2006) stated that mulching remarkably increased early fruit yield in cucumber due to in increase soil temperature.

Early yield of gafted plants decreased in control and soil solarization plots. Non-grafted Çıtırex F1 plants provided nearly 40% early yield increase compared to grafted ones in solarization plots. The effects of different rootstocks can show great differences with the same scion cultivar. Graft incompatibility and decrease in the fruit quality may appear depending on the combination of scion and rootstock (Lee 1994; Edelstein 1999).

Total yields

Statistical analysis showed that solarization resulted in significant total yield increases ($P \leq 0.01$). The average yield was 6956 kg.da^{-1}in solarization application and 5239.3 kg.da^{-1}in control plots. It was reported that combining grafting and solarization promoted plants growth and yield (Yilmaz et al. 2011). Another study, El-Nemr (2006) stated that mulching contributed to higher soil temperature for two months after transplanting and consequently this improved plant growth and yield of cucumber.

The total yield of the non-grafted plants significantly increased in the solarization plots. But the yield of the grafted plants reduced compared to non-grafted ones. While the highest yield 8932,6 kg.da^{-1} with the non grafted Çırıtex F1 and 6792,3 kg.da^{-1}with the RS 6415 + Çıtırex F1, the lowest yield 3721,9 da^{-1}in with a Çıtırex F1grafted on Ferro rootstock. The effects of different rootstocks can show great differences with the same scion cultivar. Graft incompatibility and decrease in the fruit quality may appear depending on the combination of scion and rootstock (Lee 1994; Edelstein 1999).

Total number of fruit

The differences between the total numbers of fruits on the applications were not statistically significant. The average number of fruits was 4952 da^{-1}in the solarization section, and 4668 in da^{-1} in control section. The highest number of fruits was 6332 in da^{-1}with the nongrafted Çırıtex F1, while the lowest yield was 3616 in da^{-1}with Çıtırex F1 which was grafted on Ferro F1 rootstocks (Table 2).

Average fruit weight

There was a statistically significant difference between the fruit weight on the applications. The average fruit weight obtained from the non grafted Çıtırex F1 plants was higher than grafted ones. While the high weight was 1.30 kg with non-grafted Çıtırex F1 in the solarization plot, the lowest fruit weight was 1.10 kg with grafted Çıtırex F1 on Ferro F1 in the control application.

Observation of *Fusarium oxysporum* f. sp. *melonis*

Fusarium oxysporum f. sp. *melonis* observations between solarization and control plots were statistically important. While the plant death in the solarization plot was low, the number of death plants in the control section was detected high. It is observed that the death rate of Çıtırex F1 which was grafted on RS 64-15 rootstocks was especially high and this might be caused by different rootstocks and grafting incompability. While the high soil temperature in the solarized plot decreased the plant death, the slow growth of the plants in the control plot increased the plant death (Table 2).

Studies in Israel and Turkey showed that soil solarization significantly reduced the fusarium wilt in various plants reported by Katan et al. (1976) and Yilmaz et al. (2011) respectively.

Content of soluble solids and sensory panel test in Melon (Brix)

The content of soluble solids values is not statistically different between the grafted and nongrafted plants. But sensory panel test is statistically different between the grafted and nongrafted plants. While the highest soluble solid value was obtained in nongrafted Çıtırex F1, the lowest soluble solids value was obtained with Çıtırex F1 that was grafted on RS 841 F1 cultivars. The content of soluble solid was affected by grafting and soil solarization. The fruits of grafted plants had lower total soluble solids contents than nongrafted plants. Although all rootstocks reduced soluble solid contents to a similar degree, most of the fruits harvested had an acceptable total soluble solid content for the market. These results were similar to those observed by Roberts et al. (2007) and Schultheis et al. (2008) in watermelon and by Crino et al. (2007) in melon (fig. 2)

The solarization applied on melon affected the plant growth and yield significantly. The applications of solarization provided a higher soil temperature and a faster plant growth. This increased the total yield of the melon. Although grafting is a suitable method for soil borne pathogen for melon, graft-scion incompability is still a major problem that should be overcome. Generally, in this study, grafted plants flowered late compared to the non-grafted plants and the slow growth of the grafted plants affected the ripening of the fruits. With the grafting incompability, some plant deaths and consequential low yield was observed. The combination of grafted plants with solarization applications increased the yield less than accepted. To overcome with the graft- incompability problem, new rootstocks should be bred and they should be grafted with commercial melon types instead of currently used ones.

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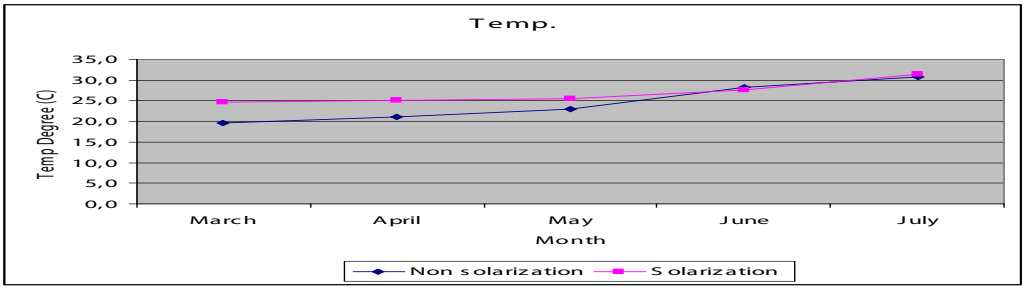

Fig. 1. Average monthly soil temperatures in solarization and non solarization from March to July 2008.

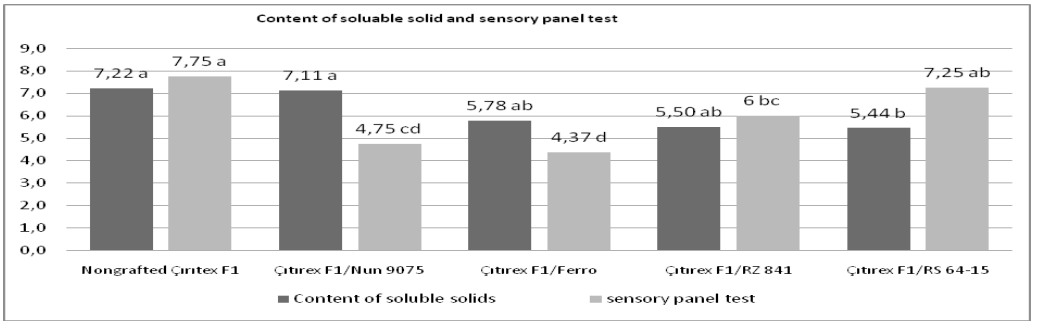

Fig. 2. Content of soluble solids and sensory panel test in melon.

Table 1. Yield characteristic of grafted and non-grafted melon plants in solarization and control.

Treatment	Flowring date (days)		Early yield (kg.da^{-1})		Total yield (Kg.da^{-1})	
	Solarization	control	Solarization	control	Solarization	control
Nongrafted Çırıtex F1	8,3 e	11,6 c	6242 a	1730 d	8932,6 a	7281,7 b
Çıtırex F1/RS 64-15	8,6 e	11,6 c	4252 b	571 e	6792,4 b	6072,3 b
Çıtırex F1/RZ 841	10,6 d	13,6 b	5132 b	2045 c	6601,7 b	4719,8 c
Çıtırex F1/Nun 9075	12,3 c	15,3 a	4911 b	1331 d	6503,5 b	4400,9 c
Çıtırex F1/Ferro	10,6 d	13,6 b	4502 b	1127 d	5948,6 b	3721,9 d
Average	10,0	13,0	5008	1361	6955,7	5239,3
SolarizationxGrafting	ns		ns		ns	
LSD $_{(0,05)}$	2,11*		995,3**		1692,9**	
CV	4,95		25,53		22,68	

Numbers in the same column followed by the same letter are not significantly different (P<0.05) according to LSD

Table 2. Plant growth characteristic of grafted and non-grafted melon in solarization and control

Treatment	Fruit number number.da^{-1}		*Fusarium oxysporum* f. sp. *melonis*	
	Solarization	control	Solarization	control
Nongrafted Çırıtex F1	6332	5133	1,0 e	2,6 d
Çıtırex F1/RS 64-15	5181	5032	11,6 b	18,6 a
Çıtırex F1/RZ 841	4179	5570	3,3 d	11,0 b
Çıtırex F1/Nun 9075	4608	3991	0,6 e	2,3 d
Çıtırex F1/Ferro	4459	3616	5,0 c	5,0 c
Average	4952	4668	4,5	7,6
SolarizationxGrafting	ns		**	
LSD $_{(0,05)}$	2291,9^{ns}		2,33*	
CV	38,93		32,56	

Numbers in the same column followed by the same letter are not significantly different (P<0.05) according to LSD

Determination of aroma compounds of grafted and ungrafted Galia C8 melon cultivar in greenhouse growing

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Keywords: Melon, grafted, aroma compounds, volatile acetates, GC-MS

Abstract

In this study, aroma compounds of grafted and ungrafted melon (Galia C-8) were investigated. Galia C-8 melon cultivar was grafted onto 7 different rootstocks [*Benincasa hispida* (BH), *Cucurbita ficifolia* (CF), *Luffa cylindrical* (LC), P 360, RS 841, TZ 148 (*Cucurbita* Hybrids) and *Cucumis melo* 'Kırkağaç 637'(KA 637)]. Aroma compounds in ripening fruits of Galia C-8 melon were determined and 24 aroma compounds were identified by GS-MS (gas chromatography-mass spectrometry). It was found that 2- methylbuthyl acetate, Hexyl acetate and Octyl acetate were the major compounds in grafted and ungrafted Galia C-8 melon cultivar. However, ethyl hexanoate was strongly positively correlated with fruity and sweet taste and the highest content of ethyl hexanoate was determined from Galia C8 /RS 841 graft combination, while others were in trace amounts. Octyl acetate, isobuthyl acetate and butyl acetate contents of ungrafted melon fruits were higher than grafted melon fruits but 2-methyl butyl acetate content was similar both ungrafted and grafted melon fruits.

INTRODUCTION

Melon is one of the most important fruit crops in Turkey. Turkey is the World's second largest melon producing country after China with 1.6 million tons (Anonymous 2011). There have been many studies on aroma compounds of melon. The most of them reported that the aroma compounds of melon cultivars were affected by maturity period, environmental factors, storage temperatures, variety, hardness (Kemp et al. 1972; Yabumoto et al. 1978; Evensen 1983; Horvat and Senter 1987; Wyllie et al. 1994; Fallik et al. 2000; Fallik et al. 2001; Beaulieu and Grimm 2001; Lamikanra and Richard 2002; Bett-Garber et al. 2003; Beaulieu et al. 2004; Beaulieu 2006; Beaulieu and Lancaster 2007)

Aroma is the one of the major determinants of fruit quality. Sugar concentration

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and aroma development depend on ripening stage which is also associated with increased respiration, ethylene production rates and softening (Pratt et al. 1977). Muskmelon (*Cucumis melo* var. *reticulatus* Naud.) is a highly appreciated quality fruit which would be of consumer interest if it would be available all the year around as high quality melon products. The flavor of muskmelon is a very complex mixture which was studied by several authors in order to identify the volatile components (Scalzo et al. 2001).

There has been an increase in popularity of grafting technique on muskmelon in agricultural production because grafting of melon increased its disease resistance to fusarium wilt, but melon's (*Cucumis melo* L.) quality has been more serious inferior than its own-rooted melon. They reported that 142 volatile compounds were separated in ripe muskmelon (56 esters, 27 alcohols, 3 aldehydes, 17 ketones, 21 alkenes and 18 other substances) and the total aromatic compounds and characteristic aromatic esters contents decreased in grafted melons than the own-rooted melon. The delightful fruit aroma in grafting melon was lower and thinner in comparison to own-rooted (Xiao et al. 2010).

Approximately 150 compounds have been identified in the volatile fraction of muskmelon. The esters, important for the fruity note, called "positive" compounds, were considered as the sum of all the compounds or separately, as acetates, ethyl esters, ethyl-2-methyl butanoate (important for the fresh fruity note) and ethyl acetate (responsible for a bad note) (Maorse and Visscher 1987).

The alcohols, important for the fermented note, called "negative" compounds, were also considered as their sum or separately, as ethanol. The main compound found in the head-space and methanol considered as a marker of the pectin degradation (Scalzo et al. 2001). Several non-acetate esters such as ethyl 2-methyl propanoate, ethyl butanoate, ethyl 2-methyl butanoate and ethyl hexanoate were negatively (often strongly) correlated with cucurbit and ethyl hexanoate was strongly positively correlated with fruity and sweet taste. Hardness was positively and strongly correlated with aromatic acetates and all aromatic (benzyl) compounds (Beaulieu and Lancaster 2007).

TSS and aroma are the most important factors determining fruit quality and consumer's preference (Shalit et al. 2001). The concentration of sugar and flavorings are changing depending on maturation period. It has been reported that these substances are associated with respiratory, the amount of ethylene production and softening of fruit (Pratt et al. 1977).

The aim of this study was to determinate the aroma compounds of grafted and ungrafted Galia C-8 melon cultivar.

MATERIALS AND METHODS

Plant Material

The Galia C-8 melon cultivar was used as scion and *Benincasa hispida* (BH), *Luffa cylindrica* (LC), Kırkağaç 637 (K637), *Cucurbita ficifolia (CF),* RS 841, P 360, TZ 148 were used as rootstocks. Ungrafted Galia C-8 was used as control. Seedlings were grafted by the tongue approach grafting technique that was described by Lee (1994).

The grafted plants grown in the greenhouses were transplanted to plastic house. Plants were fertilized with 250 kg N/ha, 80 kg P_2O_5/ha and 300 kg K_2O/ ha, as recommended by Zuang (1982). The experimental design was a completely randomized block design. Each treatment was replicated 4 times with 8 plants in each replicate. Plants were grown with (100-50) x 50 cm spacing. Fruits were harvested in ripening stage.

GC/MS Analysis

Solid Phase Micro extraction (SPME) apparatus was used for the extraction of melon aroma. Fresh melons were peeled and cut and the seeds were removed. The fruit flesh was homogenized in a blender and 50 g of the homogenate was put in a glassware and coated with paraffin and 1 g NaCl was added then put on a stirrer for the extraction as described by Fallik et al. (2001).

Aroma compounds of the melons were analyzed by GC-MS. A Shimadzu QP 5050A apparatus equipped with CPSil5CB (25 m x 0.25 mm i.d., 0.4 µm film thickness) fused-silica capillary column was used. The injector temperature was 250 ºC, set for splitless injection. The column temperature was 60 ºC//5 ºC/min//260 ºC for 20 min. Mass spectra were taken at 70 eV. Mass range was between m/z 30-425. A library search was carried out using the Wiley GC-MS Library and in-house Baser Library of Essential Oil Constituents. The mass spectra were also compared with those of reference compounds and confirmed with the aid of retention indices from published sources.

RESULTS AND DISCUSSION

The SPME GC-MS analysis method was used to identify the aroma compounds of Galia C-8 F1 fruits that grafted onto different rootstocks and ungrafted. In this study, 24 aroma compounds were determined. As shown in Table 1, the main volatile compounds in samples which are octyl acetate, hexyl acetate, 2-methyl butyl acetate, (z)-3-hexenyl acetate, isobuthyl acetate (except KA 637, BH and TZ 148), butyl acetate (except KA 637 and TZ 148), 3-octenyl acetate, heptyl acetate (except CF and LC), (Z)-5-decenyl acetate (except LC), respectively. However, especially ethyl acetate was determined only CF and propyl acetate, ethyl-2-methyl butyrate, Amyl acetate, isoamyl acetate, ethyl hexanoate (ethyl hexanoate was

strongly positively correlated with fruity and sweet taste (Beaulieu and Lancaster 2007), and the highest content of ethyl hexanoate was determined from Galia C8 /RS 841 graft combination in present study), phenylethyl acetate, ethyl butyrate, phenylpropyl acetate, decenyl acetate, nonly acetate, octanol, benzyl acetate, (E)-5-decenyl acetate and (E)-5- dodecenyl acetate were determined very small or trace amounts. However, Octyl acetate, isobuthyl acetate and butyl acetate contents of ungrafted melon fruits were higher than grafted melon fruits but 2-methyl butyl acetate content was similar both ungrafted and grafted melon fruits. Fallik et al. (2001) reported that during maturity, 2-methylbutyl acetate, hexyl acetate, butyl acetate, 3-hexenyl acetate and isobutyl acetate levels increased when the peel color turned from green to light yellow. Beaulieu (2006) reported that fifty-five compounds were integrated and used to assess volatile changes through cantaloupe maturation and during fresh-cut storage.

The aroma compounds of grafted melon fruits depended on the compatibility between both of scion and rootstock. As a result of the present study has been showed using suitable rootstocks were not the adverse effects on the aroma compounds of grafted melon.

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Table 1. Aroma compounds of grafted and ungrafted Galia C-8 melon cultivar.

Retention Time	Aroma Compounds	Control (Ungrafted)	BH	P 360	RS 841	CF	LC	KA 637	TZ 148
2.11	Ethyl acetate	tr	tr	tr	tr	2.6	tr	tr	tr
2.40	Propyl acetate	tr	-	-	tr	tr	tr	-	-
3.26	İsobuthyl acetate	10.0	tr	8.8	5.4	5.0	7.2	tr	tr
3.34	Ethyl butyrate	tr	-	tr	1.5	1.5	tr	-	-
3.78	Butyl acetate	5.8	3.0	5.1	4.4	3.8	4.1	tr	tr
4.15	Ethyl-2-Methyl butyrate	tr	-	tr	tr	tr	tr	-	-
4.63	Amyl acetate	tr	-	-	-	-	-	-	-
4.86	**2-Methyl Butyl acetate**	**24.7**	**27.0**	**25.7**	**19.9**	**26.3**	**23.6**	**21.6**	**22.2**
5.30	İsoamyl acetate	tr	-	tr	-	tr	tr	-	-
7.44	Ethyl hexanoate	-	-	-	0.4	tr	-	-	-
7.66	(z)-3-hexenyl acetate	7.9	5.8	5.7	9.2	6.0	6.6	4.7	5.4
7.87	**Hexyl acetate**	**36.1**	**45.2**	**30.5**	**42.2**	**40.8**	**42.5**	**41.2**	**40.3**
9.49	Octanol	tr	tr	1.1	0.5	tr	tr	0.9	tr
10.66	Heptyl acetate	2.5	2.4	2.2	2.1	tr	tr	2.5	2.2
11.81	Benzyl acetate	tr	0.9	0.5	0.5	tr	tr	tr	tr
13.14	3-octenyl acetate	3.1	3.2	2.4	2.7	1.9	1.5	3.0	2.4
13.60	**Octyl acetate**	**45.2**	**33.9**	**42.9**	**34.1**	**40.9**	**46.1**	**41.0**	**44.5**
14.51	Phenylethyl acetate	-	0.3	-	tr	-	-	-	-
16.48	Nonly acetate	tr	0.1	0.1	-	-	-	-	-
17.84	Phenylpropyl acetate	tr	0.5	0.2	0.2	tr	tr	tr	0.2
18.78	(Z)-5-decenyl acetate	2.8	3.5	3.2	1.8	1.1	tr	3.4	2.3
18.92	(E)-5-decenyl acetate	0.6	0.6	0.7	0.4	tr	tr	0.7	0.6
19.34	Deceny acetate	1.0	0.8	1.2	1.0	0.5	tr	1.3	0.9
24.13	(E)-5-dodecenyl acetate	0.2	0.1	0.4	0.1	tr	tr	0.3	0.2

tr: trace

Changes in carbohydrates and organic acids in leaves and mesocarp tissues during melon (*Cucumis melo* L.) fruit development

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Keywords: Fructose, glucose, sucrose, galactose, raffinose, citric acid, malic acid

Abstract

Levels of carbohydrates and organic acids were examined in leaves (source) and mesocarp tissues (sink) of two melon genotypes during fruit development. Total soluble sugars in source leaves were much lower in the early stage of fruit development and increased significantly in the late stage of fruit development. Hexoses (glucose plus fructose) and sucrose made up the bulk of the soluble sugars in leaves. Malic acid was the main organic acid in leaves, the level of malic acid in leaves was higher than citric acid during fruit development. Total soluble sugars in mesocarp tissues were significantly higher than in leaves. Total soluble sugars in mesocarp tissues increased gradually during fruit development. The increase in total soluble sugars mainly resulted from the accumulation of fructose, glucose and sucrose. Sucrose showed a dramatic increase in later stage of fruit development. Levels of galactose and raffinose were much lower. Levels of organic acids in fruits were very low. Citric acid in mesocarp tissues increased during fruit development while malic acid decreased. Citric acid was the main organic acid in mature melon fruit. In our study, low levels of soluble sugars in leaves in the early stage of fruit development indicated that an increase in sink demand enhanced photoassimilate export from source leaves.

INTRODUCTION

Melon is one of the economically important and widely cultivated vegetable crops in the world. Great changes in chemical composition occur during melon fruit development. The balance between sugars and organic acids is mainly responsible for the taste of most fruit however in sweet melon that have low organic acid content, fruit quality is determined primarily by sweetness alone (Yamaguchi et al. 1977).

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Many efforts have been made to study carbohydrate metabolism in fruits (Wang et al. 1996; Flores et al. 2001; Bower et al. 2002; Vargas et al. 2008). It is known that the export of carbohydrates from photosynthesizing leaves (source) provides the substances for fruit (sink) development. Little information has been known on the chemical changes in both leaves and fruits simultaneously. Our study aims to gain a better insight into the carbohydrates and organic acids changes in both source leaves and sink tissues during melon fruit development.

MATERIALS AND METHODS

Plant materials

C. melo 'IVF501' (orange flesh, thick skin with yellow smooth surface) and 'IVF638' (white flesh, thin skin with white smooth surface) were grown in plastic tunnel in the Institute of Vegetables and Flowers of the Chinese Academy of Agricultural Sciences in Beijing as an autumn crop under standard management. Flowers were tagged at anthesis and one fruit per plant were allowed to develop. Mature leaves and fruits from the same node were collected on 0, 8, 16, 24 and 32 days after anthesis. 5 fruits were harvested at each sampling date. Flesh mesocarp was taken from the center-equatorial portion of each fruit. Samples were immediately frozen in liquid nitrogen and stored at -80°C for analysis.

Analysis of sugars and organic acids

Soluble sugars and organic acids were analyzed with an Agilent 7890A gas chromatograph / 5975C mass spectrometer (GC/MS, Agilent Technology). A total of 12 carbohydrates and 8 organic acids were identified and quantified (Not all data is shown in the present study).

Statistical analysis

All the statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Total metabolites (soluble sugars plus organic acids) in source leaves were much lower in the early stage of fruit development and increased significantly in the late stage of fruit development (Fig. 1 A, B). Total soluble sugars increased from 24 days and 16 days after anthesis for IVF501 and IVF638 respectively. Levels of glucose, fructose, sucrose and galactose showed a distinct increase on 32 days after anthesis (Fig. 1 C, D). Hexoses (glucose plus fructose) and sucrose made up the bulk of the soluble sugars in leaves (85.38% and 86.09% of total soluble sugars for IVF501 and IVF638 on 32 days after anthesis), which was consistent with previous studies (Zhang et al. 2010; Fu et al. 2011). Levels of galactose and raffinose were lower. The content of total organic acids in leaves didn't change significantly during

fruit development (Fig. 1 A, B), malic acid was the main organic acid in leaves, the level of malic acid in leaves was higher than citric acid during fruit development (Fig. 1 E, F).

Levels of total soluble sugars in mesocarp tissues were significantly higher than in leaves (Fig. 2). Total soluble sugars in mesocarp tissues increased gradually during fruit development (Fig. 2 A, B). The increase in total soluble sugar levels mainly resulted from the accumulation of fructose, glucose and sucrose (Fig. 2 C, D). Fructose, glucose and sucrose made up 92.12% (for IVF501) and 92.27% (for IVF638) of total soluble sugars on 32 days after anthesis. Sucrose showed a dramatic increase in later stage of fruit development. Levels of organic acids were very low in fruits. Concentrations of total organic acids declined slightly during fruit development, while on a whole fruit basis, the content of all organic acids increased (Fig. 2 E, F). The total amount of organic acids in mesocarp tissues was 2.17 (5.23% of total metabolites) and 1.77 (6.46% of total metabolites) mg g^{-1} FW for IVF501 and IVF638 after fruit maturity. Citric and malic acids were the most important organic acids in fruits (Fig. 2 G, H). Citric acid in mesocarp tissues increased during fruit development while malic acid decreased during fruit development (Fig. 2 G, H). Citric acid was the main organic acid in mature melon fruit, which is consistent with previously studies (Villanueva et al. 2004; Zhang and Li, 2005). It is well known that fruit development is dependent largely upon the supply of photoassimilates imported into the fruit via the phloem from leaves (Janoudi et al. 1993). In our study, low levels of soluble sugars in leaves in early stage of fruit development indicated that an increase in sink demand enhanced photoassimilate export from source leaves.

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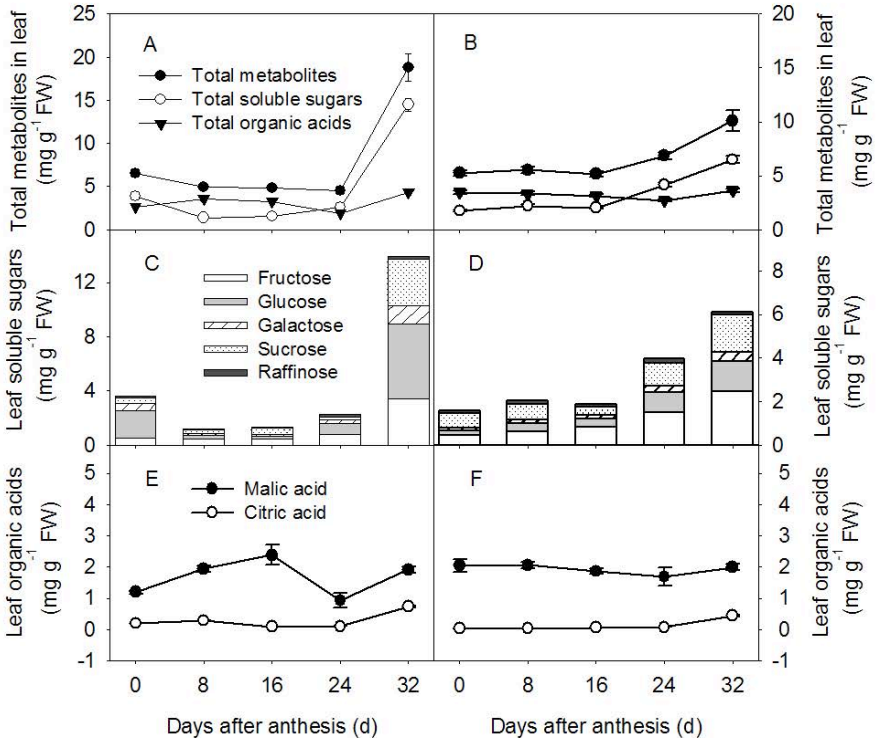

Fig. 1 Changes in total metabolites (soluble sugars plus organic acids), soluble sugars and organic acids in source leaves during fruit development of IVF501 (A, C, E) and IVF638 (B, D, F).

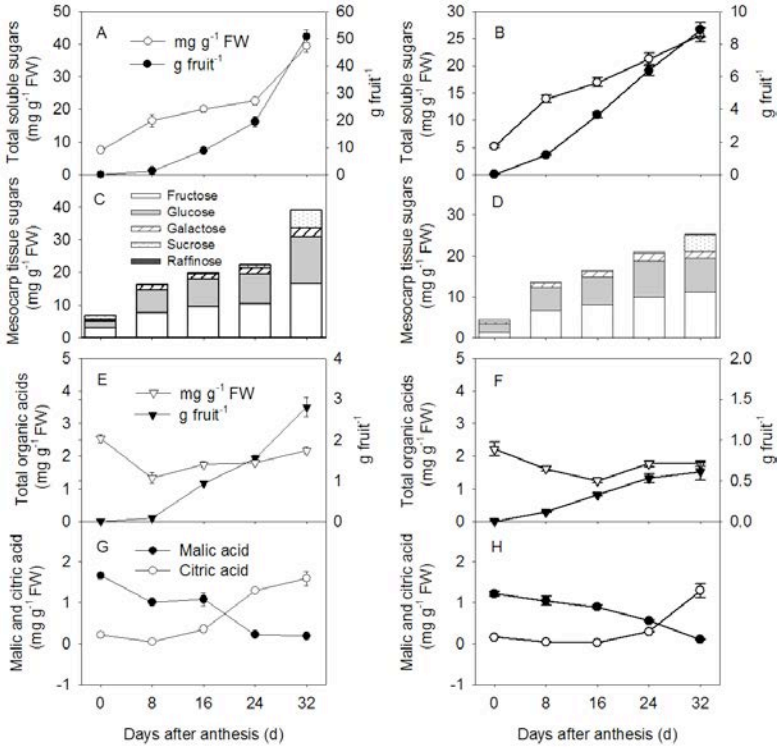

Fig. 2 Changes in soluble sugars and organic acids in mesocarp tissues during fruit development of IVF501 (A, C, E, G) and IVF638 (B, D, F, H).

Characterization of gene expression and protein accumulation in melon ethylene receptors

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Melon cultivar 'Vedrantais' (*Cucumis melo* L. var. *cantalupensis*) is a typical climacteric fruit and the ripening phenomena dramatically proceed within two to three days after initiation of ripening. Ethylene has critical roles in ripening of climacteric fruit and ethylene receptor is an important factor located front line of the signal transduction pathway. It is known that ethylene receptor genes construct multigene family. Three ethylene receptor genes, CmETR1, CmETR2 and CmERS1 have been identified in melon. However their molecular characterization during fruit development is still unclear.

We analyzed melon ethylene receptors (CmETR1, CmETR2, CmERS1) for pattern of gene expression and protein accumulation of various tissues, fruit enlargement and ripening stages of the climacteric 'Vedrantais' melon. Additionally, the connection with ethylene was studied with 1-methylcyclopropen, a gaseous ethylene action inhibitor.

In this study, we suggest that ripening initiation is involved in the change of expression levels of each ethylene receptor before or after ripening ethylene production on the basis of gene expression analysis and that the expression levels of ethylene receptors are adjusted by both ethylene dependent and independent systems.

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Inheritance of andromonoecy in *Tibish*

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Keywords: Melon, sex expression, *Cucumis melo*, genetic control, monoecy

Abstract

In melon, the presence of stamens in female flowers is controlled by the recessive allele *a*, while the absence of stamens in the female flowers is controlled by the allele a^+ (or *A*). *Tibish* group of melon is characterized by small, ovoid fruits which are harvested before maturity and consumed raw in salad, like cucumber. The plants have male and perfect flowers, characteristics of andromonoecy. The F_1 progenies between Tibish and monoecious cultivars belonging to the *flexuosus* group were andromonoecious. In F_2 and backcross progenies between *Tibish* and 'Védrantais' (andromonoecious cultivar belonging to the *cantalupensis* group), monoecious plants were observed indicating that a genomic region independent from the locus a/a^+ was involved. The segregations observed in F_2 progenies between *tibish* and 3 cultivars belonging to the *flexuosus* group could be explained by two linked loci. The symbols *A-2* and *A-3* are proposed.

INTRODUCTION

Melon (*Cucumis melo*) is cultivated throughout the world for different kind of fruits. Two main groups correspond to the non-sweet types (for instance *conomon*, *chate*, *flexuosus*, *acidulus*) and the sweet types (for instance *cantalupensis*, *inodorus*, *ameri*). On a typical melon plants, there are more than 20 female flowers which will give only 4 or 5 mature fruits due to a very strong competition between fruits. In cultigroups with non-sweet fruits, the fruits are harvested before maturity and the competition between fruits is less severe. By increasing the female flower number it could be possible to increase the total yield. Breeding hermaphrodite cultivars, with perfect flowers at each node on the main stem and the lateral branches, belonging to cultigroups where fruits are harvested before maturity, as in *flexuosus* or *tibish* groups, could be a possibility to increase earliness and yield.

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Wild melons are monoecious and many modern cultivars are andromonoecious. A few accessions from China are hermaphrodite. From these last accessions, breeders have been able to develop gynoecious lines. Two main genes are involved in the genetic control of these phenotypes: locus *a* controls the presence (allele *a*) or absence (allele a^+ or *A*) of stamens in female flowers (Rosa 1928) and locus *g* controls the presence of one type (allele *g*) or two types of flowers (allele g^+ or *G*) on one plant (Poole and Grimball 1939).The combinations of these two independent loci generate four phenotypes: monoecious (a^+- g^+g^+), andromonoecious (*aa* g^+-), gynoecious (a^+a^+ *gg*) and hermaphrodite (*aa gg*).

Four types of melons are cultivated in Sudan: sweet melons, snake melon (*C. melo* var. *flexuosus*), a salad melon known locally as 'Tibish' (C. *melo* var. *tibish*), and a melon type used for its edible seeds known locally as 'Seinat'. True wild melons known locally as 'Humaid' are present in central, northern and western parts of Sudan. The *tibish* type is characterized by small (300-500 g), ovoid smooth fruits, light green with dark green stripes, five placentas, white firm flesh, non sweet at maturity. It is andromonoecious.

To breed hermaphrodite *flexuosus* and *tibish* lines an hermaphrodite accession from China was crossed with cultivars belonging to both types. The observed segregations with *tibish* did not fit the expected ones. It prompted the study of the inheritance of sex expression in *tibish*.

MATERIALS AND METHODS

Plant material

Two accessions of *tibish* are used, namely Jebel Kordofan 4 and Khurtagat 15. Both were collected from Kordofan area in 2006 and were andromonoecious. They were crossed with three monoecious accessions of *flexuosus* melon, namely 'Alimin' and 'Silka', which are commercial cultivars of snakemelon in Sudan, and PI 222187 from the Afghanistan and with 'Védrantais', an andromonoecious Charentais line (obt. Vilmorin).

Sex expression

Opening flowers were examined daily to observe the presence of pistillate flowers, with only female organs like stigma, or perfect flowers with both female organs and stamens. Segregation ratios in F_2 and BC populations were tested for goodness of fit to theoretical ratio with Chi square test.

RESULTS

Allelism of andromonoecy

The F_1 progeny of the cross between *tibish* Khurtagat 15 and 'Védrantais', both andromonoecious, was andromonoecious as expected (Table 1). Monoecious

plants were observed in the F_2 and BC progenies indicating that andromonoecy in *tibish* was not controlled by the locus a/a^+ and that Khurtagat 15 had the allele a^+.

Inheritance of andromonoecy in *tibish*

The F_1 progenies of the five crosses between both andromonoecious *tibish* accessions and the three monoecious *flexuosus* accessions were clearly andromonoecious (Table 1) indicating that andromonoecy is dominant. In the F_2 progenies, more andromonoecious plants were observed than monoecious in opposition with the dominance of monoecy controlled by the a^+ allele.

Taking in account that Khurtagat 15 had the allele a^+, different hypothesis were tested, with the symbols *A-2* and *A-3* for the dominant alleles in *tibish*:

H1 = One dominant gene for andromonoecy in *tibish* independent from the locus *a* and epistatic on a^+ i.e. *tibish* [a^+/a^+ *A-2*/*A-2*] and *flexuosus* [a^+/a^+ *A-2*$^+$/*A-2*$^+$]. The observed segregation on the pooled F_2 (380 andromonoecious *versus* 199 monoecious) did not fit the expected 3 andromonoecious *versus* 1 monoecious (χ^2 = 27.11, Prob <0.001).

H2 = Two independent dominant genes, each sufficient for andromonoecy, in *tibish* independent from the locus *a* and epistatic on a^+ i.e. *tibish* [a^+/a^+ *A-2*/*A-2 A-3*/*A-3*] and *flexuosus* [a^+/a^+ *A-2*$^+$/*A-2*$^+$ *A-3*$^+$/*A-3*$^+$]. The observed segregations did not fit the expected 15 andromonoecious *versus* 1 monoecious (χ^2 = 781.35, Prob <0.001).

H3 = Two independent dominant genes, both necessary for andromonoecy, in *tibish* independent from the locus *a* and epistatic on a^+. The observed segregations did not fit the expected 9 andromonoecious *versus* 7 monoecious (χ^2 = 20.703, Prob <0.001).

H4 = Two linked dominant genes, both necessary for andromonoecy, in *tibish* independent from the locus *a* and epistatic on a^+. The recombination frequency between the two loci *A-2* and *A-3* was estimated by the maximum likelihood method and varied between 0.12 and 0.44 on the five F_2 and was estimated at 0.21 on the pooled F_2. With this last value, the observed segregations of the five F_2 between *tibish* and *flexuosus* and also of the F_2 and BC between *tibish* and 'Védrantais' fitted the expected segregations.

DISCUSSION AND CONCLUSIONS

Monoecy in *C. melo* has always been considered under a monogenic dominant control (Rosa 1928; Poole and Grimball 1939; Kubicki 1969; Kenigsbuch and Cohen 1990) with the recessive allele *a* for andromonoecy. This is the first work to study inheritance of andromonoecy using *tibish* genotypes. Andromonoecy is clearly dominant to monoecy and another genomic region than the locus *a* is involved. It seems that two linked dominant genes are necessary for this phenotype. We

propose the names *Andromonoecy-2* (symbol *A-2*) and *Andromonoecy-3* (symbol *A-3*) for these genes. The genotypes of the lines studied here could be 'Védrantais' [*aa A-2*$^+$*A-2*$^+$ *A-3*$^+$*A-3*$^+$], *flexuosus* [*a*$^+$*a*$^+$ *A-2*$^+$*A-2*$^+$ *A-3*$^+$*A-3*$^+$], *tibish* [*a*$^+$*a*$^+$ *A-2A-2 A-3A-3*]. Gene *a* corresponds to an ACC synthase (*CmACS-7*) inactive for the allele *a* and active for the allele *a*$^+$ (Boualem et al. 2008). Further study is needed to know if the dominant genes correspond also to enzymes involved in the ethylene pathway.

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Table 1. Phenotypes observed for sex expression (monoecy or andromonoecy) in melon in crosses between *tibish* (Khurtagat 15 or Kordofan 4) and monoecious accessions of *flexuosus* melon, namely 'Alimin' and 'Silka', which are commercial cultivar of snakemelon in Sudan, and PI 222187 from the Afghanistan and with 'Védrantais', an andromonoecious Charentais line (obt. Vilmorin).

Genotypes	Number[z]		H1[y]		H2[y]		H3[y]		H4[y]	
	Andro	Mono	Ratio[x]	Prob[w]	Ratio[x]	Prob[w]	Ratio[x]	Prob[w]	Rec[v]	Prob[w]
F_1 (Védrantais x Khurtagat 15)	15									
F_2 (Védrantais x Khurtagat 15) ⊕	17	7	3: 1	63.7	61:3	<0.001	43:21	70.4		73.0
BC (Védrantais x Khurtagat 15) x Védrantais.	16	7	1: 1	6.1	7:1	0.9	5:3	48.4		98.5
Silka		14								
Alimin		25								
PI 222187		27								
Kordofan 4	19									
Khurtagat 15	15									
F_1 (Silka x Kordofan 4)	17									
F_1 (Alimin x Khurtagat 15)	15									
F_1 (PI 222187 x Kordofan 4)	16									
F_1 (PI 222187 x Khurtagat 15)	13									
F_1 (Silka x Khurtagat 15)	16									
F_2 (Silka x Kordofan 4) ⊕	134	66	3:1	0.9	15:1	<0.001	9:7	0.22	0.18	67.7
F_2 (Alimin x Khurtagat 15) ⊕	45	20	3:1	28.0	15:1	<0.001	9:7	3.5	0.12	53.8
F_2 (PI 222187 x Kordofan 4) ⊕	55	40	3:1	0.01	15:1	<0.001	9:7	74.7	0.44	11.4
F_2 (PI 222187 x Khurtagat 15) ⊕	74	35	3:1	8.6	15:1	<0.001	9:7	1.4	0.15	61.5
F_2 (Silka x Khurtagat 15) ⊕	72	38	3:1	2.1	15:1	<0.001	9:7	5.2	0.21	97.4
F_2 pooled	380	199	3:1	<0.001	15:1	<0.001	9:7	<0.001	0.21	98.8

[z] Observed number of andromonoecious and monoecious plants in different progenies

[y] Various hypothesis for the genetic control of andromonoecy in *tibish*. H1 = one dominant gene; H2 = two independent dominant genes, each sufficient; H3 = two independent dominant genes, both necessary; H4 = two linked dominant genes, both necessary.

[x] Expected ratio of segregation according to the hypothesis H1 to H3. [w] Probability of the χ^2 for the expected ratio

[v] Recombination fraction estimated by the maximum likelihood method.

Induction of tetraploid muskmelon and changes of its characteristics

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Keywords: Muskmelon, tetraploid, mutation breeding

Abstract

Three diploid varieties were treated by removing growth point of seedlings and dripping 0.2% colchicines onto the stem tips to induce tetraploid muskmelon. The results showed that the tetraploid mutation rate of netted melon M066 was 54.4%, that of smooth rind melon such as M072 and M0544 were 20.0% and 23.3% respectively. Compared to autotetraploid with original diploid, the length of lateral branch internode was shorter, the size of corolla and leaf were larger, the number of chloroplast per guard cell increased. But the number of perfect seeds per fruit, the stainability of pollen grains, fruit weight and the index of fruit-type decreased. The flesh qualities of soluble solid content declined, but its thickness was same as original diploid. These results demonstrated the mutagenic effects were different according to genotypes and there were significant changes in physiological and biochemical characteristics between autotetraploid and diploid.

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The resources recycle and development trend for Oriental melon landraces in Jilin Province

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Keywords: Oriental melon, landraces, Jilin Province, breed characteristics, technical specifications

Abstract

Jilin oriental melon is rich in resources, many local varieties, "Bali sweet" oriental melon as a landraces representative, Shuangyang district of Changchun city in Jilin Province has already formed the main landraces, becomes the local farmers' income the main way. Due to the special geographical position and Jilin Province ecological climate environment, temperature gap between day and night, "Bali sweet" oriental melon dry matter accumulation, good quality, commonly known as the "eight miles still fines". At the same time the fertile soil, clean water and characteristics of cultivation techniques for "Bali sweet" oriental melon's green, pollution-free production to create a good natural condition. Through to the "Bali sweet" oriental melon variety screening; Health seed breeding; plant diseases and insect pests prevention and control technology research; this paper summarizes the perfect "Bali sweet" oriental melon varieties of safe production technology and high quality standard procedures, this paper expounds the oriental melon landraces resources recycle and development trend.

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Germplasm selection on melon with resistance to *Fusarium* wilt and *Monosporascus* root rot for melon rootstocks

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Keywords: Grafting, soil-borne diseases, *Fusarium oxysporum* f. sp. *melonis, Monosporascus cannonballus,* soluble solid content

Abstract

The objectives of this study are to select melon rootstocks for resistance against *Fusarium oxysporum* f. sp. *melonis* (*Fom*) and *Monosporascus cannonballus*, and investigate the compatibility between rootstock and the scion. The experiment was conducted at National Institute of Horticultural & Herbal Science in Suwon, Korea (37°18'23''N, 126°58'40''E) in a randomized block design during year 2011. Plants of 65 melon accessions were evaluated in a soil, artificially infested *Fom* race 1 isolated from diseased melon plants. The screening of melon germplasm with resistance to *M. cannonballus* was conducted on infested greenhouse. The four accessions namely, 'K134068', 'K133069', 'Wondae' and 'PI 414723' were selected to serve as rootstocks with resistance to both these pathogens. Yield and quality of fruits harvested from 'Earl's elite' (Muskmelon, *Reticulatus* Group) grafted onto the selected melon rootstocks were found comparable to or better than those of non-grafted melons. However, no fruit fermentation was observed when 'Homerunstar' (Honeydew type, Inodorus Group) was grafted with selected melon rootstocks, unlike when it was grafted onto 'Shintozwa' (*Cucurbita* spp.) rootstock where fruit fermentation was observed. The selected melon germplasm with resistance to *Fusarium* wilt and *Monosporascus* root rot is considered to be potential and valuable source in breeding of melon rootstocks. Further breeding programs by combining the desirable characteristics such as disease resistance, cold and salt tolerance would address the needs of new hybrid cultivars and producing melon production successfully under greenhouse conditions.

INTRODUCTION

Soil-borne diseases aggravated by continuous cropping cause severe yield loss in melon cultivation of the major production area in Korea. The soil-borne pathogen

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Fusarium oxysporum f. sp. *melonis* (*Fom*) is believed to be most destructive disease of melon (Evcil and Yalcin 1997; Lee 1994; Oda 1995; Cohen et al. 2002). *Fom* attacks melon at any growth stage, even before sprouting, but mainly when the fruit is ripe (Mas et al. 1981; Martyn and Gordon 1996). This causes either slow wilting accompanied by progressive yellowing, or a sudden wilting without prior yellowing or other related symptoms may also appear. *M. cannonballus* was reported to induce sudden wilting in melon which also has become a major production problem worldwide (Bruton 1998; Martyn and Miller 1996). Root infection and damage occur at all developmental stages of, but, increase in water demand during fruit development and maturation can lead to vine collapse due to loss of water-uptake capacity (Bruton 1998).

Breeding new cultivars for disease resistant is time consuming and cannot guarantee the resistance against new races of pathogens. On the other hand, grafting onto resistant rootstocks may enable the control of soil-borne diseases (Lee 1994; Oda 1995). Hence, combined breeding programs could be applied to prevent the occurrence of soil-borne diseases (McCreight et al. 1993).

Grafting is an important technique for sustainable production of fruit-bearing vegetables in Korea, Japan, and European countries where land is being used intensively (Lee 1994; Oda 1995). Grafting in melon cultivation makes possible increase in disease resistance and tolerance to environmental stresses such as soil salinity and low temperature. The use of rootstocks also increases adaptability of melon cultivars against different type of soil conditions including resistance to soil-borne diseases and which contribute to total production yield and water use efficiency (Caruso et al. 1996; Yano et al. 2002). The available rootstock species for melon grafting are melon, pumpkin (*Cucurbita* spp.) and wax gourd (*Benincasa hispida*) (Traka-Mavrona et al. 2000). It is reported that the *Cucurbita* rootstock can influence performance of scion cultivars of melon in terms of plant growth (Ruiz and Romero 1999), fruit quality, yield, and wilt incidence (Ruiz et al. 1997; Traka-Mavrona et al. 2000; Nisini et al. 2002). The effects grafted plants depend on compatibility between rootstock and the scion, the existing environmental conditions, and the cultivation. This is the reason why Lee (1994) suggests *Cucumis melo* is a suitable rootstock for melon.

The objectives of this study are to select melon rootstocks for resistance against *Fusarium oxysporum* f. sp. *melonis* (*Fom*) and *Monosporascus cannonballus*, and investigate the compatibility between rootstock and the scion.

MATERIALS AND METHODS

The experiment was conducted at the National Institute of Horticultural & Herbal Science in Suwon, Korea (37°18’23’’N, 126°58’40’’E). A total of 65 melon accessions were screened to identify potential resistance against *F. oxysporum* f. sp. *melonis* race 1 and *M. cannonballus.*

Screening against *F. oxysporum* f. sp. *melonis* race 1 and *M. cannonballus*

Plants of 65 melon accessions were evaluated in soil artificially infested with *Fom* race 1 which was isolated from diseased melon plants. Isolates of *Fom* 1 were grown on patato dextrose agar (Difco) in plastic petri dishes of size 5.5 cm diameter at 25ºC for 8 days. Sterile distilled water (5 ml) was added per petri dish, and colonies were scraped off using a sterilized glass rod. The suspension was filtered through two layers of sterile cheesecloth and conidia was adjusted to 1×10^6 conidia mL^{-1} using a hemacytometer. The roots of the 20-day-old plants were dipped into 1×10^6 conidia mL^{-1} for one minute and later transplanted to pots. Disease ratings were recorded 3 weeks after inoculation.

Screening of melon germplasm with resistance to *M. cannonballus* was conducted under infested greenhouse. Melon plants were transplanted (July 9, 2011) in a randomized complete block design with three replication per germplasm. Each bed was of size 190 cm and intra-row spacing was set at 45cm.

Compatibility between the rootstock and scion

Two melon cultivars of 'Homerunstar' and 'Earl's elite' were grafted onto selected rootstocks, 'K134068', 'K133069', 'Wondae', 'PI 414723' and 'Shintozwa' (*Cucurbita* spp.). 'Homerunstar' and 'Earl's elite' are the most common cultivars being harvested between July and August in Korea. The former is a honeydew type melon with creamy-white color and oblong shape, while the latter is a muskmelon with round shape.

Both the rootstock and scion were sown at the same time on March 31, 2011 for synchronization of grafting time. Grafting work was done on April 8, 2011 at cotyledon stage. The splice grafting technique was used in which eight old watermelon seedlings were used for grafting (Lee et al. 2010). The grafted seedlings were transferred into a mist room maintained at relative humidity of 95% for seven days where in relative humidity was gradually decreased later for acclimatization one week before transplanting.

Melon seedlings were transplanted in a greenhouse in row with black polyethylene mulching films on May 7, 2011. The experimental design consisted of randomized blocks with three replicates. A total of 7 plants per replication were grown horizontally at spacing of 3m x 0.5m (row-row x plant-plant) for each rootstock combination and cultivars. The cultivation was practiced as per method described of Rural Developmental Administration (2005).

The statistical analysis was done SAS software (SAS Institute, 1995). The data from all the experiments were subjected to ANOVA and Duncan's multiple range tests to determine the statistical significance of differences between treatments.

RESULTS AND DISCUSSIONS

Out of total 65 melon germplasm screened as rootstock, thirty five germplasm exhibited significant resistance against *Fom* race 1 and eleven germplasm showed high resistannce to *M. cannonballus*. The four accessions, 'K134068', 'K133069', 'Wondae' and 'PI 414723' were selected to serve as rootstocks having resistance to both pathogens (Table 1).

Yield and quality of fruits harvested from 'Earl's elite' (Muskmelon, Reticulatus Group) and 'Homerunstar' (Honeydew type, Inodorus Group) grafted onto selected melon rootstocks were observed. Grafting in 'Earl's elite' utilizing selected melon rootstocks was comparable to or better than those of non-grafted melon (Table 2). There are several conflicting reports (Proietti et al. 2008; Flores et al. 2010) regarding the role of grafting on changes in fruit quality in terms of advantages and disadvantages. The reasons for these differences in the previous reports might be attributed to different production environments and type of agricultural practice, kind of rootstock/scion combinations, and harvesting period. 'Homerunstar' grafted onto the selected melon rootstocks had resulted in absence of fruit fermentation, whereas fruit fermentation was observed when it was grafted onto 'Shintozwa' (Table 3).

Although the same cultural treatments, such as fertilizer application and irrigation were practiced, the present results were found to be comparable and contradictory to previous information which showed that *Cucurbita* spp. rootstock cause a remarkable deterioration in taste of melons (Lee 1994; Oda 1995), It could be suggested that the agents associated with fruit quality were translocated to the scion through the xylem.

Therefore, these selected melon germplasm with resistance to *Fusarium* wilt and *Monosporascus* root rot identified would serve as potential and valuable source for further breeding of melon rootstocks by combining other desirable characteristics such as disease resistance, cold and salt tolerance to address the needs of new hybrid varieties and producing melon production successfully under greenhouse conditions.

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Table 1. Disease severity of melon germplasm against *Fom* race 1 and *M. cannonballus*.

Accession	Disease severity[z]		Accession	Disease severity	
	Fom race 1	*M. cannonballus*		*Fom* race1	*M. cannonballus*
K134068	HR	HR	Seolnaehyang	HR	S
K134069	HR	HR	V-3-6	HR	S
Wondae	HR	HR	Earlsace	MR	MR
PI 414723	HR	HR	Acur	S	HR
Irannetmelon	HR	MR	Busan no.914	S	HR
Dalaman	HR	MR	Busan no.920	S	HR
Hwangkeumchamoe	HR	MR	Unknown 3	S	HR
Romans	HR	MR	Unknown 4	S	HR
Sageumok	HR	MR	Unknown 5	S	HR
Unknown 1	HR	MR	Unknown 6	S	HR
Unknown 2	HR	MR	PMR Honeydew	S	SR
B1	HR	SR	Unknown 7	S	SR
Baekdangkwan	HR	SR	05M40	S	S
Seonghwanchamoe	HR	SR	Charentais Fom 1	S	S
Cheongpisokwa	HR	SR	Chunhyang	S	S
Chogambaekok	HR	SR	Unknown 8	S	S
Daryang no.1	HR	SR	05M15	S	-
Julchamoe	HR	SR	05M28	S	-
Seonghwanchamoe	HR	SR	Acur Badem	S	-
05M41	HR	S	Busan no.912	S	-
05M42	HR	S	Busan no.928	S	-
Charentais Fom 2	HR	S	Busan no.951	S	-
Eunchoen	HR	S	Earlshappy	S	-
Gam	HR	S	Gamkwanilho	S	-
Gamro	HR	S	Giallo Canaria	S	-
Geumnodagieuncheon	HR	S	Iranmelon	S	-
Hong	HR	S	Papais	S	-
Icheon	HR	S	Superstar	S	-
Joseonchamoe	HR	S	Unknown 9	S	-
Korea no.18	HR	S	Unknown 10	S	-
Korea no.2	HR	S	Veedrantais	S	-
New melon	HR	MR	West	S	-
Oknaehyang	HR	S	Homrunstar	S	S

[z]Disease severity: HR=highly resistant, MR=moderately resistant, SR=slightly resistant, S=susceptible

Table 2. Fruit quality of ‘Earl’s elite’ (Muskmelon, *Reticulatus* Group) grafted onto different rootstocks.

Rootstock	Fruit weight (kg)	Fruit length (mm)	Fruit width (mm)	Soluble solid content (°Bx)	Net formaion[z]
K134068	1.4 ab	151.4 ab	136.4 ab	14.6 a	3.1 a
K134069	1.5 a	155.2 a	137.9 ab	13.8 a	3.5 a
Wondae	1.4 ab	151.4 ab	133.4 a	14.2 a	3.3 a
PI 414723	1.3 ab	147.3 ac	131.7 ab	14.2 a	3.9 a
Shintozwa	1.2 b	138.3 c	131.3 ab	14.8 a	3.5 a
non-grafted	1.2 b	144.1 bc	129.3 b	13.7 a	3.1 a

[z] Net formation : 1 good ~ 9 poor

Table 3. Fruit quality of ‘Homerunstar’ (honeydew type melon) grafted onto different rootstocks.

Rootstock	Fruit weight (kg)	Fruit length (mm)	Fruit width (mm)	Soluble solid content (°Bx)	Fermentated fruit (%)	Appearance rate[z]
K134068	1.5 a	158.5 a	134.8 a	16.0 a	6	4.3 a
K134069	1.4 ab	155.7 a	133.6 a	16.0 a	6	3.9 ac
Wondae	1.4 ab	155.6 a	130.1 ab	15.7 a	0	3.8 ac
PI 414723	1.5 a	160.3 a	135.1 a	15.8 a	0	4.2 ab
Shintozwa	1.3 b	150.8 a	126.8 b	16.2 a	81	3.5 c
non-grafted	1.3 b	153.1 a	126.4 b	15.9 a	6	3.6 bc

[z] Appearance rate : 1 poor ~ 5 good

Fusarium resistant screening and horticultural characteristics of Korean oriental melon (*Cucumis melo* L.) accessions

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Keywords: Melon, *Cucumis melo*, Korean melon, *Fusarium oxyporum*

Abstract

The incidences of *Fusarium* wilt in melon caused by *Fusarium oxysporum* f. sp. *melonis* (FOM) has markedly increased in some melon production area in the world. The foliage wilt by FOM causes decrease in yield of melon. The objective of this study was to screen *Fusarium* resistant genotypes in Korean melon germplasm and to investigate the horticultural characteristics of oriental melon. This investigation was on FOM resistant screening of 32 Korean melon accessions, carried out at the Plant Protection Research Institute in Adana, Turkey. Among Korean melon, accession No. 8, 21 and 27 had resistance to FOM race 0, 1, K1 (race 1) and K2 (race 2), showing over 75% survival ratio. Because accession No. 27 'Romance' was commercial F1 hybrid cultivar, it can be used directly for commercial cultivation. Since this experiment was accomplished with seedling inspection, field study with mature plants will be conducted. Horticultural traits as fresh weight and length and flesh thickness of oriental melon fruit were smaller than those of melon such as *reticulatus* and *inodorus* types.

INTRODUCTION

Melon (*Cucumis melo* L.) was originated from Africa and has a long history of cultivation in Africa and the Middle East. Korea has long history of cultivation for oriental melon. Oriental melon (*Cucumis melo* L. var. *makuwa* Makino) is an important fruit vegetable in Korea. The soil fungus *Fusarium oxysporum* f. sp. *melonis* (FOM) has caused Fusarium wilt of melon (Lee 1994). This fungus penetrates through the root system, invades the vascular elements and invokes wilting and plant death (Gordon and Martyn 1997). FOM isolates have been classified in four pathogenic races. Risser et al. (1976) established four physiological races of FOM, races 0, 1, 2, and 1, 2. The objective of this study was to screen the resistant

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accessions to FOM and determine some fruit characteristics among oriental melon germplasm of Korea.

MATERIAL AND METHODS

This study was carried out at Plant Protection Institute of Ministry of Food, Agriculture and Livestock and Department of Horticulture, Faculty of Agriculture University of Çukurova in Adana, Turkey. For screening experiments, seeds of 32 oriental melon and five melon accessions were sown at plastic box in May 18th and the roots (3-5 mm in length) of seedlings were inoculated by soaking with spore suspensions (10^6 spores/ml) of FOM race 0, 1, K1 (race 1 isolated from Korea) and K2 (race 2 isolated from Korea) in May 28th 2008. FOM K1 and K2 were isolated and cultured in Korea. Inoculated seedlings were transplanted in small plastic boxes filled with peat-moss. These boxes were placed in climate room which was set 18 ^{o}C during night, 25 ^{o}C during day and 12 hours light in a day. Samples were evaluated according to disease syptoms. The scoring was recorded by counting the number of dead or survived seedlings determined by observing the surface of secondary roots and the hand-sections of the vascular bundle of 10-20 plants per genotype. Thirty two genotypes were from National Horticultural Research Institute of Korea and five genotypes were from Çukurova University (originated INRA, France) (Table 1).

Horticultural characteristics were investigated at the Department of Horticulture, Faculty of Agriculture, University of Çukurova in Turkey. A total of 32 oriental melon germplasm and cultivars in this study were collected from Korea and evaluated for morphological characteristics. Five horticultural traits such as fruit weight (g), fruit length (cm), fruit diameter (cm), fruit thickness of outer layer of pericarp (cm) and soluble solid content ($^{\circ}$Bx) were measured to generate a quantitative data file. Seeds were sown in plug trays filled with perlite and peat (1:2) on May 11th, 2008. Twenty one days later, ten seedlings of each genotypes were transplanted into field condition under drip irrigation with spacing of 2 m x 0.5 m, between and within rows, respectively, on June 2nd 2008. The first harvest was on 5th August and the last harvest was on 12th August. Some morphological characteristics of fruits were determined according to a protocol of International Union for the Protection of New Varieties of Plants.

RESULTS AND DISCUSSIONS

Oriental melon accessions used in the screening for resistance to Fusarium wilt are shown in Table 1. The results of FOM screening are presented in Table 2. Accession No. 19 was not germinated in this study and melon accessions from No. 199 to No. 203 (Table 2) were used as controls to compare the activity of FOM fungus and the extent of resistance of oriental melon. The accession No.

203, 'Isabelle' (Table 1) is known to have resistance to Fusarium wilt (Villeneuve 2008). Among oriental melons, accession No. 8, 21 and 27 showed resistance to Fusarium wilt showing over 75% survival rate (Table 2 and Fig. 1). Accession No. 27, 'Romance' is a commercial F1 hybrid cultivar and, thus, this accession can be cultivated in FOM-contaminated field. It needs time for other accessions to be used as commercial breeding materials. This experiment was conducted with seedlings and further study using mature plants in field condition is needed to confirm consistency of this result and to investigate characteristics such as yield and quality of fruits in resistant accessions.

Such horticultural traits as fruit weight, length and flesh thickness of oriental melon fruits were smaller than those of melon such as *reticulatus* and *inodorus* types (Table 3 and Fig 2). The shape of oriental melon fruits were ellipse in shape compared to that of melon. The fruit shape of *reticulatus* type was globular. The skin and flesh of *chinensis* type were greenish and fruit sugar contents of chinensis type were lower than that of *makuwa* type.

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Table 1. Common names, types and seed sources of accessions.

Accession No.	Accessions	Seed source	Melon types*	Common name
1	05M40	Open Pollinated	*makuwa*	Oriental melon
2	05M41	Open Pollinated	*makuwa*	Oriental melon
3	05M42	Open Pollinated	*makuwa*	Oriental melon
4	05M28	Open Pollinated	*chinensis*	Oriental melon
5	Chunhyang	Open Pollinated	*makuwa*	Oriental melon
6	Euncheon	Open Pollinated	*makuwa*	Oriental melon
7	Meok	Open Pollinated	*makuwa*	Oriental melon
8	Gam	Open Pollinated	*chinensis*	Oriental melon
9	Icheon	Open Pollinated	*chinensis*	Oriental melon
10	Hong	Open Pollinated	*chinensis*	Oriental melon
11	Joseon	Open Pollinated	*chinensis*	Oriental melon
12	Gaeguri	Open Pollinated	*chinensis*	Oriental melon
13	Korea no.2	Open Pollinated	*chinensis*	Oriental melon
14	Korea no.18	Open Pollinated	*chinensis*	Oriental melon
15	Julchamoe	Open Pollinated	*chinensis*	Oriental melon
16	PMR Honeydew	Open Pollinated	*inodorus*	Melon
17	Unknown1	Open Pollinated	*inodorus*	Melon
18	V-3-7	Open Pollinated	*chinensis*	Oriental melon
19	New melon	Open Pollinated	*makuwa*	Oriental melon
20	Hwanggeum	Open Pollinated	*makuwa*	Oriental melon
21	Hwanggeum No.9	Open Pollinated	*makuwa*	Oriental melon
22	Daryang No. 1	Open Pollinated	*makuwa*	Oriental melon
23	Gamro	Open Pollinated	*chinensis*	Oriental melon
24	Unknown	Open Pollinated	*chinensis*	Oriental melon
25	B1	Open Pollinated	*makuwa*	Oriental melon
26	B2	Open Pollinated	*makuwa*	Oriental melon
27	Romance	F1 hybrid	*reticulatus*	Melon
28	Earl's Ace	F1 hybrid	*reticulatus*	Melon
29	Earl's Happy	F1 hybrid	*reticulatus*	Melon
30	Superstar	F1 hybrid	*inodorus*	Melon
31	Papaiseu	F1 hybrid	*inodorus*	Melon
32	Geumssaragi euncheon	F1 hybrid	*makuwa*	Oriental melon
199	Charantais Fom-1	Open Pollinated	*cantalupensis*	Melon
200	Charantais Fom-2	Open Pollinated	*cantalupensis*	Melon
201	Charantais T	Open Pollinated	*cantalupensis*	Melon
202	Margot	-	*cantalupensis*	Melon
203	Isabelle	-	*cantalupensis*	Melon

*Melon types were separated based on Yi et al. (2004).

Table 2. The effects of FOM race 0, 1, K1 and K2 on survival rate (%) of oriental melon seedlings.

AN*	0	1	K1	K2	AN*	0	1	K1	K2
1	0	0	0	100	20	83	100	7	57
2	23	0	0	0	21	87	86	89	100
3	0	0	0	0	22	67	71	14	9
4	0	0	0	0	23	56	70	79	59
5	0	0	0	11	24	6	9	13	0
6	100	7	15	0	25	17	0	21	11
7	0	0	0	0	26	33	0	0	23
8	100	100	100	100	27	86	66	62	90
9	0	0	0	80	28	13	0	0	0
10	7	77	31	14	29	3	0	0	6
11	0	0	0	0	30	0	0	0	86
12	73	67	83	10	31	0	0	0	0
13	32	10	3	74	32	0	0	0	12
14	73	77	0	0	199	0	0	0	0
15	50	52	63	0	200	0	0	0	0
16	0	0	0	0	201	0	0	0	100
17	0	0	0	0	202	0	0	0	100
18	76	0	0	0	203	100	100	100	100

*AN: Accession number.

Table 3. Horticultural characteristics of oriental melon accessions.

CN	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit flesh thickness (cm)	Sugar content (°Bx)
1	542.7±69.5	14.9±1	8.6±0.4	1.8±0.1	15.3±2.0
2	629.0±70.9	13±0.7	9.6±0.5	2.2±0.4	15.7±1.3
3a	615.7±65.1	15.5±0.7	8.8±0.5	2.1±0.2	18.0±1.6
3b	315.3±24.7	8.9±0.9	8.0±0.6	1.6±0.1	17.8±1.0
4	722.7±92.4	9.1±0.4	12.0±0.9	3.1±0.3	10.0±2.1
5	317.2±30.9	11.2±0.6	7.3±0.5	1.6±0.1	14.4±1.0
6	739.8±134.8	14.1±1.1	9.9±0.7	2.2±0.3	16.1±1.0
7	393.2±69.9	10.8±0.6	8.0±1.0	1.6±0.1	16.4±1.1
8	390.5±38.4	14.5±1.0	7.4±0.3	1.4±0.2	8.3±1.1
9	1044.0±147.3	20.4±1.1	10.1±0.6	2.5±0.3	7.4±0.7
10	702.3±248.2	13.2±2	10.1±1.1	1.9±0.3	9.3±0.9
11	539.5±138.4	14.9±1.5	8.6±0.8	1.9±0.1	8.9±1.1
12	390.5±70.5	11.8±0.8	7.6±0.6	1.7±0.2	11.3±1.1
13	856.0±60.8	17.8±1.1	9.5±0.3	2.2±0.2	8.3±0.8
14	635.7±41.0	14.4±1.1	8.9±0.5	2.1±0.2	8.6±2.0
15	21.3±3.7	3.8±0.2	3.4±0.2	0.4±0.1	6.3±0.3
16	864.0±125.3	15.3±2.1	10.8±1.9	1.9±0.6	5.3±2.1
17	2549.3±855.8	17.7±2.7	16.1±3.9	3.9±0.4	8.1±2.2
18	731.5±116.8	13.5±1.5	10.1±0.7	2.1±0.1	8.8±1.4
20	622.2±88.5	14.2±0.5	9.5±0.8	2.1±0.3	11.0±2.2
21	485.3±79.8	12.6±1.1	8.8±0.3	2.0±0.2	7.1±3.2
22	617.3±118.8	13.0±1.0	9.3±0.8	2.2±0.3	8.6±2.0
23	524.2±123.2	12.9±1.5	7.7±0.3	1.8±0.2	9.2±0.9
24	531.2±85.4	13.7±0.7	8.7±0.7	1.8±0.2	10.9±1.3
25	563±105.7	13.5±1.3	8.6±0.7	2.0±0.2	9.3±1.1
26	406±83.6	12.7±2.7	7.5±0.6	1.7±0.2	16.3±1.0
27	1323.2±144.7	13.0±0.9	14.2±0.6	3.3±0.2	8.7±0.9
28	1251.5±356.4	13.5±0.8	13.4±1.5	3.3±0.4	7.5±1.7
29	1810.5±206.1	14.9±0.5	15.5±0.8	4.1±0.2	9.7±1.9
30	2820.5±313.1	19.4±0.7	17.2±0.6	4.7±0.3	13.8±1.2
31	1743.2±326.9	20.4±2.6	15.8±4.7	3.5±0.5	11.7±1.8
32	493.5±72.6	13.5±1.9	7.7±1.2	1.9±0.3	13.8±0.7

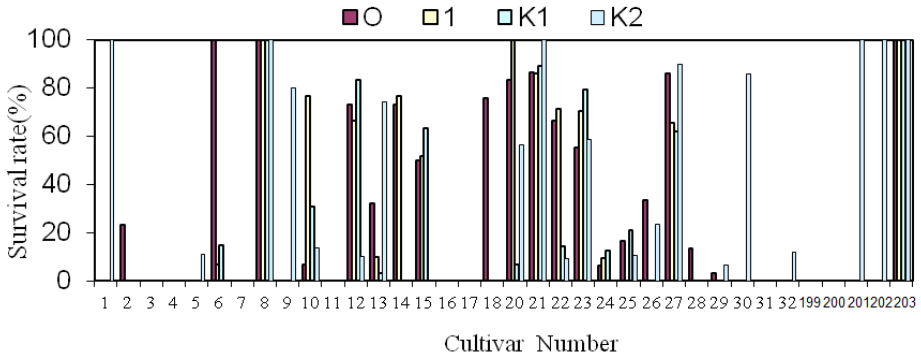

Fig. 1. The survival rate of oriental melon by infection of FOM race 0, 1, K1 and K2 seedlings.

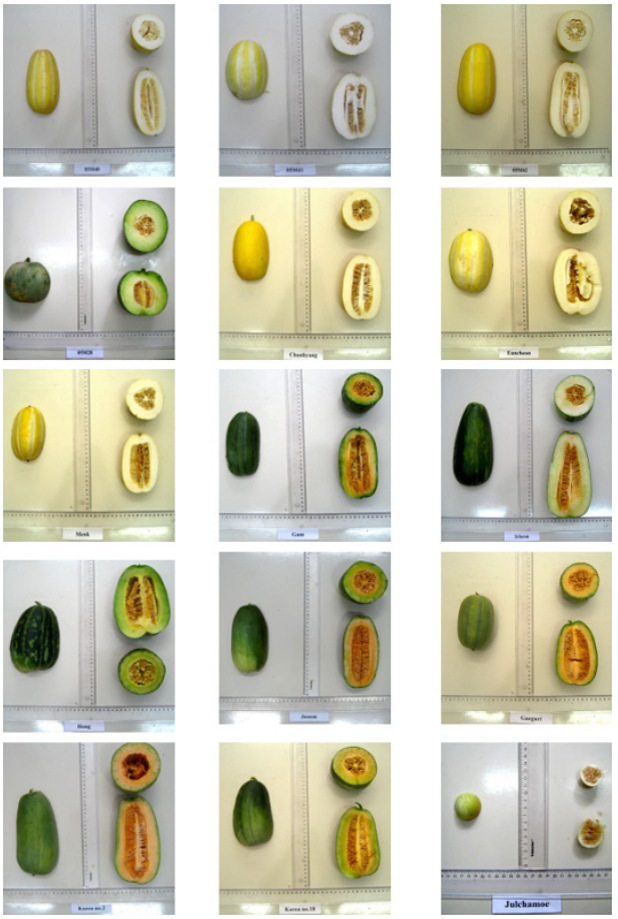

Fig. 2. The photographs of oriental melon fruits. The names of accession are 05M40, 05M41, 05M42, Chunhyang, Euncheon, Meok, Gam, Icheon, Hong, Gaeguri, Korea no. 18, Julchamoe, PMR Honeydew, Unknown1, V-3-7, Hwanggeum, Hwanggeum No.9, Daryang No. 1, Gamro, Unknown, B1, B2, Romance, Earl's Ace, Earl's Happy, Superstar, Papaiseu, and Geumssaragi Euncheon. The length of ruler is 50 cm.

Fig. 2 continued.

Differential response of *Cucumis melo* L. to *Fusarium oxysporum* f.sp. *melonis* race 1. 2

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Keywords: Melon, Fusarium wilt, race-specific resistance, artificial inoculation.

Abstract

Fusarium wilt incited by *Fusarium oxysporum* f.sp. *melonis* (FOM) causes important losses in melon crops world wide. Four physiological races have been identified namely 0, 1, 2 and 1.2. Race 1.2 was further subdivided in pathotype Y causing plant yellowing, and pathotype W that induces plant wilting and death without previous yellowing. In most of the cases the resistance to race 1.2 has been described as recessive, polygenic, and not race specific. However, some evidences on race specific effects within resistance to FOM race 1.2 have been reported. In this work we study these effects and try to assess whether they are due to a race specific resistance in 14 melon accessions having some level of resistance to FOM race 1.2, as well as from 'Charentais Fom-1' (resistant to races 0 and 2), 'Charentais Fom-2' (resistant to races 0 and 1), and 'Dinero F_1' (with partial resistance to FOM race 1.2). Melon seedlings of these accessions were artificially inoculated with six FOM isolates (3 from pathotype Y and 3 from pathotype W) using 'tray immersion' procedure. 'Kogane Nashi Makuwa', 'BG-5384', 'Shiro Uri Okayama', 'C-211', and the control 'Dinero F_1' showed a high level of resistance to all the FOM isolates. However some genotype x isolate effects were also detected. 'Baza' when inoculated with isolate FOM 9302, and 'Korça when inoculated with FOM *37mls.1.2W* showed a resistance level similar to 'Dinero F_1', but not when they were inoculated with other isolates. These results are characteristics for race-specific resistances and might confirm the presence of such a resistance to FOM race 1.2 in melon.

INTRODUCTION

Melon Fusarium wilt, induced by the soil borne fungus *Fusarium oxysporum*

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f.sp. *melonis* (L&C) Synd. & Hansen (FOM), is one of the major biological threats to melon crops around the world (González-Torres et al. 1994) and is one of the most difficult to control. Four physiological races (0, 1, 2 and 1.2) of FOM have been identified based on their reaction to a set of differential genotypes (Risser et al. 1976). Resistance to races 1 and 2 is conferred by the single dominant genes *Fom-2* and *Fom-1*, respectively. Both genes also confer resistance to race 0 (Risser et al. 1976). Recently, Oumouloud et al. (2010) described a recessive resistance to races 0 and 2, controlled by *fom-4* in melon cv. 'Tortuga'.

Partial resistance to race 1.2 was shown to be polygenic and not race-specific (Perchepied et al. 2005; Chikh-Rouhou et al. 2011), while in the breeding line 'BIZ', two recessive genes were shown to confer full resistance (Herman and Perl-Treves 2007). However, Perchepied and Pitrat (2004) reported small race-specific effects on quantitative and polygenic resistance.

This work examines the reaction of 14 melon accessions that have been previously reported as having some level of resistance to FOM race 1.2 (Chikh-Rouhou et al. 2011) when inoculated with 6 different race 1.2 isolates, and tries to assess if a differential response to FOM race 1.2 may be due to race-specific effects

MATERIALS AND METHODS

The plant material used in these experiments included 14 melon accessions that have shown some level of resistance to FOM race 1.2 in previous experiments (Chikh-Rouhou et al. 2011), together with the differential genotypes: 'Charentais-Fom-1', which is resistant to FOM races 0 and 2; 'Charentais-Fom-2', which is resistant to races 0 and 1; and the hybrid F_1 'Dinero' (obt. Syngenta) which carries the genes *Fom-1* and *Fom-2* and is resistant to both pathotypes of race 1.2.

FOM isolates *37mls*, *9302*, and *0502*, belonging to the pathotype 1.2 W, and *0501*, *0125*, and *24ml*, belonging to the pathotype 1.2 Y, were used for inoculum preparation following Chikh-Rouhou et al. (2011). All the FOM isolates were collected by Dr. González-Torres in infected melon fields of Almeria and Murcia (SE of Spain).

Fungi were grown in potato dextrose broth on a rotary shaker for 10 days at room temperature. Conidia were harvested by filtration through an autoclaved nylon mesh. Spore concentration was determined using a haemocytometer and adjusted to the appropriate density by diluting with sterile distilled water.

Trays containing ten-day-old melon seedlings growing in sand were dipped into 'Hoagland' nutrient solution containing a suspension of conidia (3×10^6 spores / mL). The trays were then placed in a growth chamber with 14 hours of light per day. Light intensity was 1,300 µEinstein/m^2 s, and temperature was maintained at 26/20 °C (day/night). Ten to fifteen plants were used for each accession–isolate combination.

For the statistical analyses, the values of the area under the disease progress curve (AUDPC) were used. The AUDPC integrates both the intensity of symptoms and the time taken between inoculation and expression of symptoms. The AUDPC was calculated according to the formula proposed by Perchepied and Pitrat (2004): $AUDPC = \sum_i[(x_i + x_{i+1} - 2) / 2] (t_{i+1}-t_i)$, with i = scoring period 1 to 4 , x_i = mean of the symptom scores for disease, and $t_{i+1} - t_i$ = the number of days between scoring date i and scoring date i + 1.

RESULTS AND DISCUSSION

'Kogane Nashi Makuwa', 'C-211', 'BG-5384' and 'C-40' have been described as having a partial and race non specific resistance to FOM race 1.2 (Risser and Rode 1973; Chick-Rouhou et al. 2008), and they showed a high level of resistance in this experiment whatever the FOM isolate used for inoculation (Table 2), supporting the statement of Pitrat et al. (1996) that many of the resistance sources to FOM race 1.2 come from the Far East.

However some genotype x isolate effects could be detected in our experiment (Table 1). 'Baza' inoculated with isolate *9302* showed a level of resistance similar to that of the resistant control 'Dinero', but 'Baza' was more susceptible than 'Dinero' when inoculated with the other isolates. Similarly 'Korça' behave as resistant as 'Dinero' only when inoculated with isolate *37mls* (Table 2).

Perchepied and Pitrat (2004) reported some minor race-specific effects of resistance to FOM race 1.2, that may account for the results presented here. Moreover, within the FOM isolates that overcome the resistance controlled by *Fom-1* and *Fom-2* may exist genetic differences that have not been detected yet.

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Table 1. Sum of squares, F values and probabilities for a two factor (isolate, and accession) variance analysis.

Sources	Degrees of freedom	Sum of squares	F	P [x]
Model	101	90754.359	27.954	S
Accession	16	93150.976	181.143	S
Isolate	5	1343.130	8.358	S
Isolate x Accession	80	17805.56	6,925	S
Error	1727	268101,402		
Total	1734	471155.430		

[x] S: Significant, $P \leq 0.05$; ns: Not significant, $P \leq 0.05$

Table 2. AUDPC mean values ± SD, for different melon accessions inoculated with six *Fusarium oxysporum* f.sp. *melonis* isolates.

Accession	**FOM isolate(*)**					
	37mls (1.2W)	*9302* (1.2W)	*0502* (1.2W)	*0501* (1.2Y)	*0125* (1.2Y)	*24mls* (1.2Y)
F_1 Dinero	0.00 ± 0.0 a	3.50 ± 2.0 a	0.00 ± 0.0 a	0.00 ± 0.0 a	11.87 ± 20.3 ab	7.00 ± 6.0 a
C-211	4.81 ± 7.5 ab	6.70 ± 4.5 ab	17.23 ± 6.3 cd	5.83 ± 3.7 a	28.54 ± 22.3 def	9.86 ± 5.6 ab
C-40	6.14 ± 6.3 ab	21.40 ± 5.0 abc	12.65 ± 9.7 cd	4.66 ± 4.7 a	0.77 ± 2.3 a	5.38 ± 9.1 a
Korça	7.87 ± 5.6 ab	33.92 ± 8.2 ghi	10.76 ± 5.6 bcd	25.84 ± 5.8 cde	22.22 ± 9.9 bcd	23.33 ± 6.3 cd
BG5384	10.16 ± 2.0 ab	12.60 ± 7.8 abc	0.00 ± 0.0 a	15.45 ± 8.2 b	13.02 ± 6.7 abc	5.25 ± 4.5 a
Kogane Nashi Makuwa	11.31 ± 14.8 ab	7.80 ± 4.3 ab	34.41 ± 10.7 cd	14.63 ± 4.9 b	2.62 ± 6.0 a	21.26 ± 10.4 cd
NC44082	12.92 ± 8.2 ab	22.75 ± 5.1 def	12.11 ± 4.4 cd	22.75 ± 6.5 bc	18.42 ± 5.4 bcd	21.80 ± 7.3 cd
Charentais Fom-1	13.00 ± 6.1 ab	23.18 ± 5.5 def	2.33 ± 5.7 ab	17.00 ± 5.5 bc	31.17 ± 13.3 def	23.00 ± 7.0 cd
Amarillo Oval Tardio	13.16 ± 5.3 ab	14.80 ± 9.2 bcd	17.23 ± 4.3 cd	22.45 ± 8.8 bc	18.75 ± 8.0 bcd	19.25 ± 8.0 cd
C-181	14.22 ± 16.6 ab	18.66 ± 7.9 cde	9.69 ± 6.4 bc	19.54 ± 5.8 bc	38.57 ± 13.7 ef	16.15 ± 4.1 bc
Mollerusa	15.08 ± 5.2 ab	25.77 ± 8.6 efg	17.20 ± 11.6 cd	22.75 ± 7.2 bc	22.28 ± 13.9 bcd	21.38 ± 5.6 cd
Mochuelo	16.50 ± 17.2 ab	34.12 ± 3.9 ghi	18.8 ± 4.4 cd	26.09 ± 8.3 cde	16.45 ± 9.1 bcd	19.65 ± 5.2 cd
Encin4078	18.00 ± 19.1 ab	39.84 ± 5.6 i	21.00 ± 7.8 cd	28.58 ± 7.4 de	39.80 ± 9.8 ef	18.84 ± 6.4 cd
CA13111C	19.09 ± 12.7 ab	31.50 ± 10.0 fghi	18.25 ± 7.1 cd	33.54 ± 6.2 e	17.13 ± 7.0 bcd	25.66 ± 5.2 cd
Charentais Fom-2	20.00 ± 16.4 ab	36.75 ± 4.2 hi	19.25 ± 4.5 cd	22.75 ± 10.7 bc	37.27 ± 8.7 ef	27.56 ± 7.8 d
Baza	20.90 ± 21.7 ab	11.66 ± 5.2 abc	14.00 ± 6.1 cd	27.12 ± 7.1 de	42.50 ± 12.2 f	22.88 ± 5.4 cd
Rajado	25.03 ± 18.5 b	30.62 ± 7.7 fgh	19.92 ± 3.6 cd	22.75 ± 3.1 bc	27.63 ± 11.8 cde	22.07 ± 5.0 cd

(*) For each accession means followed by different letters are significantly different according to Tukey b test b ($P \leq 0.05$).

Conservation, characterization and evaluation of melon genetic resources carried out by Vegetable Research and Development Station Bacau, Romania

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Keywords: *Cucumis melo,* local population, quality

Abstract

Our study starts from the urgent problem of continuing loss of biodiversity recorded to absolutely all plant species, including *Cucumis melo* L. A large collection of melons is held at the Vegetable Research and Development Station, Bacau. The main objective of this study was (1) to save endangered precious resources, by collecting local native populations and old - primitive varieties, under risk in order to reduce genetic erosion and save these materials in order to be used in breeding, (2) to evaluate accessions in order to keep and introduce the valuable material in breeding programs. This study presents information on a few collected accessions of *Cucumis melo* from North East Moldova region of Romania. Characterization and evaluation of morphological plant characteristics of *Cucumis melo* genotypes were accomplished (plant height, fruit length, fruit diameter, shape index, fruit weight, seed weight, flesh weight). Fruit quality and consumer acceptability in melon are strongly related to the concentration of soluble solids content and titratable acidity, accumulation of water and total dry matter. Flesh color is another important feature taking into account when assessing fruit quality. Some promising genotypes were identified with superior plant characteristics, such as fruit size, fruit weight, early maturation and high content of soluble solids. The promising genotypes which have superior important plant characteristics were successfully utilized in breeding programs that are aimed to improve the quality and the yield of species.

INTRODUCTION

In Romania, muskmelon represents a highly appreciated annual species and is cultivated especially in Southwest Plain of the country, the Danube Plain and Dobrogea. In the past 20 years, an expansion of protected culture was reported. The aim of this research was to conduct a complex study on conservation and utilization

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of melon resources. The intensive activity of genetic improvement and also the use of technological development of horticultural inputs, have lead to the replacement of many cultivars (Gupta 2010). Cultivar replacement is reported to be the main cause of genetic erosion around the world. The present study envisages identifying the quality profile of biological material in order to reintroduce old cultivars, in local cultivation systems, including their commercialization on small niche markets, and their exploitation in breeding programs.

MATERIALS AND METHODS

The experiments presumed two different stages: (1) collection and conservation of biological material (2) evaluation of accessions in order to characterize and evaluate the resources for introduction in breeding programs.

The biological material consisted on *cantalupensis* and *inodorus* melons. *Cantalupensis* type includes melons with deep ribs outside, either a smooth pericarp or a suber net. The fruits have strong fragrance and flavor. *Inodorus* are known as winter melons and are clearly distinguishable due to the absence of flavors.

Collecting and conservation activities prioritize autochthonous plant germplasm. Our country has an enormous genetic diversity but unfortunately due the chaotic development in the last decades and lack of founds regarding conservation of genetic resources, important losses were recorded in all species. Despite these inconveniences conservation activities are developed in research stations (with the purpose to use the collected material in breeding programs) and in the Gene Bank of Romania. Long, medium and short term conservation is ensured through conventional storage facilities, such as fields, glasshouses, as well as seeds chambers.

Characterization of accessions: entering in our collection, all material is endowed with passport descriptors, such as genus, species, accession name, collecting site and date. There were accomplished phenological observations and biometrical measurements.

Physiological investigations were performed on mature fruits. The physiological changes concerned respiration rate, endogenous ethylene content, flesh conductibility, total dry matter, water and minerals, soluble dry matter, titratable acidity and β carotene. Respiration intensity ($mgCO_2$/kg/hour) was assessed in the detached fruits by measuring the CO_2 volume using RIKEN analyzer.

The endogenous ethylene content was determined by GS 9000 series. Ten ml of gas were analyzed from the seminal cavity using plot fused silica 10 m x 0.53 mm coated with carboplot P7, DF=25.0. The ethylene content was expressed in ppm.

The electrical conductibility was tested in four different fruit areas (peduncle, apical, near skin, and near seminal cavity). The tissue conductibility was expressed in µS/cm.

The determination of total dry matter substance was carried out by weighing the fresh vegetal material, drying it for 24 hours at 105°C, cooling it outside and then weighing again the dry vegetal material. The obtained results were expressed in percentage. The difference till 100% represents the water content.

The soluble dry matter content was determined using refractometer method and the results expressed as a percentage.

The most important pigment in melon flash, responsible for orange color (β carotene), was extracted in petrol ether and determined using a spectrometer at λ=415 nm. The content of β carotene was expressed in mg/100 g.

RESULTS AND DISCUSSION

The intensity of the respiration process reflects the degree of biodegradation that occur in melons fruits. Respiration is used as an indicator for assessing the maturity, the physiological state of tissues or biodegradation rate of reserve substances. Cantaloupe melons have a higher respiratory intensity, indicating higher rates of biodegradation. For this reason it is recommended to consume it soon after harvest. At *inodorus* melons climacteric peak may occur much later than any of the other *cantalupensis* melons, or absent altogether. Low intensity values obtained at *inodorus* melons respiration is similar with a longer storage of these melons.

Melons are climacteric fruits, characterized by increased ethylene synthesis during ripening process. The highest average content of endogenous ethylene was in *cantalupensis* melons being 5.03 times higher than in *inodorus* melons. In *cantalupensis* melons endogenous ethylene content ranged from 19.13 ppm to 29.56 ppm. At *inodorus* melons climacteric may lack or may occur much later recorded the lowest endogenous ethylene content: 4.82 ppm. Variation of endogenous ethylene content for this group of melons was from 4.12 ppm to 5.35 ppm. Between endogenous ethylene content and intensity of fruit respiratory was found a highly significant correlation.

Study of flash conductibility has a particular importance in assessing the optimal timing of harvesting, transport and estimation of duration of storage life of melon fruit. Increase of flash conductivity is the result of plasma membrane damage. During maturation melon flash become more vulnerable to injuries. The electrical tissues conductivity of cantaloupe melons at full maturity was: 1,322.50 μS/cm. *Cantalupensis* melons had a faster maturation rate, flesh texture was very juicy and required special attention during handling in order not to cause damage that would lead to deterioration of fruit quality. Due to low electrical conductivity *inodorus* melons were suitable to postharvest and allowed good flavor retention properties after one month storage at a temperature of 20-22 °C. During the storage the value of electrical conductivity increased slightly. At harvest moment conductivity of *inodorus* melons was 1.65 times lower in comparison with cantaloupe melons. The

water content of fruit varied inversely with total solids content and ranged from 88.91% at *cantalupensis* melons to 90.54% at *inodorus* melons.

Soluble dry matter content is an index of physiological importance in determining the quality of melon fruit. In addition to flavor, fruit sweetness is one of the first quality indexes of consumers. A highly significant positive correlation was found between soluble solids content and total solids content. *Inodorus* melons registered 1.02 times smaller content of soluble solids than cantalupensis genotypes. Melon fruit acidity is an index with a particular importance in determining taste. It was found a decrease of acidity titratable value with maturation.

Flesh color and uniformity are important characteristics for evaluating the quality of melon fruit. The flesh predominant pigment is β-carotene (Vavich and Kemerer 1950). We registered a varied pigmentation from white, greenish white, dull white, yellow to deep orange depending on the amount of carotene in melon fruits.

Cantalupensis fruits have the most intense process of respiration, a high content of endogenous ethylene, and the highest level of tissue conductivity, they are recommended for use immediately after maturation. Analyzed genotypes of *inodorus* group registered the lowest respiratory intensity and endogenous ethylene content so the fruits are suitable to postharvest and storage over a long period.

ACKNOWLEDGEMENTS

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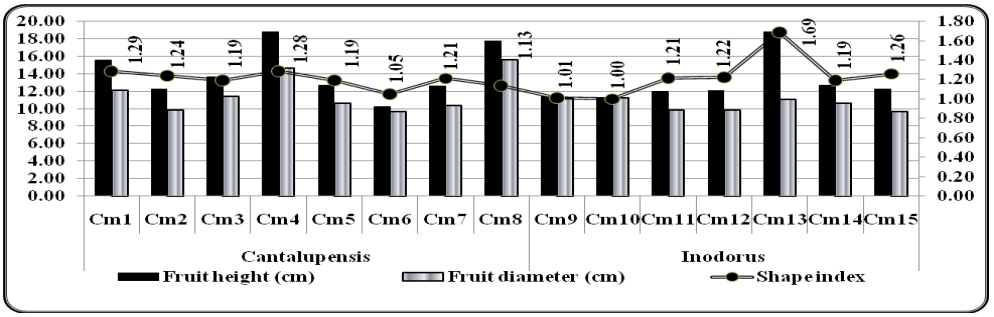

Fig.1. Fruit length and diameter.

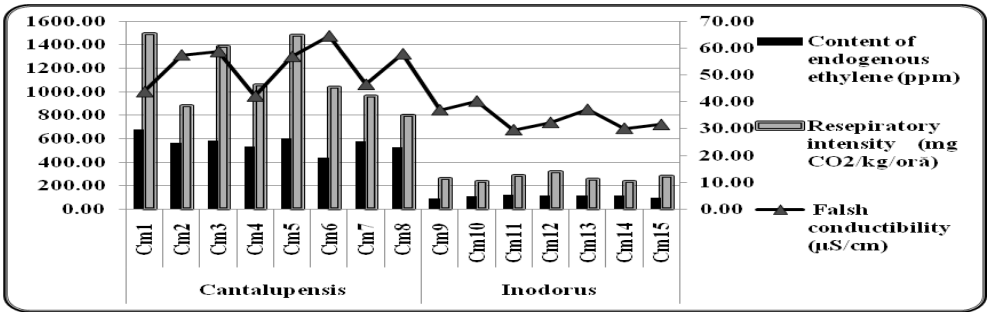

Fig. 2. Respiration intensity (mg CO2/kg/hour), flash conductibility (µS/cm) and endogenous ethylene content (ppm).

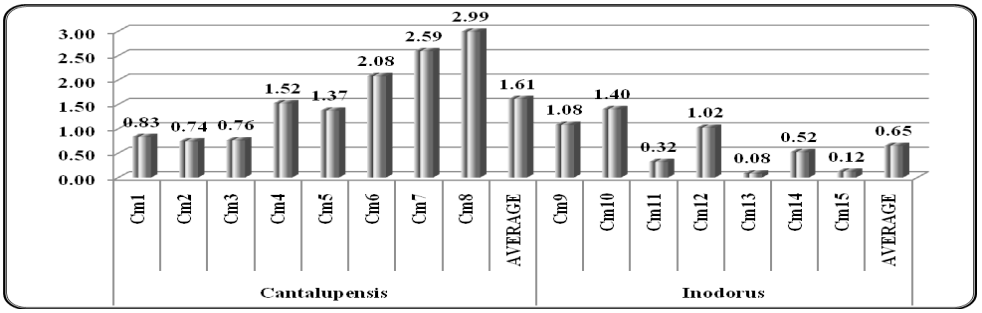

Fig. 3. Variation of β carotene content (mg/100g).

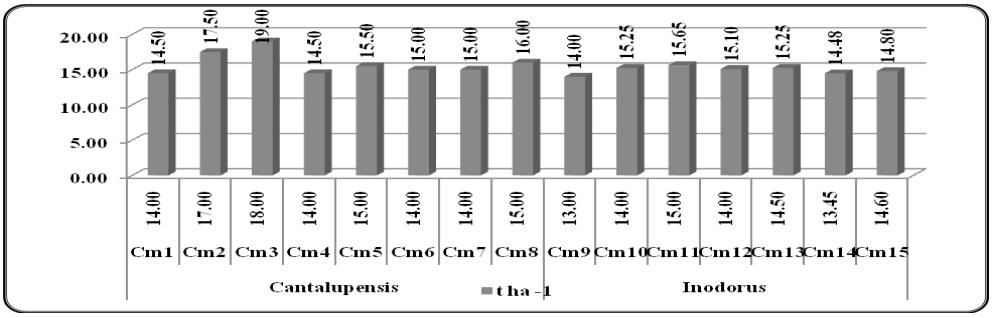

Fig. 4. Variation on yield (t ha $^{-1}$).

Studies on genetic relationship analysis and purity identification of hybrids of melon cultivars by SRAP markers

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Keywords: SRAP, melon, genetic relationship analysis, hybrid seed purity

Abstract

Genetic relationship analysis of twelve melon cultivars and the identification of hybrid seed purity of a melon cultivar were analyzed with SRAP technology. Our results show that 13 out of 40 SRAP primers were screened and 78 polymorphic bands were amplified, with a polymorphic band percentage of 50%. Clustering analysis showed that the value of genetic variation indexes of 12 melon cultivars based on the SRAP data varied from 0.263 to 0.921, and they could be classified into 2 groups and 5subgroups by UPGMA phylogenetic analysis. Primers Me15-Em7 and Me3-Em16 were selected for SRAP identification of 100 single seeds of 'Honglvzaocui', and the purity was 77%. This study would provide information for genetic relationship analysis and identification of hybrid seed purity of melon cultivars.

INTRODUCTION

Melon (*Cucumis melo* L) is a kind of fruit with good color, smell and taste, which is cultivated widely in China and abroad. The melon germplasm resources and variation type are extremely rich; germplasm resources are the basis of breeding and biological research. The hybrid genetic authenticity is an important indicator of seed quality, which directly affects melon production and quality. Therefore, the study of melon genetic relationships and hybrid seed identification have important application value. There are already some reports on the identification of melon genetic relationships and hybrid seed purity tests, mainly using RAPD, ISSR, RFLP and SSR, while a few based on SRAP markers (Liu et al. 2002; Baudracco-Arnas and Pitrat 1996; Katzir et al. 1996). SRAP (sequence-related amplified polymorphism), a PCR-based marker system described by Li and Quiros (2001), applies unique primers for DNA amplification and polymorphism detection. (Yang et al. 2009). In this study, upon the research of 12 domestic and foreign melon cultivars, we explore the validity of the SRAP marker system for studying genetic relationships and seed

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purity in this species; on the other hand, we determine the genetic relationships of the melon cultivars and provide a reference for the melon germplasm collection and preservation.

MATERIALS AND METHODS

Materials

Melo cultivars for genetic relationship analysis and "Honglvzaocui" melon seeds for seed purity tests were provided by the Horticultural Research Institute Shanghai Academy of Agricultural Sciences (Table 1).

Reagents

Reagents for DNA extraction, PCR and agarose were purchased from Shanghai Sangon Biological Engineering Co., Ltd. The primers used are listed in Table 2.

DNA extractions

The genomic DNA was extracted from leaf tissue using a modified CTAB protocol (Zhang et al. 2007). DNA was quantified by electrophoresis in 1% agarose gels, and diluted to 10 ng·μl^{-1} with 1×TE buffer and stored at 4℃ or -20℃ for further use.

PCR amplification (SRAP)

Each PCR reaction mixture of SRAP (total volume is 20 μL) consisted of 2.0 μl of 25 mmol·L^{-1} $MgCl_2$, 2.0 μl of 10 × PCR buffer, 0.4μl of 10 mmol·L^{-1} dNTPs, 0.2 μl of 5 U·μl^{-1} Taq DNA polymerase, 3.0 μl for each of 0.1 μmol·L^{-1} primers, 3.0 μl of 10 ng·μl^{-1} DNA, and 6.4 μl of sterilized double distilled water. The PCR amplification was performed on a thermal cycler (PTC-100) with an initial denaturation at 94℃ for 5 min, and 5 cycles of 1 min denaturation at 94℃; 1 min annealing at 35℃, 1.5 min extension at 72℃; and 35 cycles, 94℃ for 1 min, 50℃ for 1 min and 72℃ for 1.5 min. A final extension was set up at 72℃ for 10 min (Ma 2008). PCR products were separated in 2.5% agarose gels (containing ethidium bromide) at 120 V for 1.5h. The EQ 170-8060 gel imager of Gel Doc™ was used for observation and image analysis.

RESULTS AND ANALYSIS

DNA extraction results and detection

The agarose gel electrophoresis detected that genomic DNA was of high purity, which met the SRAP technical requirements (Fig. 1).

SRAP primer selection and polymorphism analysis

13 out of 40 pairs of primer combinations gave clear and reproducible

amplification patterns. 39 out of 78 reproducible bands amplified using these 13 pairs of primer combinations were polymorphic in the 12 melon cultivars, with an average polymorphism rate of 50%, The average of bands amplified with each primer combination was six, which indicates that the SRAP could detect more genetic loci and improve polymorphism detection. The presence or absence of bands was scored as binary code (1 and 0). Figure 2 shows a SRAP-PCR amplification result with Me2-Em8 and Me15-Em12 primer pairs in the 12 melon cultivars.

Genetic relationship and cluster analysis

The genetic similarity coefficient between pairs of varieties based on Dice similarity within the cultivars was evaluated (Table 3). The average genetic similarity coefficient (S_{ij}) among all 12 cultivars was 0.592, ranging between 0.263 and 0.921, which indicated that a considerable genetic diversity existed in the cultivars and their genetic relationships were complex. The highest S_{ij} (0.921) between No. 2 and 3 showed less genetic variation and close genetic relationship, while the lowest S_{ij} (0.263) was observed between No.1 and 11, suggesting a remarkable genetic variation.

A dendrogram (Fig. 3) using UPGMA based on the similarity coefficient divided the 12 melon cultivars into five groups (S_{ij} is 0.67). Group 1 contained 6 cultivars (No. 1, 2, 3, 4, 5); group 2 contained 1 cultivar (No. 12); group 3 contained 4 cultivars (No. 6, 7, 8, 9); group 4 contained 1 cultivar (No.10); group 5 contained 1 cultivar (No. 11). It was found that cultivars from the same breeding institute were clustered together, suggesting closer genetic relationships.

SRAP polymorphism analysis of melon hybrid combinations and its parental lines

In order to study if the SRAP markers could be used for the identification of hybrid purity of melon, this experiment adopted the best polymorphic 13 pairs of primers to amplify the “Honglvzaocui” hybrid and its parental lines. The result showed that four pairs could generate polymorphism between F_1 and parental samples, Me15-Em7, Me3-Em16, Me2-Em7 and Me14-Em9. Figure 4 shows the amplification result of primers Me15-Em7 and Me3-Em16. As the arrow indicates, the female parent lacks a specific band present in the male parent; while the band can be generated on “Honglvzaocui” hybrid seeds, which allows to separate hybrids from the female parent. Taking advantage of these two primer combinations, it was easy to perform “Honglvzaocui” hybrid purity testing in 100 seed, showing that the hybrid seeds purity was 77%, while the field morphological identification result was 80%. Identification of SRAP polymorphism and the field morphological in line with the rate of 96.25%.

CONCLUSION AND DISCUSSION

SRAP markers should be used in plant genetic diversity analysis, genetic linkage mapping, germplasm identification, gene mapping and comparative genomics and other areas. Ferriol et al (2004) illustrated that the polymorphism detected was 66.2% using 11 SRAP primer combinations and 47 pumpkin accessions. Wang et al (2004) built a molecular genetic linkage map of cucumber with SRAP technology and mapped important agronomic traits as Lateral Branch and First Flower Node. Yu et al (2005) used the SRAP-labeled technology for the molecular identification of 22 tomato varieties. It was found that 1 pair of SRAP primers resulted in eight polymorphic loci. In this study, SRAP markers technology was used for genetic relationship analysis in melon. 13 pairs of polymorphic primer combinations were selected. Seventy-eight bands were amplified in 12 melon cultivars, 39 of which were polymorphic The average similarity coefficient was 0.592. The 12 melon cultivars were divided into 5 groups with UPGMA cluster analysis while the similarity coefficient was 0.67. The results showed that the genetic relationship depended mostly on the plant material and its sources, indicating that SRAP markers could reflect the genetic relationship and genetic differences between different varieties more accurately at the molecular level.

Due to not timely isolation strict, artificial emasculation, or other reasons, mixing with the female parent inbred line seed during hybrid production process often happens, which will produce a serious loss. Therefore it is necessary to test purity before packaging. Melon hybrid purity identification has been reported, but previous works used SSR and RAPD (Ai et al. 2005; Liu et al. 2006; Li et al. 2008). This work discusses the SRAP marker for identification of purity of melon hybrids, on the basis of genetic analysis using 13 primer combinations on the "Honglvzaocui" seeds and its parental lines. It was found that there were 4 primer combinations that were polymorphic between the parents with good reproducibility. This work illustrates that SRAP markers are efficient and reliable for identification of the purity of melon hybrids.

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Table 1. Source and names of the plant material.

No.	Material name	Source	No.	Material name	Source
1	04-38	Independent breeding	7	09-1-3	Shandong, China
2	QJ1	Independent breeding	8	09-2-1	Shandong, China
3	A01-1	Independent breeding	9	09-2-2	Shandong, China
4	M09-14	America	10	M09-7	America
5	A01-2	Independent breeding	11	M09-13	America
6	09-1-1	Shandong, China	12	04-39	Independent breeding

Table 2. Sequence of SRAP primers used in this study.

No.	Sequence	No.	Sequence
Me2	5’TGAGTCCAAACCGGAGC-3’	Em7	5’GACTGCGTACGAATTCAA-3’
Me3	5’TGAGTCCAAACCGGAAT-3’	Em8	5’GACTGCGTACGAATTCTG-3’
Me13	5’TGAGTCCAAACCGGTGT-3’	Em9	5’GACTGCGTACGAATTGAT-3’
Me14	5’TGAGTCCAAACCGGTCA-3’	Em12	5’GACTGCGTACGAATTGTC-3’
Me15	5’TGAGTCCAAACCGGTAC-3’	Em13	5’GACTGCGTACGAATTGGT-3’
		Em14	5’GACTGCGTACGAATTCAG-3’
		Em15	5’GACTGCGTACGAATTAGC-3’
		Em16	5’GACTGCGTACGAATTCGG-3’

Table 3. Genetic variation indexes of the 12 melon cultivars.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.868	1										
3	0.868	0.921	1									
4	0.684	0.763	0.737	1								
5	0.684	0.711	0.711	0.684	1							
6	0.579	0.658	0.579	0.632	0.579	1						
7	0.605	0.658	0.658	0.605	0.605	0.816	1					
8	0.447	0.500	0.500	0.553	0.605	0.658	0.789	1				
9	0.605	0.684	0.711	0.711	0.711	0.737	0.789	0.737	1			
10	0.447	0.526	0.526	0.553	0.657	0.500	0.605	0.658	0.684	1		
11	0.263	0.316	0.395	0.342	0.447	0.447	0.421	0.553	0.579	0.605	1	
12	0.684	0.711	0.658	0.526	0.632	0.579	0.632	0.526	0.605	0.605	0.316	1

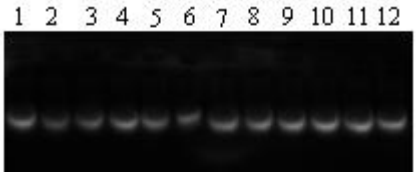

Fig.1. Agarose gel electrophoresis of total DNAs extracted from melon cultivars.

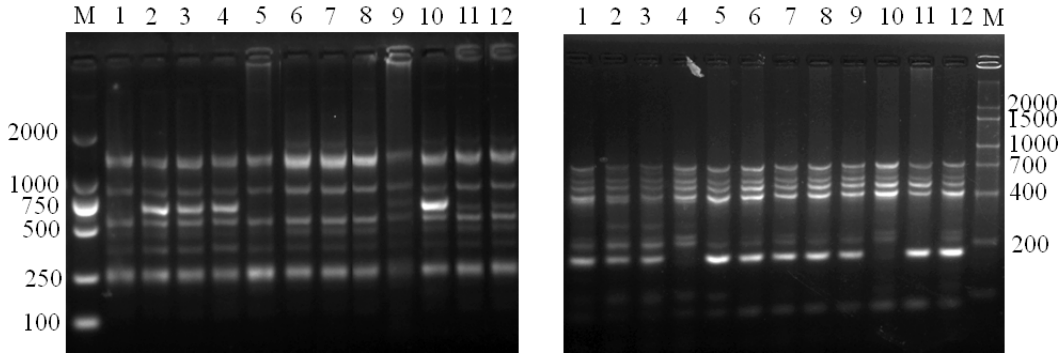

Fig. 2. SRAP-PCR amplified patterns obtained with primers Me2- Em8 (A) and Me15- Em12 (B) in 12 melon cultivars.

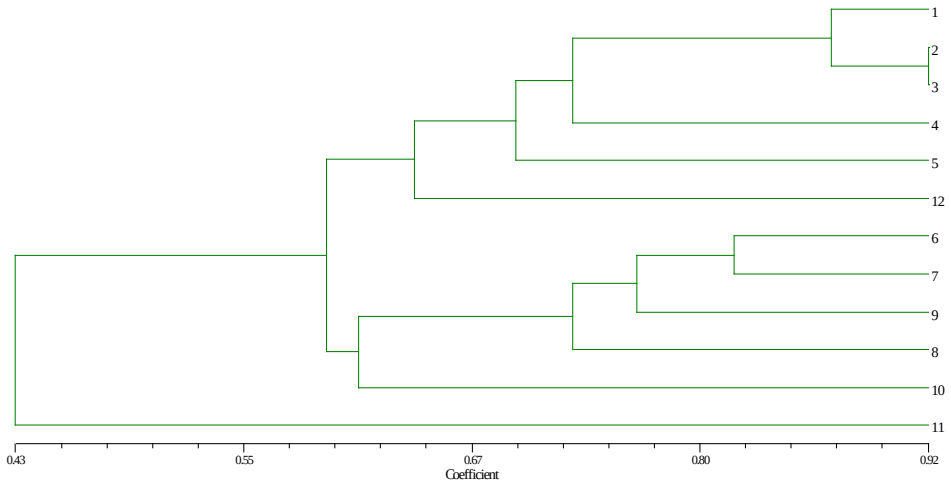

Fig. 3. Dendrogram of 12 melon cultivars obtained using the UPGMA method.

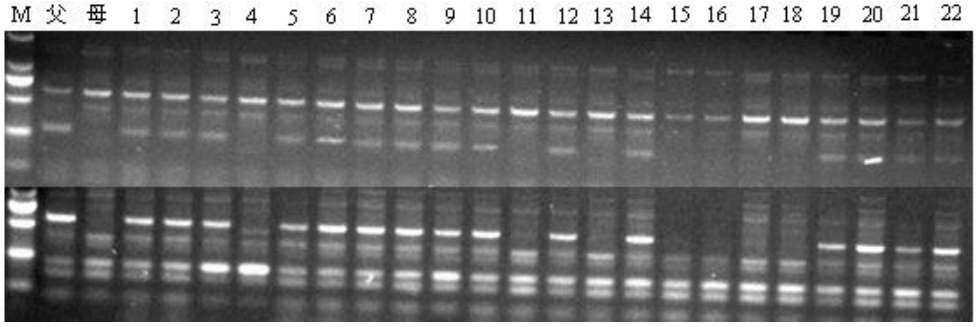

Fig. 4. SRAP profile of the melon hybrid “honglvzaocui” amplified by Me15-Em7 (A) and Me3-Em16 (B) primer pairs.

Notes: 1-22, hybrid seeds; 4, 11, 13, 15, 16, 17 and 18 represent female parent inbred lines

Determination of resistant local varieties to Fusarium wilt *(Fusarium oxysporum* f.sp. *melonis)* with molecular markers

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Abstract

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis* (*F.o.m*), is a soil-borne disease of melon (*Cucumis melo* L.). *Fusarium oxysporum* f. sp. *melonis* (F.o.m) causes serious economic losses in melon.Two dominant resistance genes have been identified, *Fom*-1 and *Fom*-2, which provide resistance to races 0 and 2, and races 0 and 1, respectively. The most effective control measure is the use of resistant varieties. We mainly tested 44 local melon local varieties from Egean region and different parts of Turkey to determine their resistance to Fusarium wilt with artifical inoculation (Races FOM 0, FOM 1, FOM 2, FOM 1,2 pathogen isolates inoculated to melon seedlings roots) and for *Fom1* and *Fom2* genes using SCAR (Sequence Characterized Amplified Region) and CAPS (Cleaved Amplified Polymorphic Sequences) molecular markers. Results of artifical inoculation tests and molecular markers tests for race FOM 2 were not consistent. According to morphological and molecular results, we were able to detect some resistant varieties for race FOM 0 and FOM 1. These varieties are: TR-40559 (Malatya, var. *cantalupensis)*, TR-70699 (Manisa, var. *inodorus*), TR-61613 (Muğla, var. *inodorus*) and TR-69891 (Kırkağaç, var. *inodorus*). It can be stated that these varieties are usable in future fusarium wilt resistant breeding programs.

INTRODUCTION

Melon (*Cucumis melo* L.) is a valuable and economically important crop grown in all over the world. It is a member of the genus *Cucumis*, in the family Cucurbitaceae (Wang et al. 1997). Melon is a diploid (2n=2x=24) which has an estimated genome size of 450 Mb (Arumuganathan and Earle 1991).

Melon is a crop that has an economic importance in both Turkey and the world. According to FAO statistics (2006), melon production in the world was 1.3 million ha area. In 2006, melon production in the world was 26.7 million tones. The most important share in production belongs to China (51%), Turkey (6%), USA (5%) and Spain (4%), respectively (FAOSTAT, 2009). The melons are grown in open fields and under low tunnel in Turkey. The open field melon growing provinces

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are Ankara, Manisa, Diyarbakir, Balikesir and Cankiri (Yilmaz et al. 2011).

Turkey is rich in melon genetic resources due to its diverse geographical and ecological situation. Harlan (1951) described Turkey as a microcentre for many landraces including *Cucurbitaceae* species, like *C. melo* (subtropical and tropical Africa) (Sari et al. 2008). Melon local varieties are grown by farmers for their flavour, taste and performance in extreme conditions.

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis* (*F.o.m*), is a soil-borne disease of melon (*Cucumis melo* L.). *Fusarium oxysporum* f. sp. *melonis* (F.o.m) causes serious economic losses in melon. Since the first case of Fusarium wilt was reported in New York in 1930 (Chupp 1930 a, b) it has been found in many melon growing areas worldwide, including North America (Leach 1933; Leach and Currence 1938; Leary and Wibur 1976; Martyn et al. 1987), Europe and Asia (Quiot et al. 1979; Sherf and Macnab 1986), with reports as severe as 100% of yield losses (Benoit 1974; Sherf and Macnab 1986). Four races of *F. oxysporum* f. sp. *melonis* have been identified (0, 1, 2, and 1,2). Resistance to *F. oxysporum* f. sp. *melonis* races 0 and 1, and races 0 and 2 is controlled by two independent genes *Fom-2* and *Fom-1*, respectively (Risser and Mas 1965; Risser 1973; Risser et al. 1976; Robinson et al. 1976). However FOM race 1,2 overcomes these resistance genes.

An effective control for this pathogen is through host resistance (Joobeur et al. 2004). Traditional artificial inoculation methods are used to evaluate resistance to fusarium wilt it is a time consuming process, unreliable and susceptible plants may escape detection (Burger et al. 2003; Joobeur et al. 2004). For this reason, MAS (Marker Assisted Selection) has an important role in plant breeding. Molecular markers tightly linked to fusarium wilt resistance genes are highly valued in melon breeding.

Many researches have been carried out to find tightly linked molecular markers to fusarium wilt resistant genes. Brotman et al. (2005) reported two cleaved amplifed polymorphic sequence (CAPS) markers mapped at a distance of 2.8 cM from the *Fom-1* locus. Oumouloud et al. (2008) also reported three sequence characterized amplifed region (SCAR) markers linked to the *Fom-1* locus. More recently, one sequence tagged site (STS) and two CAPS markers have been determined linked to *Fom-1* locus (Tezuka et al. 2009). Wang et al (2000) found linked CAPS and SCAR markers for the *Fom-2* gene.

The aim of this study was to evaluate the presence of *Fom-1* and *Fom-2* genes in some local melon genotypes via molecular markers and to determine the efficiency of these markers and melon genotypes for future fusarium wilt resistant breeding programmes.

MATERIALS AND METHODS

Material

The melon material used in this study was provided by National Gene Bank of Turkey, Aegean Agricultural Research Institute (AARI, Izmir, Turkey) and USDA (United States Department of Agriculture). This material consisted of 44 accessions which mainly belong to the *inodorus* and *cantalupensis* botanical varieties, from different regions of Turkey.

Screening for resistance to F.o.m with artificial inoculation

The screening for resistance to 4 races of F.o.m (race 0, race 1, race 2 and race 1,2) was carried out by artificial inoculation using the isolates provided by Wageningen Enstitute (Holland) in West Mediterranean Agricultural Research Institute (BATEM, Antalya, Turkey), with three replicates.

Seed of the accessions was treated with 1 % Sodium Hipocloride solution at 3 min after sown in the greenhouse. The trial was designed according to a randomized plot design with three replications. 5 plants of every accession, a susceptible control and a resistance control for each race were tested. Also, an uninoculated negative control was used. Seedlings after washed with tap water were dipped with a conidial suspension of 10^6 spores/ml for 5 minutes. Treated seedlings were planted in 180x165 mm pots. Seedlings were incubated at 23-25° C and 60-65 humidity in a climate chamber. 28 days after the inoculation, plants were examined according to susceptible/resistance reaction. Assessment was made according to Armstrong (1978).

DNA extraction

Four weeks after sowing, young leaves were collected from seedlings of each accession. Genomic DNA was isolated according to Doyle & Doyle (1981). DNA was quantitified using a Nanodrop and quality was checked by 1% agarose gel. DNA was diluted to 30 ng/ul to carry out PCR amplifications.

Molecular Screening

In this study, we used SCAR markers (Wang et al. 2000) for *Fom-2* gene, and CAPS (Brotman et al. 2005; Tezuka et al. 2009), SCAR (Oumouloud et al. 2008) and STS (Tezuka et al. 2009) markers for *Fom-1* gene (Table 1 and table 2). The PCR protocols for each marker were carried out as reported in the literature.

Table 1. Primers used for determination of the *Fom1* gene.

Marker	Marker Type	Restriction Enzyme	References
NBS1- CAPS	CAPS	NcoI	Brotman et al. (2005)
62-CAPS	CAPS	Alw26I	Brotman et al. (2005)
SB17645	SCAR	-	Oumouloud et al. (2008)
SV01574	SCAR	-	Oumouloud et al. (2008)
SV061092	SCAR	-	Oumouloud et al. (2008)
S-TAG/GCC-470	STS	-	Tezuka et al. (2009)
C-TCG/ GGT-400	STS	EcoRI	Tezuka et al. (2009)
CAPS2	STS	Taq1	Tezuka et al. (2009)

Table 2. Primers used for determination of the *Fom2* gene.

MARKER	MARKER TYPE	REFERENCES
AM	SCAR	Wang et al. (2000)
FM	SCAR	Wang et al. (2000)

RESULTS AND DISCUSSION

Artificial Inoculation Results

In our study, 44 melon accessions were artificially inoculated with 4 F.o.m races (FOM 0, FOM 1, FOM 2, FOM 1,2). According to the races, the resistance status of genotypes is given in table 3. The responses of genotypes to F.o.m. race 0 and race 1 inoculation were similar. TR-30744 (Gaziantep), TR-40559 (Malatya, var. *cantalupensis*), TR-61613 (Muğla, var. *inodorus*), TR-70699 (Manisa, var. *inodorus*), TR-40503 (Van, var. *cantalupensis*) and TR-69891 (Kırkağaç, var. *inodorus*) were found to be resistant to FOM race 0 and race 1. TR-61578 (Aydın, var. *inodorus)*, TR-64044 (Denizli var. *inodorus)* and TR-30744 (Gaziantep) were resistant to F.o.m race 2. All genotypes were found to be susceptible to F.o.m race 1,2. Only one genotype, TR-30744 (Gaziantep) was resistant to F.o.m race 0, F.o.m race 1 and F.o.m race 2, but susceptible to F.o.m race 1,2.

Table 3. Melon accessions used in this study, their origin, botanical variety, and their reaction when artificial inoculated with F.o.m. races 0, 1, 2 and 1,2.

NO	Genotype	Origin	Botanical variety	FOM0	FOM1	FOM2	FOM1,2
1	TR-61578	Aydın	*inodorus*	S	S	R	S
2	TR-61582	Aydın	*inodorus*	S	S	S	S
3	TR-61583	Aydın	*inodorus*	S	S	S	S
4	TR-61601	Aydın	*inodorus*	S	S	S	S
5	TR-62000	Denizli	*inodorus*	S	S	S	S
6	TR-61999	Denizli	*inodorus*	S	S	S	S
7	TR-64044	Denizli	*inodorus*	S	S	R	S
8	TR-69022	Eskişehir	*inodorus*	S	S	S	S
9	TR-30744	Gaziantep		R	R	R	S
10	TR-62038	İzmir	*inodorus*	S	S	S	S
11	TR-62742	İzmir	*inodorus*	S	S	S	S
12	TR-70685	İzmir	*inodorus*	S	S	S	S
13	TR-66005	Kütahya	*inodorus*	S	S	S	S
14	TR-40559	Malatya	*cantalupensis*	R	R	S	S
15	TR-70698	Manisa	*inodorus*	S	S	S	S
16	TR-70699	Manisa	*inodorus*	R	R	S	S
17	TR-61676	Muğla	*cantalupensis*	S	S	S	S
18	TR-61613	Muğla	*inodorus*	R	R	S	S
19	TR-46489	Sivas	*inodorus*	S	S	S	S
20	TR-40503	Van	*cantalupensis*	R	R	S	S
21	TR-69891	Kırkağaç	*inodorus*	R	R	S	S
22	PI-167266	Adana	*momordica*	S	S	S	S
23	PI- 169302	Hasanbey	*inodorus*	S	S	S	S
24	PI-169303	Altınbaş	*inodorus*	S	S	S	S
25	PI-169307	Topatan	*momordica*	S	S	S	S
26	PI-169333	Kırkağaç	*inodorus*	S	S	S	S
27	PI-169336	Topatan-İstanbul		S	S	S	S
28	PI-176502	Mollaköy	*inodorus*	S	S	S	S
29	PI-179247	Bağrıbütün	*cantalupensis*	S	S	S	S
30	PI-179251	Çobanalmaz	*inodorus*	S	S	S	S
31	PI-344305	Kış Kavunu	*inodorus*	S	S	S	S
32	PI-344306	Çıtır Kavun	*inodorus*	S	S	S	S
33	PI-344307	İzmir-Dilim	*inodorus*	S	S	S	S
34	PI-344308	Sarı kavun	*cantalupensis*	S	S	S	S
35	PI-344309	Karakavun		S	S	S	S
36	PI-344315	ÇanakkaleKışlık	*inodorus*	S	S	S	S
37	PI-344326	Çumra	*cantalupensis*	S	S	S	S
38	PI-344330	Diyarbakır	*cantalupensis*	S	S	S	S
39	Kırkağaç 589	Kırkağaç 589	*cantalupensis*	S	S	S	S
40	ÇEŞME	Çeşme	*inodorus*	S	S	S	S
41	ÇEŞME	Çeşme	*inodorus*	S	S	S	S
42	BUCA	Buca	*inodorus*	S	S	S	S
43	KARABURUN	Karaburun	*cantalupensis*	S	S	S	S
44	KARABURUN	Karaburun	*Cantalupensis*	S	S	S	S

S: Susceptible R: Resistant

Molecular Results

To detect resistant genotypes to FOM race 0 and race 1, we used AM and FM SCAR markers which are 1,7 and 3,3 cM far from *Fom-2*. We have not obtained any PCR products in all tested genotypes with the FM SCAR marker, therefore, this marker was not useful for *Fom-2* selection in our study. In contrast, for the AM marker we obtained bands of 1,783bp and 1,683 bp in the susceptible and the resistant genotype, respectively.

Out of the 44 tested genotypes, 4 genotypes (TR-40559, TR-70699, TR-61613 and TR-69891) and the positive control were found to have *Fom-2* and gave the same result for both molecular and inoculation analysis. The genotype TR-40503 was found to be resistant to FOM race 0 and race 1 according to the inoculation test, but not in the molecular test (Table 4). Wang et al (2000) have shown that AM correctly predicted disease outcome of 41 out of 45 diverse genotypes tested and FM predicted 37 of 45 genotypes correctly. These results indicate that AM marker can be useful for detecting the presence of the *Fom-2* gene in a variety of populations as a tool for MAS.

Table 4. Melon accessions used in this study, their reaction after artificial inoculation with F.o.m. races O and race1 and the results of AM marker screening.

NO	Genotype	Artificial Inoculation	AM Marker	NO	Genotype	Artificial Inoculation	AM Marker
1	TR-61578	S	S	24	PI-169303	S	S
2	TR-61582	S	S	25	PI-169307	S	S
3	TR-61583	S	S	26	PI-169333	S	S
4	TR-61601	S	S	27	PI-169336	S	S
5	TR-62000	S	S	28	PI-176502	S	S
6	TR-61999	S	S	29	PI-179247	S	S
7	TR-64044	S	S	30	PI-179251	S	S
8	TR-69022	S	S	31	PI-344305	S	S
9	TR-30744	R	not tested	32	PI-344306	S	S
10	TR-62038	S	S	33	PI-344307	S	S
11	TR-62742	S	S	34	PI-344308	S	S
12	TR-70685	S	S	35	PI-344309	S	S
13	TR-66005	S	S	36	PI-344315	S	S
14	TR-40559	R*	R*	37	PI-344326	S	S
15	TR-70698	S	S	38	PI-344330	S	S
16	TR-70699	R*	R*	39	Kırkagac 589	S	S
17	TR-61676	S	S	40	ÇEŞME	S	S
18	TR-61613	R*	R*	41	ÇEŞME	S	S
19	TR-46489	S	S	42	BUCA	S	S
20	TR-40503	R*	S	43	KARABURUN	S	S
21	TR-69891	R*	R*	44	KARABURUN	S	S
22	PI-167266	S	S		S. Control	S	S
23	PI- 169302	S	S		R. Control	R*	R*

R: Resistant S: Susceptible

While S-TAG/GCC-470 marker is 0 cM a far away from *Fom-1* locus, we have not obtained PCR products of this size. This might be due to different alleles for resistance to races 0 and 2 that originated independently in different lineages of the melon phylogeny (Tezuka et al. 2009). STS marker S-TAG/GCC-470 and CAPS markers C-TCG/GGT-400 and CAPS2 were developed in *Cucumis melo* var. *Reticulatus, Cucumis melo* var. *Makuwa* and var. *Conomon (Asian varieties)* botanical varieties. But all tested local genotypes in this study belong to *inodorus, cantalupensis* and *momordica* botanical varieties.

Of the two markers 62-CAPS and NBS1-CAPS developed by Brotman et al. (2005), NBS1-CAPS gave the expected results in the 33 genotypes, assessed with artifical inoculation. Seven out of 40 genotypes, TR-64044, TR-30744, TR-70699, TR-46489, PI-167266, PI-344309 and PI-344326 gave different results for artificial inoculation of FOM race 2 and molecular analysis with NBS1-CAPS. Only TR-61578 was detected as resistant to FOM race 2 by NBS1-CAPS marker. CAPS marker 62-CAPS mostly gave mis-predicted results when compared to artificial inoculation tests (table 5).

The three SCAR markers developed by Oumouloud et al (2008) did not give the same results than with the inoculation test to FOM race 2. Among these markers, $SV01_{574}$ SCAR marker gave correct results in 30 genotypes. TR-61578 and TR30744 were determined to have *Fom-1* with $SV0_{1574}$ SCAR marker (Table 5).

The differences between artificial inoculations and molecular markers linked to *Fom-2* genes may support the hypothesis that different genotypes have different alleles for resistance to Fusarium wilt races 0 and 2 that originated independently in different lineages of the melon phylogeny (Oumoloud et al. 2008).

Table 5. Melon accessions used in this study, their reaction after artificial inoculation with F.o.m. races 2 and the results of the markers used for determination of the *Fom-1* gene.

NO	Genotype	Race 2	SB17-645	SV01-574	SV06-1092	62-CAPS	NBS-CAPS	CAPS2	CAPS-400
1	TR-61578	R	S	R	R	R	R	S	R
2	TR-61582	S	n.t.	n.t.	n.t.	n.t.	n.t.	S	R
3	TR-61583	S	R	S	R	Het.	S	S	R
4	TR-61601	S	R	S	R	n.t.	S	S	R
5	TR-62000	S	R	S	R	R	S	S	R
6	TR-61999	S	R	S	R	Het.	S	S	R
7	TR-64044	R	R	S	R	R	S	S	R
8	TR-69022	S	R	S	R	R	S	S	R
9	TR-30744	R	R	R	R	S	S	S	R
10	TR-62038	S	R	S	R	S	S	S	R
11	TR-62742	S	R	S	R	Het.	S	S	R
12	TR-70685	S	n.t.	n.t.	n.t.	R	S	S	R
13	TR-66005	S	R	S	R	R	S	S	R
14	TR-40559	S	R	S	R	R	S	S	R
15	TR-70698	S	R	S	R	R	S	S	R
16	TR-70699	S	R	R	R	S	R	S	R
17	TR-61676	S	R	R	R	R	S	S	R
18	TR-61613	S	n.t.	n.t.	n.t.	n.t.	n.t.	S	R
19	TR-46489	S	S	R	R	S	R	S	R
20	TR-40503	S	n.t.	n.t	R	n.t.	n.t.	S	R
21	TR-69891	S	R	S	R	R	S	S	R
22	PI-167266	S	R	S	R	Het	Het	S	R
23	PI- 169302	S	S	S	S	R	S	S	R
24	PI-169303	S	R	S	R	R	S	S	S
25	PI-169307	S	R	S	S	R	S	S	S
26	PI-169333	S	R	R	S	R	S	S	R
27	PI-169336	S	S	R	R	R	S	S	R
28	PI-176502	S	S	S	R	R	S	S	S
29	PI-179247	S	R	R	R	S	S	S	R
30	PI-179251	S	S	S	R	R	S	S	R
31	PI-344305	S	R	S	R	R	S	S	R
32	PI-344306	S	R	R	R	S	S	S	R
33	PI-344307	S	S	S	R	S	S	S	R
34	PI-344308	S	S	S	R	S	S	S	R
35	PI-344309	S	R	S	R	S	R	S	R
36	PI-344315	S	R	S	R	R	S	S	R
37	PI-344326	S	R	S	R	S	R	S	R
38	PI-344330	S	S	S	R	n.t.	n.t.	S	R
39	KIRKA.589	S	R	R	R	R	S	S	R
40	ÇEŞME	S	R	S	R	R	S	S	R
41	ÇEŞME	S	R	R	R	R	S	S	R
42	BUCA	S	R	S	R	R	S	S	R
43	Karaburun	S	R	S	R	R	S	S	R
44	Karaburun	S	S	S	R	R	S	S	R

R: Resistant, S: Susceptible, Het: Heterozigous, n.t.: not tested

CONCLUSION

Plant genetic resources are the most important source for breeding new cultivars in modern and traditional breeding programs. In addition, local varieties are grown by farmers for long time in the same location, so they have adaptation to abiotic and biotic stess conditions.

We detected some resistant local varieties for race FOM 1. These varieties, TR-40559 (Malatya, var. *cantalupensis),* TR-70699 (Manisa, var. *inodorus*), TR-61613 (Muğla, var. *inodorus*) and TR-69891 (Kırkağaç, var. *inodorus*) can be used in future breeding programs. The results of artifical inoculation tests and molecular markers for race FOM 2 were not consistent. This might be explained because the markers linked to the resistance gene for FOM race 2 were developed in different melon germplasm. This result shows that more research is needed for each sub class of melon to develop molecular markers thightly linked to resistant genes to fusarium wilt.

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Determination of BATEM's melon pure lines for resistance to ZYMV

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Abstract

Almost all cultivars of melon which are used in greenhouse production are foreign origin F1 hybrid in Antalya. Local hybrid melon cultivars are required to increase production to reduce imports in the seed sector. But, many local cultivars are susceptible to virus diseases. This also leads to serious losses in the production.

Viral diseases cause important economic losses throughout the world. More than 35 viruses have been isolated from cucurbits. These viruses constitute complex and dynamically changing problems. Most commercial melon varieties are susceptible to the viral pathogens. Potyviruses form the largest and the most economically significant group of plant viruses. Severe losses in melon production areas are due to potyvirus infection, including *watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV) and papaya ringspot virus (PRSV) infected melon plants may show vine decline, reduced or absent yield and fruit quality defects. In this study 60 Batı Akdeniz Research Instute (BATEM) melon pure lines were tested for resistance to ZYMV in Kocayatak test compartments. Virus isolates were mechanically inoculated to the cotyledons at the first true melon leaf stage of the plant by rubbing carborundum-dusted with extracts from 1g of infected plant material prepared in 0.02M phosphate buffer (pH:7.0) and added activated carbon. Soft Scorching was used as an alternative method of transmission of virus to the plants. After inoculation cotyledons were washed using tap water. Plants were left to grow in 16 h light/8 h dark at 25 ^{o}C. Presence or absence of virus symptoms (mosaic, yellowing, shoestring leaves, stunting, and fruit and seed deformation.) was scored for each plant 3 weeks after inoculation. The accessions, developed visual symptom, were recorded as susceptible and symptom less plants resistant.*

The results show that 31 melon pure lines were resistant and 29 lines were susceptible to ZYMV. As a result, the resistant lines can be successfully used in breeding studies.

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INTRODUCTION

Turkey is one of the important melon producing countries in the world. In the world melon production, our country has come in second place after China (FAO, 2010). Cultivated melon consists of inodorous melon group (85%) (Kirkagac, Hasanbey, Yuva etc.) and the rest (15%) is composed by reticulatus and cantalupensis types (i.e. Ananas, Galia etc.) in Turkey (Abak 2001).

Viral diseases cause important economic losses throughout the world. More than 35 viruses have been isolated from cucurbits (Provvidenti 1996). These viruses constitute complex and dynamically changing problems as described by Nameth et al. (1986). Most commercial melon cultivars are susceptible to the viral pathogens. Potyviruses form the largest and the most economically significant group of plant viruses (Riechmann et al. 1992). Severe losses in melon production areas are due to potyvirus infection, including *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV) (Davis and Mizuki 1987; Provvidenti and Gonsalves 1984). Infected melon plants may show vine decline, reduced or absent yield and fruit quality defects.

Virus infections caused by nonpersistently transmitted viruses are difficult to prevent by insecticide application (Raccah 1986), which is the most frequent measure used by growers. Therefore, incorporation of genetic resistance in the host plant is a valuable alternative. Commercial cultivars of melons carrying resistance to *cucumber mosaic virus* (CMV) and PRSV are available (Lecoq et al. 1998). However, resistant cultivars to ZYMV or WMV are not commercially available (Danin-Poleg et al. 2000; Pitrat and Lecoq 1984; Gilbert et al. 1994; Gray et al. 1988).

The objective of the present work was to search for novel sources of resistance to ZYMV, in Batem's *C. melo* germ plasm collection, and to use this resistance sources in further breeding studies.

MATERIALS AND METHODS

In the study 60 melon accessions including inodorous and cantalopensis groups were evaluated for resistance to ZYMV (Table 1). Screening tests were carried out at Batı Akdeniz Agricultural Research Institute. Virus isolate ZYMV-Ad (Gene bank Ac. N. JF 317296.1) was used for the study. Virus isolate was propagated by mechanically (Soft spongy pad) inoculation onto the susceptible melon plants. Virus isolate was mechanically inoculated to the cotyledons at the first true leaf stage of the plant by rubbing carborandum-dusted with extracts from 1 g of infected plant material prepared in 0.02 M phosphate buffer and containing 0.1% 2-mercaptoethanol (pH:7.0) and added activated carbon.

Soft Scorching was used as an alternative mechanically inoculation method. Firstly, to prepare virus isolate, infected plant materials were pounded with phosphate tampon (1/9) in the mortar by pestle without using abrasive carborandum dust. This isolate were absorbed by Soft spongy pad. Then, Soft Scorching absorbed virus isolate was rubbed over leaf surface. Thus, leaf surface was abraded and

inoculated by Soft Scorching (Fig. 1).

After inoculation cotyledons were washed using tap water. Plants were left to grow in 16 h light 8 h dark at 25 ^{o}C temperature conditions. Presence or absence of virus symptoms was scored for each test plant 3 weeks after inoculation. The plants developed visual symptoms were recorded as susceptible and the plants without symptoms were accepted as resistant.

Symptom evaluation was according to Yilmaz et al. (1994) the using a scale of 0 to 9 (0: No symptom, 1: very weak vein clearing, 2: very weak mosaic symptom on the leaf, 3: weak vein clearing with weak mosaic, 4: moderately mosaic and light yellowing, 5: mosaic symptoms on the leaves and shorten in plant height, 6: severe mosaic and mild deformations on the leaves with shorten in plant height, 7: severe mosaic, mottling, moderately deformation of leaves, 8: severe mosaic, filamentous appearance of leaves, moderately fruit deformations, 9: severe dwarfing of the plants, very severe mosaic and yellowing, severe fruit deformation, filamentous appearance of leaves).

RESULTS AND DISCUSSIONS

It might be concluded that the damage caused by abrasive application during rubbing was not noticed in ZYMV transmission by using of the spongy pad technique.

For this reason this technique could be accepted as a new inoculation method. Using this technique without carborundum dust provides 8 times faster inoculation than classic mechanic inoculation technique.

The typical ZYMV symptoms were observed on the hosts 7 days after inoculation. ZYMV symptoms were evaluated as to (1-9) scale after 21 days inoculation. The plant showing symptoms between 0 to 1 scales was accepted as a resistant to ZYMV virus.

If the virus symptoms were observed on even one of the plants among the 10 plants, this plant was accepted as a susceptible to this virus infection. The resistant plants were carried to out of the screenhouse and inspected until harvesting stage. The plants that are not showing virus symptom until harvest time were accepted as resistant plants to ZYMV infection (Fig. 2).

The results show that 31 melon pure lines were resistant and 29 lines were susceptible ZYMV. As a result, the resistant lines can be successfully used in breeding studies. Additional work is now needed to introduce resistance genes in adapted types and release cultivars with good horticultural quality and resistance to ZYMV.

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Table 1. Results of-inoculation of *C. melo* with ZYMV.

Accession	Susceptıble/Resıstance	Accession	Susceptıble/Resıstance
TF1/1	S	TF 61	S
TF 4	S	TF 62	R
TF 5	R	TF 64	R
TF 6	R	TF 65	R
TF 8/2	R	TF 66	R
TF 9	S	TF 67	S
TF 10	R	TF 69	R
TF11	R	TF 70	S
TF 12	R	TF72	S
TF 14	S	TF 74	R
TF 15	S	TF 78	S
TF 18	S	TF 81	R
TF 19	S	TF 82	R
TF 28	S	TF 84	S
TF 29	S	TF 85	S
TF 30	S	TF 87	S
TF 31	S	TF 88	R
TF 33	R	TF 89	R
TF 34	S	TF 90	R
TF 35	R	TF 92	S
TF 37	R	TF 93	R
TF 40	S	TF 94	R
TF 41	S	TF 96	R
TF43	R	TF 99	R
TF46	R	TF 101	R
TF 52	S	TF 104/1	R
TF 53	S	TF 105/1	R
TF 56	S	TF 115/1	S
TF 57	R	V59	S
TF 60	R	TF 109	S

Fig. 1. Process of inoculation using Soft Scorching.

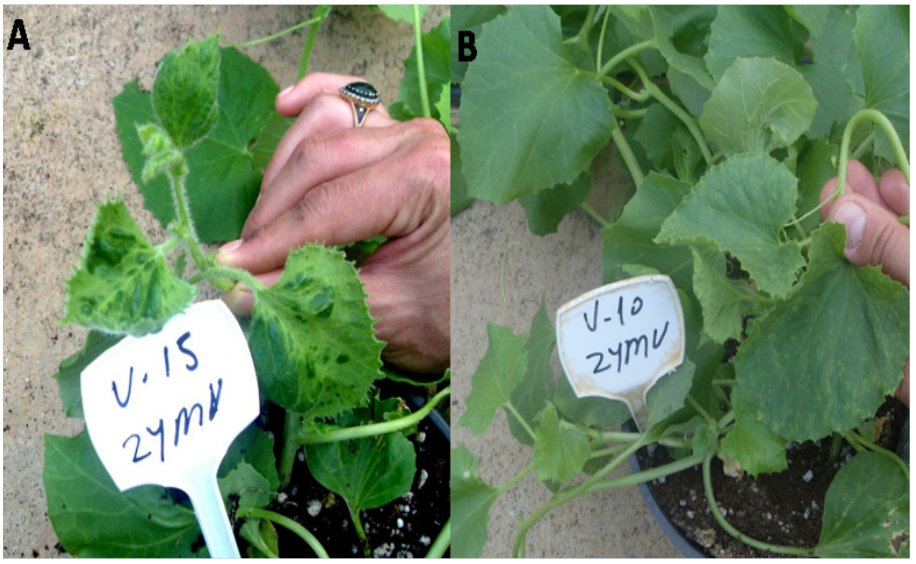

Fig. 2. Young sensitive melon plants with typical ZYMV symptoms (A) and resistant plants without any symptoms (B).

The determination of several physical, gravimetric and frictional seed properties of melon cultivars

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Keywords: Characterization, *Cucumis melo* L., physical properties

ABSTRACT

Seedling and classification machines are still under specific development for most of the seeds commonly used in agriculture. Those machines well adapted to the plant physical requirements for breeding. Therefore, determination of physical properties of seeds is a very important subject to breeding for mechanization. Several physical, gravimetric, and frictional seed properties of six open pollinated melon (*Cucumis melo* L.) cultivars (Kırkağaç 589, Altınbaş 637, Vilmorin Depuis 1743, Mühürlü, Hasanbey, Ananas) were determined and compared in terms of 1000 unit mass, width , length, thickness, roundness, sphericity, bulk angle, specific bulk density, density, moisture content, mineral matter content, swelling capacity, and static fraction angle (aluminium, stainless steel, galvanized sheet metal, rubber, glass, MDF). The obtained data for the six melon cultivars, which might be useful to engineers in the equipment design, were generally found to be statistically different. These differences could be attributed to the individual characteristics of these cultivars.

INTRODUCTION

Determination of technical properties of plant species or cultivars used as biological material is important in order to attain successful results in many fields such as designing, producing, operating all the agricultural tools and machines such as harvester, transplanter, sowing machine, spraying machine, and increasing productivity and for carrying out post-harvest activities such as storage, drying, grinding, milling, packaging, freezing (Mohsenin 1980; Aviara et al. 1999; Davies 2010). Specific plant properties should be considered in breeding studies based on mechanization (Abeels 1994). With this study it is aimed to help (i) design and production of various machines and systems, (ii) breeding studies based on mechanization for melon. Therefore in this study various dimensional and

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structural features of six melon open pollinated cultivars (Kırkağaç 589, Altınbaş 637, Vilmorin Depuis 1743, Mühürlü, Hasanbey and Ananas) which are grown commonly in Turkey were determined. Similarities and differences among these cultivars were identified.

MATERIALS AND METHODS

In order to determine some of the technical properties; Kırkağaç 589, Altınbaş 637 Vilmorin Depuis 1743, Mühürlü, Hasanbey, and Ananas melon cultivars were used in the study. In order to measure seeds' width, length, and thickness, a digital caliper (Mitutoyo, USA) with 0.01 m sensitivity was used. Roundness and sphericity values were done according to Işık (2003):

$$Roundness = \frac{r}{R} \qquad \phi = \frac{(LWT)^{1/3}}{L}$$

Where; *r*, curvature radiues of the sharpest edge (mm); *R*, mean diameter of the product (mm); *Φ*, sphericity (%); *L*, length of the biological material (mm); *W*, width of the biological material (mm); *T*, thickness of the biological material (mm).

Density and specific bulk density values were determined according to Işık (2003). In determination of static fraction angles of seeds, a system that is sensitive towards slipping was used. This system shows the angle where melon seeds begin to slip with a scale. Static fraction angles were measured on 6 different surfaces composed of aluminum, stainless steel, galvanized sheet metal, rubber, glass, and MDF materials. Angle of repose was calculated with the equation:

$$BA = \tan^{-1}(\frac{T_b}{r_b})$$

Where: *BA*, bulk angle (°); T_b, height of bulk (mm); r_b, mean radius of bulk base (mm).

Swelling capacity of seeds were carried out by keeping seeds in 24±1 °C distilled water and measuring them in 30 minutes intervals. Moisture content was done according to Işık and Alibas (2000):

$$W_s = W_t - W_m \qquad N_{yb} = \frac{W_s}{W_s + W_m} 100 \ [\%]$$

Where: W_s, weight of water in biological material (g); W_m, weight of dry matter in biological material (g); W_t , total weight of biological material (g); N_{yb}, moisture content according to wet basis [%].

Mineral matter content was done according to Uylaşer and Başoğlu (2000):

$$Organic = \frac{HKT - FKT}{FKT} 100 \quad [\%] \qquad Mineral = 100 - Organic \quad [\%]$$

Where: *HKT*, air dry weight (g); *FKT*, oven dry weight (g); *Organic*, amount of organic substance within biological material [%]; *Mineral*, amount of mineral substance within biological material [%].

RESULTS

Dimensional, structural, and frictional properties of six melon cultivars used in the study are given in Table 1. Several technical properties of seeds were determined with various measurement and calculation methods. It is noteworthy, that there was no difference among cultivars studied regarding their seed roundness value. While differences existed among melon cultivars regarding the geometrical length values at 5% importance level, for all other properties, there was a statistical difference at 1% importance level.

Weight gain values of melon cultivars based on time are given in Fig. 1. According to this, it was determined that the seed which has the greatest water uptake capacity was Mühürlü, followed by Ananas, Vilmorin Depuis 1743, Kırkağaç 589, Hasanbey, and Altınbaş 637 seeds. It was determined that Mühürlü, Altınbaş 637, and Vilmorin Depuis 1743 cultivars reach maximum water inlet capacity at 270th minute, while Hasanbey cultivar reached at 360th minute, and Kırkağaç 589 and Ananas cultivars reached at 420th minute.

Table 1. Seed dimensional, structural, and frictional properties of melon cultivars.

Seed properties	Cultivars[z]					
	Kırkağaç 589	Altınbaş 637	Vilmorin Depuis 1743	Mühürlü	Hasanbey	Ananas
1000 unit mass**(g)	39.97^{b}	37.93^{b}	30.67^{c}	45.13^{a}	32.00^{c}	37.00^{b}
Width** (mm)	4.90^{a}	4.70^{abc}	4.31^{c}	4.86^{a}	4.42^{bc}	4.71^{ab}
Length * (mm)	10.60^{bc}	10.25^{bc}	9.38^{c}	11.89^{a}	9.92^{bc}	10.69^{ab}
Thickness** (mm)	1.94^{a}	1.62^{b}	1.47^{b}	1.47^{b}	1.58^{b}	1.60^{b}
Roundness^{ns} (%)	0.23	0.23	0.23	0.21	0.23	0.22
Sphericity** (%)	0.44^{a}	0.42^{a}	0.41^{a}	0.37^{b}	0.42^{a}	0.41^{ab}
Bulk Angle** (°)	19.09^{bc}	17.83^{bc}	16.72^{c}	23.97^{a}	18.37^{bc}	19.90^{b}
Specific Bulk Density** (kg.m^{-3})	898.20^{b}	866.19^{bc}	954.57^{a}	909.83^{ab}	839.18^{c}	839.24^{c}
Density**	0.8982^{b}	0.8662^{bc}	0.9546^{a}	0.9098^{b}	0.8392^{c}	0.8392^{c}
Moisture Content** (% yb)	6.90^{a}	6.61^{d}	6.23^{f}	6.72^{b}	6.45^{e}	6.65^{c}
Mineral Matter Cont.** (%)	3.67^{a}	3.59^{c}	3.34^{e}	3.65^{ab}	3.42^{d}	3.63^{b}
Swelling Capacity (%)**	41^{cd}	39^{d}	42^{c}	49^{a}	40^{cd}	45^{b}
Static Fraction Angle (°)						
Aluminium**	24.18^{ab}	23.61^{b}	22.75^{bc}	25.63^{a}	21.44^{c}	22.64^{bc}
Stainless Steel**	22.89^{cd}	24.36^{b}	22.07^{de}	27.25^{a}	21.57^{e}	23.28^{c}
Galvanized Sheet Metal**	25.47^{b}	25.50^{b}	21.30^{d}	27.10^{a}	22.97^{c}	26.63^{ab}
Rubber**	28.89^{ab}	30.20^{a}	25.61^{c}	28.99^{a}	26.94^{bc}	28.58^{ab}
Glass**	20.01^{c}	20.64^{bc}	19.69^{c}	29.61^{a}	22.30^{b}	20.18^{c}
MDF**	30.07^{d}	33.89^{b}	32.24^{bc}	39.49^{a}	31.78^{cd}	31.57^{cd}

Means followed by the same letter in a line are not significantly different at P<0.01** and P<0.05*; ns = non-significant.

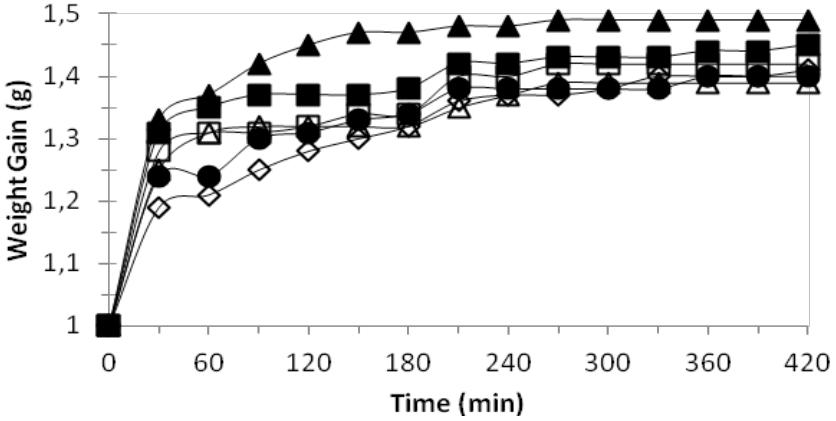

Fig. 1. Weight change of melon cultivars seeds based on water uptake: ◊, Kırkağaç 589; Δ, Altınbaş 637; □, Vilmorin Depuis 1743; ▲, Mühürlü; ●, Hasanbey; ■, Ananas.

DISCUSSION

It was determined, that differences exist among melon cultivars regarding their seed properties analyzed in the study. In the previous studies; specific physical and structural seed properties of many species such as cowpea (Yalçın 2007; Kabas et al. 2007; Davies and Zibokere 2011), pea (Yalçın et al. 2007), broad bean (Hacıseferoğulları at al. 2003), rape (Çalışır et al. 2005), corn (Coskun et al. 2006), flax (Coşkuner and Karababa 2007), or sugar beet (Dursun et al. 2007) were determined and similarities and differences between cultivars were found.

It might be concluded that properties analyzed should be considered in designing of and producing various machines and systems, conducting post-harvest activities and breeding studies based on mechanization. Moreover, seed properties which were analyzed in this study were found to be parameters that can be utilized in distinguishing genetic resources from each other.

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Resistance to two-spotted spider mite in melon genotypes

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Keywords: *Cucumis melo*, cucurbitacin, defense responses, peroxidase, polyphenol oxidase, *Tetranychus urticae*

Abstract

Two-spotted spider mites (*Tetranychus urticae* Koch, Acari: Tetranychidae) are frequent pests that can cause serious damage to many crops including melons during hot dry weather. In order to finding resistance sources, 33 melon cultigens collected from different parts of Iran, were evaluated by antixenosis (choice test at six leaf stage) against two-spotted spider mite. Analysis of variance revealed highly significant differences among cultigens. Based on the results of the screening, four extreme susceptible (Garmsari, Zard-Khareji, Bargney and Sabz) and four extreme resistant (Dastanbu1, Darunghermez, Zard-Jalali and Kharbozeh) genotypes were selected for further study. Antibiosis data were collected by assessing fecundity of mated female mites on leaf discs in the laboratory. The number of eggs laid by each female per day showed significant differences between genotypes. The fewest eggs (1.38 and 1.56) were laid on Darunghermez and Dastanbu1 and the most eggs (4.3 and 4) were laid on Garmsari and Zard-khareji genotypes, respectively. The activity of two enzymes (peroxidase and polyphenoloxidase) and content of cucurbitacin B and D were also measured after infestation in susceptible and resistant genotypes. The enzymes increased in response to the pest until four days in the resistant genotypes. No significant correlation was observed between amount of these metabolites and resistance to the mite. The resistant melon genotypes identified in this study appear to be good sources for *T. urticae* control in breeding programs.

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Prostigmata), is one of the most important pest in many crops especially in greenhouse conditions (Hussey and Scopes 1985). TSSM damages plant cells and tissues by piercing them and sucking cell contents (Martinez-Ferrer et al. 2006). Acaricides are commonly used to control TSSM, but their continued use is not sustainable. The use of resistant cultivars when available presents an alternative control option.

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Knowledge of the resistance mechanisms in the genus *Cucumis* against TSSM has been primarily derived from studies on the cucumber (Scully et al. 1991; Balkema-Boomestra et al. 2003) and in some case on melon (Mansour et al. 1987; East et al. 1991). Enzyme activity induced by arthropod feeding and pathogen infection has been shown to play an important role in plant resistance. Insect feeding has been shown to increase peroxidase (POX) and polyphenol oxidase (PPO) activity (Bi et al. 1997). Among secondary metabolites, cucurbitacins in particular have been shown to be involved in both direct and indirect plant defense systems (Balkema-Boomestra et al. 2003). The objectives of our studies were to identify sources of resistance to TSSM in Iranian melon genotypes that display antixenosis and antibiosis properties, and to determine whether enzymes and cucurbitacins are associated with the resistance observed.

MATERIALS AND METHODS

TSSM were maintained as a colony on common bean leaves for three generations. Thirty- three melon genotypes mostly collected from different parts of Iran and three commercial imported genotypes were used in this study (Table 1). Seeds of each genotype were sown in pots and kept in greenhouse (27ºC, 60-70% RH, 14:10 h /L:D). The experiments were conducted when the plants were at the sixth leaf stage. To evaluate preferential choices of TSSM, the 36 genotypes were placed in blocks of nine genotypes to comply with an alpha-lattice (0-1) design with three replicates. For each block, the youngest leaves (about 4 cm diameter) from each pot (10 leaves per genotype) were placed around three 100 cm diameter circle platforms randomly with a distance of 4 cm between adjacent leaves. Ten adult female mites were released in a Petri dish at the center of the circle in recognition of each leaf. The number of mites present on each leaf was recorded after 48 hours (Bernal and Setamou 2003). To evaluate the effect of cucurbitacin content of genotypes on their preferential choices, a sample of second leaf was taken from each genotype. Calibration standards were prepared by serial dilution of stock solutions of cucurbitacin D (Extrasynthese) and B (Bionaturis). The quantitative analysis of cucurbitacins was carried out by HPLC (Analytical, 235 nm UV detector).

TSSM fecundity was quantified using leaf disc bioassays to assess four resistant and four susceptible genotypes selected from the antixenosis tests. Plants were arranged in a randomized block design with three replicates. Three mated female TSSM were transferred to each leaf disc (4 cm^2) placed on water agar, and the number of eggs laid per female was counted for seven days. Enzyme activity also was investigated for the same eight genotypes arranged in a randomized block design with two replicates. Ten mated female TSSM were transferred to the youngest leaf of one plant in plot. At 0 (control), two, four, six and eight days after infestation, the mites were removed and the leaves were subjected to POX and PPO activity evaluation by a UV-VIS spectrophotometer (Janda et al. 2003). Data were subjected to analysis of variances and means were compared using Duncan's test (SAS, Version 9).

RESULTS AND DISCUSSION

Mean TSSM per leaf varied widely between the melon genotypes (Table 1) in antixenosis test. Genotypes 8, 6, 34 and 17 had significantly fewer female TSSM, indicating a strong antixenotic effect contrary to genotypes 11, 35, 5 and 21 which had the greatest numbers of TSSM on their leaves, indicating little or no antixenotic effect. It might be expected that a pest would prefer genotypes that are optimized for these parameters while avoiding genotypes that inhibit their survival and reproductive success (Coleson and Miller 2005). Mite preference could be due to morphological differences among the genotypes (Agrawal 2004), or as allomones that inhibit the initiation of feeding or oviposition (Flores et al. 2008).

Regression analysis showed that although there were large differences between genotypes regarding cucurbitacins contents in putative resistant and susceptible genotypes (Table 2), there was no clear relation between these compounds and resistance/ susceptibility to TSSM. In fact, plants could produce a wide variety of biochemical compounds that have potential biological effects against herbivores and insects. Balkema-Boomestra et al. (2003) showed that concentration of cucurbitacin C in the cucumber leaves is an important parameter in resistance to spider mite in cucumber. Besides, several studies indicated that other groups of cucurbitacins, such as B, C, D, E and I have potential activity against some insects and herbivores (Chen et al. 2005).

The results indicated significant differences between the mean numbers of eggs laid by each female per day ($P< 0.01$). The number of eggs laid on Darunghermez (1.38) and Dastanbu 1 (1.56) were significantly fewer than on all other genotypes (Table 3). Garmsari (4.3), Zard-Khareji (4) and Bargney (3.47) had the greatest number of eggs produced per female for the seven-day period. These results are largely in agreement with other published studies (Scully et al. 1991; Mansour et al. 1987). It is possible that biochemical mechanisms contributing to observed differences in preference and fecundity could induce specific nutrients, phytochemicals, phytoalexins, or other anti-insect and antifeedant factors present in plant leaves that affect the mite's behavior and biology (Sedaratian et al. 2008; Chatzivasileiadis et al. 2001).

Significant differences for POX and PPO activity were found between genotypes (Table 3). The data also showed that after infestation, POX activity increased in all of the resistant genotypes, while susceptible genotypes presented the least POX activity. These results are largely in agreement with other published studies (Trevisan et al. 2003; Moran 2001; Dowd 1994). POX regulates a physical or chemical response of the host to pest infestation by catalyzing the oxidation of phenolic components, lignin monomers, toxin precursors and fatty acids into the signaling pathway (Dowd 1994). PPO activity also increased in the resistant genotypes following infestation, but it remained unchanged in all susceptible genotypes (Table

3). Some authors believe that PPOs play a key role in defense against herbivores and has an anti-nutritive effect on insects, but the reaction sequence is very complex (Constable and Barbehenn 2008).

In conclusion, there is a considerable variation for resistance to TSSM in melon genotypes which might be valuable in the development and implementation of melon breeding programs. Not any relation was found with resistance to TSSM and Cucurbitacin B and D as the main known secondary metabolites in plant but the increasing of POX and PPO enzymes was obvious in response to its infestation. Further biochemical studies of these and additional genotypes should prove the mechanism of resistance.

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Determination of some melon lines against to the reaction of *Fusarium oxysporum* f. sp. *melonis* by using classical and molecular analysis

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Keywords: *Fom-1*, *Fom-2*, melon breeding, molecular marker, *cantalupensis*

Abstract

One of the most important fungal diseases in melon cultivation is *Fusarium oxysporum* f. sp. *melonis* (FOM). Races 0, 1, 2 and 1-2 of FOM have been identified. All FOM races are seen in our country. Disease resistance is controlled in melon by the genes *Fom-1* and *Fom-3* against races 0 and 2, the gene *Fom-2* against races 0 and 1. In this study, one hundred summer-type pure melon lines which belong to Bati Akdeniz Agricultural Research Institute (BATEM) were evaluated for resistance to FOM by using classical and molecular analysis methods. Root dip method was used in classical analysis. In molecular analysis, primers SSR 154 and NBS1-CAPS were used for *Fom-1* and *Fom-2* gene determination, respectively. From classical analysis, 73 of a hundred pure lines were found resistant to race 1, seventeen of them resistant to race 2 and ten lines resistant to race 1-2. Results of classical analysis and molecular analysis were found concordant for race 1. In contrast, only ten lines were determined as resistant to race 2 in molecular analysis.

INTRODUCTION

Turkey is one of the most important melon producing countries in the world. In the world melon production, our country has come in third place after China and USA (FAO, 2010). There are many diseases limiting the production of melon. Melon production worldwide is limited by fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis* (FOM) which is an important soil-borne fungal pathogen. FOM has four races, designated 0, 1, 2 and 1–2 (Mas et al. 1981, Martyn and Gordon 1996).

Three wilt-resistance genes, *Fom-1, Fom-2* and *Fom-3* have been identified in melon (Mas et al. 1981; Zink and Gubler 1985; Martyn and Gordon 1996). *Fom-1* gene confers monogenic resistance against FOM races 0 and 2 (Brotman et al. 2005, Tezuka et al. 2009) and *Fom-2* gene confers resistance to FOM races 0 and 1 (Joobeur et al. 2004, Tezuka et al. 2009). Another gene, *Fom-3,* controls resistance to races 0 and 2 (Zink and Gubler, 1985; Schreuder et al. 2000). Resistance to race

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1-2 appears to be a quantitative trait (Perchepied et al. 2005).

The most effective means to control this disease is to use resistant cultivars (Martyn and Gordon 1996). Classical and molecular analyses are used to determine resistance to *Fusarium oxysporum* f. sp. *melonis* in breeding programs. In classical analysis are used traditional artificial inoculations. This situation causes time-consuming process such as young seedlings must be uprooted, roots pruned, dipped into inoculums of appropriate spore concentration, transplanted, and symptom development monitored constantly over four weeks or longer (Zink and Thomas 1990; Wechter et al. 1995). Identification of DNA markers tightly linked to a resistance gene is a powerful and useful tool to avoid the previously mentioned drawbacks in melon breeding programs (Oumouloud et al. 2008). An immediate, direct, efficient, and practical use of linked markers is marker-assisted selection (MAS) in plant breeding programs (Zheng et al. 1999).

In our study, one hundred summer-type pure melon lines were evaluated for resistance to FOM by using classical and molecular analysis methods. In breeding program, mechanical inoculation method for classical analyses and SSR154 and NBS1-CAPS markers for molecular analysis were used for determination of resistance.

MATERIALS AND METHODS

The melon line TF 96 BATEM, resistant to FOM and a standart melon variety Ananas, susceptible to FOM, were crossed to produce populations. FOM isolates of races 0, 1, 2 and 1-2 were used for inoculation of melon plantlets.

In classical analysis, root dip method was used. FOM isolates from infected melon plants were maintained in the greenhouse. Melon plants were inoculated with this isolate and two uninoculated plants were used as control. All plants were investigated for symptom expression for 7-14-21-28 days after inoculation.

In molecular analysis, genomic DNA was extracted from young leaves by the CTAB method as described by Doyle and Doyle (1990). NBS1-CAPS marker was used for *Fom-1* gene (Brotman et al. 2005). PCR amplifications were conducted as described by Brotman et al. (2005) with some modifications. SSR154 marker was used for *Fom-2* gene (Joobeur et al. 2004). PCR amplifications were conducted as described by Joobeur et al. (2004) with some modifications.

RESULTS AND DISCUSSION

The objective of this work was to transfer resistance genes to *cantalupensis* type melons by using FOM resistant melon lines. In breeding program, classical and molecular analyses were used for determination of resistance.

Classical analysis

One-hundred F_6 lines were tested for their reaction to races 1, 2 and 1-2 were noted to be resistant (without distinguishing heterozygous from homozygous plants) or susceptible by using classical analysis. As result of work: 73 lines were determined as resistant genotypes for race 1, 17 lines were determined as resistant genotypes for race 2 and 10 lines were determined as resistant genotypes for race 1-2.

Molecular analysis

One-hundred F6 lines were tested for having the *Fom-1* and *Fom-2* genes using molecular markers linked to these genes. NBS1-CAPS primer amplified a single 250 bp band, as expected from the sequence data, for the resistant and the susceptible genotypes. A codominant polymorphism was revealed after restriction of the 250 bp fragment with *Nco*I between resistant genotypes and the other lines (Fig. 1). Ten lines were identified as homozygous resistant at the *Fom-1 locus.* SSR154 primer provided a single 299 bp band, as expected from the sequence data, for the resistant genotypes (Fig. 2). In our study, 73 plants were identified as homozygous resistant at the *Fom-2* locus.

Successful breeding program depends on the availability and quality of resistance sources, and on effective methods for inoculating the plants and assessing their response to the disease. The expression of fusarium wilt symptoms following artificial inoculation is affected by the virulence of the pathogen isolates (Namiki et al. 1998), the genetic background of the plant (Mas et al. 1981), and environmental factors such as temperature, humidity and light intensity (Cohen et al. 1996). So, the use of molecular markers linked to the *Fom-1 and Fom-2* loci would be useful for breeders.

Application of MAS requires a tightly linkage (less than 5 cM) between the marker and the gene of interest or else the use of closely linked markers flanking the targeted locus (Tanksley 1983). NBS1-CAPS marker used in our study is tightly linked (2.8 cM) to the *Fom-1 locus* (Brotman et al. 2005). SSR154 marker is very tightly linked (0.4 cM) to the *Fom-2 locus* (Joobeur et al. 2004). These markers would be useful for the introgression of the *Fom*-1 and *Fom*-2 genes in an agronomical *C. melo* genetic background. Because of SSR154 marker tightly linked to the *Fom-2 gene, the* results of classical analysis and molecular analysis were found the same for FOM race 1. However, NBS1-CAPS were mapped at 2.8 cM from the *Fom-1* gene. So, analysis NBS1-CAPS in a set of 17 genotypes resistant to race 2 of FOM revealed that these markers do not separate all the genotypes according to their resistance phenotype. In our study, only ten plants were determined as having the resistant allele at the locus. According to the study of Oumouloud et al. (2008), NBS1-CAPS did not allowed the right identification of 3 out the 8 *cantalupensis* types analyzed.

CONCLUSION

Molecular markers used in our study are helpful for marker-assisted selection in a wide range of genetic intercrosses. On the other hand, it could be possible to distinguish heterozygous from homozygous resistant plants.

ACKNOWLEDGEMENTS

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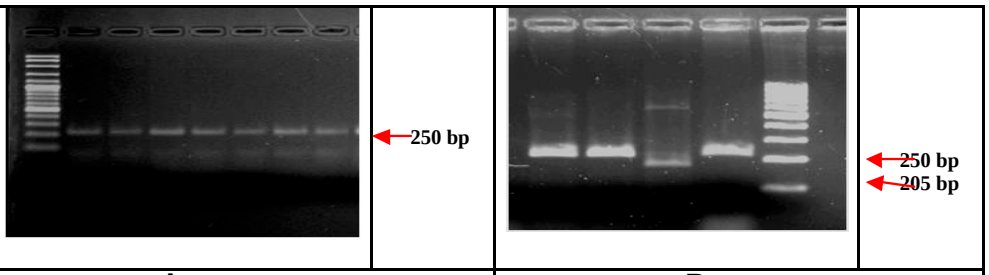

Fig. 1. Segregation of the codominant NBS1-CAPS linked to the *Fom*-1 locus (A). PCR with specific primers was followed by restriction with *Nco*I (B).

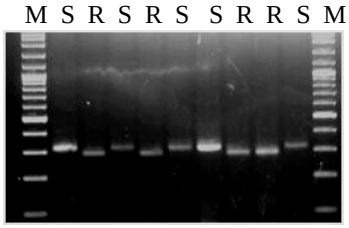

Fig. 2. Segregation of the codominant SSR154 linked to the *Fom*-2 locus. M: Size Marker, S: Susceptible, R: Resistant

Screening for salinity and drought tolerance in melons

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Keywords: *Cucumis melo*, salt stress, water stress, selection, breeding, resistance

Abstract

In this study, salinity and drought responses of 42 melon genotypes were investigated in early plant growth stage. The plants were grown in vermiculite culture with 200 mM NaCl for salinity stress and irrigation was stopped gradually for terminal-drought stress. Control plants without stress were also grown. Young plant stage (29 day-old plant) was used for screening studies. In order to identify tolerant and susceptible melon genotypes, some physiological measurements and analysis were realized: 0-5 scale evaluation for the symptomatic appearance of the plants, shoot fresh and dry weights, root fresh and dry weights, leaf number per plant, leaf area, SPAD-Chlorophyll meter reading, membrane injury index, leaf relative water content, leaf water potential, leaf osmotic potential, stomata conductance, leaf temperature, Na, K, Ca, and Cl concentrations of the shoot and root samples. For each parameter, differences in salinity and drought stresses compared to control were calculated. At the end of the study, large variations were determined in melon genotypes for their salinity and drought responses. Forty-two melon genotypes were classified as tolerant, mild tolerant and susceptible.

INTRODUCTION

Agricultural productions in arid and semi arid regions around the world are under the threat of drought and salinity due to climatic changes. These two abiotic stresses cause serious reductions in plant growth, yield and crop quality in Mediterranean region. Melon is one of the major economic crops in Turkey. It is grown in greenhouse and open field in the country. Tolerant cultivars that are developed by breeding studies can be the permanent solution for minimizing the deleterious effects of drought and salt stresses (Dasgan and Koc 2009). It is well

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known that drought and salt-tolerant accessions in a given species could exist in gene pools (Dasgan et al. 2002). The objective of the present work is screening of the domestic melon genotypes for drought and salinity, therefore saving of the resistant genotypes for future breeding programmes. This project was supported by one of the UNDP (United Nation Development Programme) programmes called "*MDG-F 1680 UN joint programme on enhancing the capacity of Turkey to adapt to climate change*" during 2009-2010.Screening for salt and drought tolerance in melon was previously reported in a few studies. Twenty melon cultigens (cultivars and breeding lines) in Israel were tested for salt tolerance by Medlinger and Pasternak (1992). All cultigens were grown in the field using drip irrigation at three salt salinity levels: electrical conductivity (EC) of 1.2, 7.5, or 14.0 $dS.m^{-1}$. Nineteen of the 20 cultigens proved to be salt-susceptible, one line was salt-tolerant at EC = 14.0. Thirty local melon genotypes in Turkey were screened for their drought (Kusvuran et al. 2012 a) and salinity (Kusvuran et al. 2012 b) tolerances at seedling stage and the tested melon materials could be assigned into three groups, including tolerant, intermediate tolerant and susceptible. In this study, we extended the screening to 42 Turkish genotypes.

MATERIAL AND METHODS

Forty-two native melon (*Cucumis melo* L.) genotypes, originating from Turkey, were used as plant material (Table 2). Melon plants were grown under salinity, drought and control treatments for 29 days during the period April 20 – May 19 under Mediterranean climate in Adana. The experiment was carried out in a greenhouse with day and night temperatures of approximately 24-28^{o}C and 18-20^{o}C, respectively, and relative humidity in the range of 60 to 70%. Seeds were directly sown into the vermiculite substrate in 2 liters plastic pots. For each genotype, 3 independent plants in one pot were used as one replication and 4 replicates for salt, drought and control treatments were used separately. Plants were irrigated by the full strength nutrient solution in ppm concentrations of 177.2 N; 52.70 P; 240.44 K; 53.46 Mg; 120.30 Ca; 3.36 Fe; 0.85 Mn; 0.45 B; 0.50 Zn; 0.10 Cu; 0.05 Mo. The amount of nutrient solution applied in the treatments was determined based on daily measured drainage fraction from the base of the pots (Dasgan et al. 2009). Range of drainage fraction was kept about 20% during the experimental period. The melon plants were grown without salt and drought treatments up to 3 true leaf stage (16 day-old plants). Then, NaCl was added with 50 mM increments on every day until the final concentration of 200 mM. Similarly drought stress were performed gradually during 4 days, later on the terminal drought was continued. Plants were subjected to the salt and drought stresses during 12 days. Control plants were grown under non-saline and non-drought conditions for the same period of time. In order to screen tolerant and susceptible melon genotypes, some physiological measurements and

analysis were realized; 0-5 scale evaluation for the appearance of shoot damage, shoot fresh and dry weights, root fresh and dry weights, leaf number per plant, leaf area, SPAD-Chlorophyll meter reading, membrane injury index, leaf relative water content, leaf water potential, leaf osmotic potential, stomata conductance, leaf temperature, Na, K, Ca, and Cl concentrations of the shoot and root samples in salt treatment, as previously described (Akhoundnejad 2011).

RESULTS AND DISCUSSION

The reason for page limitation we can only give the mean values of the screened physiological parameters of 42 melon genotypes in Table 1. In order to screen melons for salinity and drought stresses, among the 27 parameters the most relevant ones, the greater level of variation, were shoot dry weight, root dry weight, plant total leaf area, leaf water potential, stomatal conductance, K, Ca, Na and Cl concentrations in shoot and root, K/Na, Ca/Na, membrane injury index and visual appearance of damages by scale evaluation.

According to the physiological data, melon germplasm was classified into 3 groups: tolerant, moderate tolerant and susceptible (Table 2). For salinity response, 11 genotypes were tolerant, 20 genotypes were moderate tolerant and 11 genotypes were susceptible. For the drought responses, 15 genotypes were tolerant, 17 genotypes were moderate tolerant and 7 genotypes were susceptible. Only 5 genotypes were tolerant to both salinity and drought stresses. After screening of melon genotypes in young plant stage, field experiment until fruit stage with the selected tolerant genotypes is going to progressing. It might be argued that the screening for salinity and drought tolerances at early plant-growth stage may or may not produce tolerant adult plants (Dasgan and Koc 2009). The performance of young plants under stress conditions has been considered highly predictive of the response of the adult plants to the abiotic stesses (Qureshi 1990; Akhoundnejad 2011; Dasgan and Akhoundnejad 2012). The screening parameters at young plant stage in this study could contribute great effectiveness in reducing the number of genotypes in a feasible time in a large germplasm for future works including reproductive stage.

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Table 1. Physiological parameters of 42 melon genotypes grown under saline and drought stresses during 12 days (data is mean of 42 genotypes).

Screening parameters	Control	Salinity	Drought	Differences in salinity compared to control (%)	Differences in drought compared to control (%)
Plant height (cm)	26.15	11.23	11.45	-57.06	-56.21
Number of leaf (leaf/plant)	6.27	2.97	3.99	-52.63	-36.36
Shoot fresh weight (g/plant)	39.45	14.43	11.63	-63.42	-70.52
Shoot dry weight (g/plant)	3.29	1.24	1.48	-62.31	-55.02
Root fresh weight (g/plant)	13.30	6.22	6.25	-53.23	-53.01
Root dry weight (g/plant)	0.76	0.49	0.97	-35.52	27.63
SPAD reading	44.32	35.74	56.83	-19.36	28.23
Leaf area (cm^2/plant)	588.38	171.84	117.70	-70.79	-80.00
Relative water content (%)	77.85	65.82	73.57	-15.45	-5.50
Leaf water potential (-MPa)	0.02	0.04	0.09	100.00	350.00
Leaf temperature (^oC)	30.14	29.54	32.46	-1.99	7.70
Stomatal conductance (mmol $m^{-2}s^{-1}$)	104.49	90.54	50.42	-13.35	-51.75
Osmotic potential (-MPa)	1.23	2.90	1.46	135.77	18.70
Potassium in shoot (%)	4.04	2.35	5.15	-41.83	27.48
Potassium in root (%)	2.19	1.52	3.05	-30.59	39.27
Calcium in shoot (%)	1.97	2.55	2.72	29.44	38.07
Calcium in root (%)	0.56	0.38	0.60	32.14	7.14
Sodium in shoot (%)*	1.00	5.70	-	470.00	-
Sodium in root (%)*	1.13	3.11	-	175.22	-
Chloride in shoot (%)*	0.65	5.46	-	740.00	-
Chloride in root (%)*	0.561	2.54	-	352.76	-
K/Na ratio in shoot*	5.06	0.45	-	-91.11	-
K/Na ratio in root*	2.16	0.49	-	-77.31	-
Ca/Na ratio in shoot*	2.30	3.14	-	36.52	-
Ca/Na ratio in root*	0.55	0.12	-	-78.18	-
Membrane injury index (%) **	-	37.87	10.01	-	-
Scale	-	2.90	3.03	-	-

*: Salt treatment only, **: Control included for membrane injury index calculation.

Table 2. Salinity and drought responses of 42 melon genotypes classified as tolerant, mild tolerant and susceptible.

Salt stress responses of the melons			Drought stress responses of the melons		
Tolerant genotypes	Mild-tolerant genotypes	Susceptible genotypes	Tolerant genotypes	Mild-tolerant genotypes	Susceptible genotypes
CU-43	CU-2	CU-107	CU-134	CU-2	CU-43
CU-159	CU-3	CU-111	CU-159	CU-3	CU-176
CU-176	CU-130	CU-134	CU-179	CU-107	CU-230
CU-179	CU-135	CU-207	CU-202	CU-111	CU-244
CU-213	CU-151	CU-230	CU-207	CU-130	CU-246
CU-234	CU-202	CU-246	CU-208	CU-135	CU-251
CU-243	CU-208	CU-311	CU-234	CU-151	CU-280
CU-251	CU-209	CU-316	CU-311	CU-209	
CU-280	CU-235	CU-317	CU-317	CU-213	
CU-196	CU-244	CU-252	CU-196	CU-235	
Mln-9	CU-249	Mln-13	Mln-4	CU-243	
	CU-308		Mln-6	CU-249	
	CU-315		Mln-7	CU-315	
	CU-326		Mln-9	CU-316	
	Mln-1		Mln-13	Mln-1	
	Mln-4			Mln-10	
	Mln-6			Mln-11	
	Mln-7				
	Mln-10				
	Mln-11				

Functional validation of the double phenotype triggered by the melon *Vat* gene by complementation experiments

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Keywords: *Cucumis melo*, *Vat* gene, *Aphis gossypii* resistance, *Agrobacterium tumefaciens*.

Abstract

Aphis gossypii is the only species of aphid colonizing melon, causing stunting and leaf curling and transmitting non-persistent viruses. We demonstrated previously by a map-based cloning strategy that a single CC-NBS-LRR gene, named *Vat*, confers the double resistance to *A. gossypii* colonization and to unrelated viruses when transmitted by this vector. We confirm here by complementation experiments, the double function mediated by the *Vat* gene. Two transgenes carrying the *Vat* gene under the transcriptional control of its own promoter or under the control of the CaMV-35S promoter respectively were inserted via *Agrobacterium tumefaciens* into the susceptible melon cultivar 'Védrantais'. Transgenic T2 plants were evaluated for their resistance to *A. gossypii* colonization and to *Cucumber mosaic virus* (CMV) transmission by this vector, in comparison with susceptible and resistant melon cultivars. Transgenic plants carrying both transgenes exhibited the *Vat* phenotype, restricting aphid colonization and CMV infection. The *Vat* gene under the control of its own promoter induced a high level of resistance, similar to the referent resistant cultivar 'Margot', whereas the gene under control of the CaMV-35S promoter induced a slightly weaker level of resistance.

INTRODUCTION

Interactions between plant pathogens and their hosts are governed by highly specific recognition mechanisms. In most cases, resistance genes confer resistance to a single pathogen. In melon, a single resistance gene, called *Vat*, confers a double phenotype: resistance to *Aphis gossypii* colonization and to viruses when transmitted by this vector (Pitrat and Lecoq 1982). *A. gossypii* colonizes melon, causing serious

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stunting and severe leaf curling and transmits non-persistent unrelated viruses such as *Cucumber mosaic virus* (CMV) and Potyviruses. Several *A. gossypii* resistant melon accessions have been reported on which aphids quickly escape the plants, have a low biotic potential and do not transmit viruses. Different phenotypes of resistance were observed according to *A. gossypii* genotypes (Thomas et al. 2012). The *Vat* gene was isolated by map-based cloning from the Korean accession PI 161375. It belongs to the CC-NBS-LRR family; it is 6 kb long, carries 5 exons and 4 introns and encodes a predicted 1473 amino acid protein (Pauquet et al. 2004; Dogimont et al. 2007). We validate here, by complementation experiments, the double phenotype triggered by the melon *Vat* gene.

MATERIAL AND METHODS

Plant transformation

The C58C1-pch32 *Agrobacterium tumefaciens* strain was used for melon transformation and two different constructions carrying an 11 kb genomic fragment were obtained: Prom*Vat* with the binary vector pBin 19 carrying the *Vat* gene under the transcriptional control of its own promoter and 35S*Vat* with the binary vector pBin 61 carrying the *Vat* gene under the control of the 35S promoter from cauliflower mosaic virus (CaMV-35S promoter). Both constructions harbored the *nptII* selective gene for kanamycin resistance. The two *Agrobacterium* constructions were grown overnight in Luria-Bertani (LB) medium with 50 $mg.L^{-1}$ kanamycin and 20 $mg.L^{-1}$ gentamycin (for Prom*Vat*) or 5 $mg.L^{-1}$ tetracyclin (for 35S*Vat*). The Charentais type line 'Védrantais' (Vilmorin release) susceptible to *A. gossypii* colonization and to CMV infection was used for transformation experiments. Cotyledons from 4 day-old seedlings were used as explants and transformed following the protocol described by Chovelon et al. (2011).

Detection of the *Vat* and *nptII* genes by polymerase chain reaction

Genomic DNA was extracted from young leaves of putative transgenic plantlets and the presence of the transgene was checked by PCR using primers designed within the *Vat* and the *nptII* genes. The amplified products (857 pb for *Vat* and 450 pb for *nptII*) were subjected to electrophoresis and visualized by UV light after immersion in ethidium bromide solution.

Ploidy estimation and induction of parthenogenetic diploids from tetraploid plants

The ploidy level of transgenic plants was determined by flow cytometry. Samples were prepared from leaves, chopped in 1 ml of DAPI and filtered. The DNA content was analyzed with a flow cytometer (Partec) calibrated from leaves of diploid seedlings. The plant ploidy level was confirmed by phenotypic

observations: tetraploid melon plants have larger flowers, protruding stigmas, low fertility, thickened leaves, short internodes and rounder seeds than diploid plants (Ezura et al. 1992). Hermaphrodite flowers of tetraploid transgenic T0 plants were castrated before anthesis and pollinated with irradiated pollen (300 Gy). Fruits were harvested at 30 days. Parthenogenetic immature embryos were aseptically dissected from seeds and placed on culture medium for development of diploid plants (Sauton et al. 1987) at 26-28°C and 16-8h photoperiod.

Biological tests of resistance to *A. gossypii* and to *Cucumber mosaic virus* when transmitted by this aphid

Transgenic plants obtained were assessed for their resistance to *A. gossypii* and to CMV transmission by this vector according to Boissot et al. (2008). For each transgenic line, five T2 plants issued from seedlings were propagated by cuttings (10 plants from each T2 plant) and avaluated by biological tests. In each experiment ten plants of 'Védrantais' and 'Margot' were added as susceptible and resistant controls. For resistance to aphids, the differences of the index of colonization between transgenic lines and controls were analyzed by paired comparison according to Dunn procedure. For CMV resistance, the numbers of resistant and susceptible plants were compared for each transgenic line to 'Védrantais' and 'Margot' by a Khi^2 test.

RESULTS AND DISCUSSION

Melon transformation

Two independent experiments were completed with the two *A. tumefaciens* constructions Prom*Vat* and 35S*Vat* on 'Védrantais' cotyledon explants. Eleven transgenic plants, positive by PCR for the *Vat* and *nptII* genes, were obtained corresponding to a transformation rate of 1.55 % (Table 1), similar to that usually reported ranging from 0.7 to 3% (Chovelon et al. 2011). One plant transformed with Prom*Vat* was diploid and all the others were tetraploid. The diploid plant was self-pollinated and the segregation ratio for the *Vat* gene in the T1 generation was 3:1 (positive:negative by PCR) strongly suggesting the presence of a single insertion of the transgene. Parthenogenic diploid T1 plants were obtained from tetraploid T0 plants. Four T2 lines carrying the *Vat* gene under the control of its own promoter (Prom*Vat*) or under the CAMV-35S promoter (35S*Vat*) were obtained by self-pollination. T3 lines, homozygous for both transgenes, will be obtained in summer 2012.

Biological tests of resistance to *A. gossypii* and to CMV transmitted by this vector

Transgenic melon T2 plants were evaluated for their resistance to *A.*

gossypii colonization (Table 2) and to CMV transmission by this vector (Table 3) in comparison with the susceptible 'Védrantais' and resistant 'Margot' cultivars. All the transgenic melon plants, carrying both transgenes, exhibited the *Vat* phenotype restricting *A. gossypii* colonization and CMV infection. The index of aphid colonization obtained on the 35S*Vat* and Prom*Vat* lines was respectively three to six times lower than that obtained on 'Védrantais'. In the same way, CMV infection was significantly reduced on the three transgenic lines tested (68 to 100% of plants without CMV infection). The Prom*Vat* transgenic line showed a high level of resistance, similar to 'Margot' for both phenotypes. However, the two 35S*Vat* lines expressed a slightly lower level of resistance, significantly different from 'Védrantais' and 'Margot'.

CONCLUSION

The double phenotype mediated by the *Vat* gene on melon was demonstrated here by complementation experiments and biological tests. Two long size transgenes (11 kb), including the *Vat* gene, were successfully inserted by genetic transformation via *A. tumefaciens* in the susceptible 'Védrantais' cultivar and four T2 transgenic lines were obtained. Transgenic T2 line carrying the *Vat* gene under the control of its own promoter expressed a high level of resistance to aphid colonization and CMV transmission, similar to the resistant 'Margot' cultivar. The CaMV-35S promoter has been often used as a strong and constitutive promoter of the expression of many foreign genes. Surprisingly in these experiments, the two 35S*Vat* transgenic T2 lines expressed an intermediate level of resistance for both phenotypes. These results will be confirmed on T3 transgenic plants, homozygous for the *Vat* gene.

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Table 1. Melon transformation via *A. tumefaciens.*

Transgene	Number of explants	Regenerated plants	Transgenic plants obtained[Z]	Ploidy level 2n	Ploidy level 4n	Transforma-tion rate %	Number of transgenic diploid lines
Prom*Vat*	310	32	5	1	4	1.6	2
35S*Vat*	400	21	6	0	6	1.5	2

[Z] plants positive by PCR using *Vat* and *nptII* specific primers

Table 2. Resistance of transgenic T2 plants to *A. gossypii* colonization.

Cultivars and transgenes	Transgenic T2 lines	Number of plants	Index of aphids colonization[Z] (median)	Dunn group[Y]		
Margot	Control (R)	10	1.01	A	B	
Prom*Vat*	n°1	50	1.01	A		
35S*Vat*	n°2	49	2.01		B	
35S*Vat*	n°3	42	2.10		B	
Védrantais	Control (S)	10	6.64			C

[Z] index of colonization at 7 days: density of larvae estimated with 0-3 scale + ln(number of adults + 0.001).

[Y] paired comparison according to Dunn procedure (bilateral non parametric test).

Table 3. Resistance of transgenic T2 plants to CMV transmission by *A. gossypii.*

Cultivars and transgenes	Transgenic T2 lines	Number of plants tested	Number of resistant plants[Z]		Khi² compared to[Y] Margot	Khi² compared to[Y] Védrantais
Margot	Control (R)	10	10	100%		
Prom *Vat*	n°1	42	42	100%	1	<0.001
35S*Vat*	n°2	42	31	74%	0.026	<0.001
35S*Vat*	n°3	50	34	68%	0.047	<0.001
Védrantais	Control (S)	10	0	0%		

[Z] plants without symptoms of CMV infection 20 days after inoculation by *Aphis gossypii*

[Y] p-value obtained with Khi^2 Monte Carlo method: DDL=1, alpha=0.05

QTL associated with one recessive gene for powdery mildew resistance in the melon genotype TGR-1551

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Keywords: Codominant markers, *Podosphaera xanthii*, quantitative trait loci

Abstract

Resistance to powdery mildew (PM) races 1, 2 and 5 in TGR-1551 is independently controlled by one dominant and one recessive gene. A RIL population obtained from a cross between 'TGR-1551' and the susceptible Spanish cultivar 'Bola de Oro' has been evaluated for PM resistance to those three races in three different seasons (summer, fall, and spring). QTL analyses confirmed the existence of the *Pm-R* QTL associated with the dominant gene. An additional QTL, possibly associated with the recessive gene, has been also detected for PM resistance. This QTL is located on LG XII and several microsatellite markers (TJ29, CMBR111, and CMBR150) have been found to be associated with it. Differences in the percentage of variation explained by this QTL (LG XII) and the markers associated were observed among seasons.

INTRODUCTION

Powdery mildew of melon (*Cucumis melo* L.) caused by *Podosphaera xanthii* (Castagne) U Braun & N Shishkoff (Shishkoff 2000) is a limiting factor for melon production worldwide. Although fungicide application is the principal management practice, the use of resistant varieties is the better strategy for controlling this disease. Several genes for PM resistance (Pitrat 1991; Teixeira et al. 2008; Wang et al. 2011) and quantitative trait loci (QTL) associated (Perchepied et al. 2005; Fukino et al. 2008; Yuste-Lisbona et al. 2010a) have been mapped in different melon populations obtained from several genetic sources. In TGR-1551, the resistance to races 1, 2 and 5 of *P. xanthii* is independently controlled by one dominant and one recessive gene (Yuste-Lisbona et al. 2010b), but only a major QTL, *Pm-R*, associated with the dominant gene has been mapped on linkage group (LG) V (Yuste-Lisbona et al. 2010a).

The objective of this research is the evaluation of a RIL population obtained from the cross between TGR-1551 and the Spanish cultivar 'Bola de Oro' in order to detect any QTL that could be linked to the recessive gene for resistance to *P. xanthii* in TGR-1551.

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MATERIALS AND METHODS

Plant material

A RIL population (F7:F8) derived from a cross between the Zimbabwean PM resistant genotype TGR-1551 and the susceptible Spanish melon cultivar 'Bola de Oro', was used.

The *P. xanthii* isolates used in the artificial inoculations were '27' (race 1), '2204' (race 2) and 'C8 Cris' (race 5). Each isolate was kept under axenic conditions (Bertrand 1991) on cotyledons of 'Bola de Oro' for race 1, 'PMR 45' for race 2 and 'Edisto 47' for race 5. To increase the amount of conidia for the tests, the isolates were subcultured on zucchini squash cotyledons ('Diamante' and 'Negro Belleza', Semillas Fitó).

Inoculation test

This RIL population, together with the parental genotypes (TGR-1551 and 'Bola de Oro') and their F1 progeny, were evaluated in glasshouse for resistance to the three *P. xanthii* races using three plants per genotype in three environmental conditions: summer 2010 (30-35ºC day/20ºC night; 90 RIL), fall 2010 (25ºC day/10-15ºC night; 99 RIL) and spring 2011 (22-28ºC day/ 15-20ºC night; 95 RIL). Artificial inoculations were carried out following Ferrière and Molot (1988). Twelve days after inoculation, plants were scored according to the level of fungus sporulation, as follows: 0= no visible sporulation, 1= low level of sporulation, 2= moderate level of sporulation, and 3= profuse sporulation.

Extraction of genomic DNA and marker analyses

DNA was extracted from young leave tissue of each genotype using Plant DNAzol kit (Invitrogen, Germany) according to the recommendations of the supplier. Molecular evaluation of the RIL population was carried out with 260 molecular markers, most of them codominants (149 SSR, 96 EST-SSR). Some of these molecular markers have been previously reported to be linked to several resistance genes: SV01, SV06 (Oumouloud et al. 2008) AM, FM (Wang et al. 2000) SSR138, SSR154, SSR178 (Joobeur et al. 2004) to *Fusarium wilt* (*Fom-1 and Fom-2*); Marker E, Marker D (Dogimont et al. 2004) to *Aphid gossypii* (*Vat*); and PM1-SCAR, PM2-CAPS, PM3-CAPS, PM4-dCAPS, PM5-CAPS and PM6-SCAR (Yuste-Lisbona et al. 2011), linked to PM resistance. Mapping was then performed using JoinMap® 4.0 software (Van Ooijen 2006), being the markers grouped at a minimum LOD score of 4.0 and a recombination frequency value of 0.3. Kosambi mapping function (Kosambi 1944) was used to translate recombination frequency into centimorgans (cM).

In order to map powdery mildew resistance, the level of sporulation (0 to 3 scores) was used as a numerical value. QTL analyses were conducted for the three

RIL evaluations using Interval Mapping (IM) and Multiple QTL Model (MQM) mapping with MapQTL® 5.0 software (Van Ooijen 2004). A permutation test (1000 cycles) was used to determine the LOD threshold with a confidence interval of 99%.

RESULTS

In the genetic map obtained the 260 loci were distributed among 21 linkage groups. These groups were designed following the nomenclature proposed by Périn et al. (2002) and regrouped into twelve linkage groups. This map spanned a total genetic distance of 1098.48cM, with an average of 4.2cM between consecutive markers.

Two QTL were identified for the resistance to each of the three *P. xanthii* races tested and in the three evaluations. Besides the major QTL associated with the dominant resistant gene on LGV (*Pm-R*), one minor QTL, linked to the recessive gene, was identified on LG XII (Fig. 1). No significant differences were observed for QTL analysis parameters (LOD, variance, markers linked) among races tested. Differences among seasons were observed, though. The LOD scores obtained for the minor QTL ranged between 4.56 and 10.12, and explained on average around 18% of the phenotypic variation observed. The minimum values were observed in summer (LOD <6; variance <8%) and CMBR150 was the molecular marker associated with the QTL. In fall and spring, LOD scores were higher (6.52-10.12) and this QTL explained a phenotypic variance of around 17% in spring and 24% in fall. The molecular markers linked to this QTL in fall were TJ29 and CMBR111, and only CMBR111 was significantly linked in spring. These three markers flanking the minor QTL are 10cM apart from each other.

DISCUSSION

Two QTL associated with resistance to *P. xanthii* have been identified previously on LGXII. One of them is the QTL *PmXII.*1 (Perchepied et al. 2005), associated with *Pm-y* gene and controlling PM resistance to races 1, 2 and 5. Direct association of *PmXII.1* with the minor QTL reported in TGR-1551 was not possible due to the lack of common molecular markers between both genetic maps. The other QTL was identified by Fukino et al. (2008) who associated it with one of the two dominant genes for resistance to race 1 that they described previously in PMAR 5 (Fukino et al. 2004), as well as to *PmXII.1*. This QTL from Fukino et al. (2008) is strongly linked to the markers TJ29, CMBR 111 and CMBR 150, which are the same markers associated with the QTL described herein. Our results indicated that the minor QTL identified on LG XII could be associated with the recessive gene of TGR-1551 for races 1, 2 and 5, but further and more precise studies would be needed to determine if all these QTL are the same or are tightly linked in a cluster of resistance genes. Some environmental factors could be modifying the response

to PM regardless the race, since significant differences were observed herein among seasons, and no among PM races.

The recessive gene present in TGR-1551 is unique, since no recessive genes conferring resistance to more than one PM race has been reported previously in melon. This gene could confer a durable resistance to powdery mildew, such as the resistance conferred by *mlo* in barley (Jørgensen 1992). It is the first time that a QTL associated with a recessive gene conferring resistance to *P. xanthii* have been mapped in melon. Besides, the use of the markers identified as associated with this QTL would make the selection of PM resistant genotypes in breeding programs much more effective.

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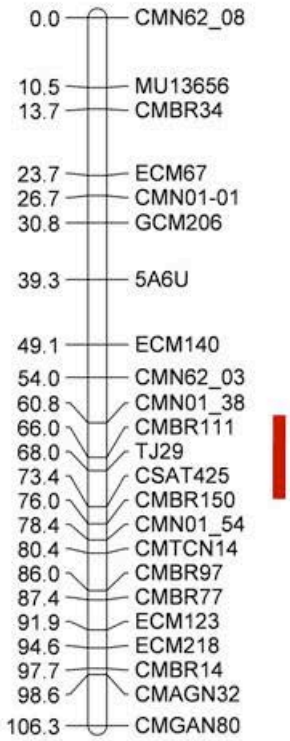

Fig. 1. Linkage group XII showing the genomic region where the QTL identified for powdery mildew resistance to races 1, 2, and 5 in TGR-1551 has been found.

Linkage map in Iranian melon (*Cucumis melo* L.) with emphasis on Fusarium wilt resistance gene

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Keywords: Iranian melon, linkage map, linkage group, Khatooni melon, Fusarium resistance

Abstract

Iran as a center of genetic diversity is one of the main producer of melon in the world. Regarding the unique characteristics of Khatooni as a main melon accession in Iran, this study aimed to constructing a linkage map with emphasis on fusarium wilt resistance gene race 1 using RAPDs and AFLPs. An F_2 population was developed through crossing the Khatooni and Charentais-Fom2. In order to develop the linkage map, 42 RAPD and 39 AFLP primer combinations were used. The results of the linkage analysis indicated that 293 polymorphic RAPDs and AFLPs fell into 15 linkage groups with a total length of 1291 cM. In this map the mean interval between markers in the constructed map was 6.38 cM. An AFLP marker, E-CA/M-CTG, with a distance of 21 cM away from fusarium wilt resistance gene race 1 was found. This is the first linkage map has been developed by using Iranian melon accession. This linkage map can be improved by further markers to obtain a high density linkage map.

INTRODUCTION

Melon (*Cucumis melo* L.*)* is one of the important and valuable species of Cucurbitaceae. According to the FAO statistics reported in 2010, Iran maintains the highest rank in production of melon next to China and Turkey. Given the fact that Iran is known to be one of the locations in the world that is important in terms of genetic diversity of melon (Robinson and Decker-Walters 1997). The present study was carried out to provide a preliminary linkage map so that it can be utilized in modern molecular and breeding studies on melon. In its attempt to achieve that

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purpose, this study placed the major emphasis on the Iranian genotype of this valuable vegetable crop. Linkage maps are viewed as a kind of basic research the results of which have been applied in a number of breeding activities. Some of the applications of linkage maps include gene tagging, map-based cloning, and marker assisted selection.

The first linkage map of melon was prepared by Baudracco-Arnas and Pitrat (1996) which 110 markers into 14 linkage groups with 1390 cM in length were mapped. Brotman et al. provided a tentative linkage map using an F_2 population. In this map which uses RFLP markers, SSRs, ISSR, RAPD, and Aphis-resistant, 107 points have been determined in linkage groups (Brotman et al. 2000). Perin et al. (2002) used two populations resulted from three Line Inboards prepared a map 1590 cM in length with 777 markers in 12 linkage groups. Oliver et al. (2001) in a genetic map specified 385 molecular markers including RFLPs, AFLPs, RAPDs, SSRs, ISSRs, SCAR, and morphological marker in 12 linkage groups with average of 3.1 cM per marker that included 1185 cM plant genome. Likewise Oliver et al. (2001), using 411 molecular markers and a morphological characteristics and employing an F_2 population, prepared a more comprehensive map. All loci were located in 12 linkage groups including 1197 cM and more anchor points (Oliver et al. 2001). In another attempt, using the two RILs and employing AFLPs, ISSRs, RFLP and disease-resistance characteristics and morphological characteristics, determined 668 loci on a map 1654 in length and in 12 linkage groups (Perin et al. 2002). Using an F_2 population produced by crossing a local accession, a linkage map was prepared in *C. melo*. This map which included 1421 cM of the plant's genome contained 179 RAPDs, AFLPs, SSRs, ISSRs, and RFLPs. The purpose of map of these markers has been to provide reference points, or anchor points, to be eventually combined with various linkage groups in some other map(s) containing these points (Silberstein et al. 2003). The principal objective of the present study was to construct a genetic map for *C. melo*, using RAPD, AFLP and morphological marker.

MATERIALS AND METHODS

Plant Material

The population, which consisted of 94 F_2 plants, were derived from cross between Khatooni (Iranian accession) and Charentais-Fom2 (French line). The steps and methods employed in the preparation of the afore-mentioned population are fully described in a separate article (Shojaeiyan et al. 2003). In order to screening of F_2 plants for resistance gene, artificial inoculation was done using spore of *F. oxysporum* f. sp. *melonis* race 1.

Genomic DNA

Genomic DNA has been extracted separately from 94 F_2 plants and the parents through the use of Genomic DNA Purification Promega Wizard Kit (Promega 1999). The quality and quantity of the DNAs were determined by a spectrophotometer, GenQuant RNA/DNA Calculator. Two DNA pools (dominant and recessive) were prepared, each containing a mixture of 10 DNA plants from those plants which had dominant and recessive reactions in artificial inoculation respectively. These two bulked DNA were used for primary marker screening in bulked segregant analysis.

Analysis of RAPDs and AFLPs

RAPDs were analyzed on the basis of the procedure proposed by Williams (Williams et al. 1990). The components of PCR reaction, the reaction quantities and the thermal cycler were set as explained in the previous article (Shojaeiyan et al. 2003). The PCR products were loaded onto the 10% acrylamide gel and electrophoresis was done through the use of CBS electrophoresis. The gels were stained with silver nitrate.

AFLPs were analyzed by following the procedure described by Buerstmayr et al. (2002), using *MseI* and *EcoRI* enzymes. Selective amplification was performed using primer combinations two/three selective nucleotides. A total of 81 polymorphic markers (42 RAPD and 39 AFLP) were obtained after screening with DNA of parents and pools. Markers showing polymorphism and association with the resistance locus were evaluated on DNA from single plants of the two pools. Finally those markers associated with the resistance trait, found through BSA, were used on the entire F_2 population to calculate the degree of linkage.

Data analysis

Given the nature of RAPDs and AFLPs, the band patterns resulted from PCR of each primer that were polymorphic among parents and F_2 population were scored on the basis of the dominant fashion (i.e., score of 1 was given to presence of band and that of 0 to the absence of it). For the data obtained from polymorphic markers, deviation test from Mendel's ratio was performed through the use of Chi-square test. For the construction of the linkage map, use was made of markers whose inheritance model was not significantly different from the expected ratio of 3:1 at .05 level of significance and the remaining markers were excluded from the data. The linkage map was categorized by means of MAPMAKER/Exp. 3.0b software (Lander et al. 1987; Lincoln & Lander 1992) and by considering LOD$\geq$3 and the maximum distance of 50 cM. For drawing the linkage map, use was made of MapChart software package, version 2.1 (Voorrips 2002).

Nomination of Markers

For the nomination of AFLPs, it has been made of the initials of the names

of restriction enzymes that have been utilized at the stage of enzyme digestion and also of the order of polymorphic bands that are alphabetically ordered from the smaller to larger fragments. As for RAPDs, the first part of the marker belongs to the name of a primer from the employment of which the associated polymorphism has been observed. This part is composed of one or two English capital letters and a two-digit figure, named after Operon primers. The second part consists of a three or four-digit figure which is separated from the first part by a decimal sign and indicates the size of the polymorphic fragments.

RESULTS AND DISCUSSION

Linkage Map

From among 120 AFLP primer combinations (which had already been screened on parents and bulks), 39 primer combinations were selected on account of their having the largest number of polymorphisms and their scorability. Also, 42 primers were selected out of 432 RAPD primers estimated in parents. Overall, out of 81 primers used for screening of the population, 293 polymorphic markers, with 3:1 Mendel segregation ratio, were obtained. The linkage analysis related them to 15 linkage groups (Fig. 1). The summary of the linkage map is presented in Table 1. The present map is 1291 cM in length and includes groups with minimum length of 16.6 cM and maximum length of 234 cM. There were four groups (LG1, LG2, LG4 and LG5) more than 100 cM long and four groups (LG8, LG13, LG14 and LG15) less than 50 cM long which constituted the largest and the smallest linkage groups in the map respectively. An AFLP marker, E-CA/M-CTG, with a distance of 21 cM away from fusarium wilt resistance gene race 1 was found in LG1. The range of markers associated with each group varied from 4 to 67 markers. The mean distance of the marker in linkage groups was estimated at 6.38 cM, which is indicative of the relative saturation of the map. The procedure employed for the calculation of the average distance was that proposed by Remington et al. (1999). The characteristic feature of the average distance between the adjacent markers which is referred to as the concentration of the map varied depending on the length of the group and the number of markers in each group. The linkage group 9 that is 62.9 cM long and that has 20 markers has found to be more concentrated than other groups. High saturation has always been one of the features taken into account in the saturation of genetic maps. Yet, observing 9 large distances in 8 linkage groups (LG1, LG2, LG3, LG4, LG7, LG10, LG12 and LG13), each more than 25 cM long, shows the extent to which the map of the above-mentioned groups has been saturated.

This is the first linkage map of melon in which Iranian germplasm has been used and the improvement of this map, particularly with multiple allelian markers which have been adequately preserved in the genome of the melon groups can enhance the validity of the map. Future research in this area needs to aim at achieving

a high degree of transparency and concentration in the construction of genetic maps in a manner which results in an increase in their validity and saturation.

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Table 1. Number of markers and length for each linkage group of the melon linkage map.

LG	Length (cM)	No. of markers	cM/marker	LG	Length (cM)	No. of markers	cM/marker
LG1	103.7	19	5.75	LG9	62.9	20	3.31
LG2	226.6	44	5.27	LG10	80.5	17	5.03
LG3	85	19	4.72	LG11	58.1	5	14.52
LG4	234	67	3.54	LG12	75.5	13	6.29
LG5	126.1	29	4.5	LG13	28.3	4	9.43
LG6	66.3	16	4.42	LG14	35.1	5	5.77
LG7	72.8	22	3.46	LG15	16.6	6	3.32
LG8	19.5	7	3.25				

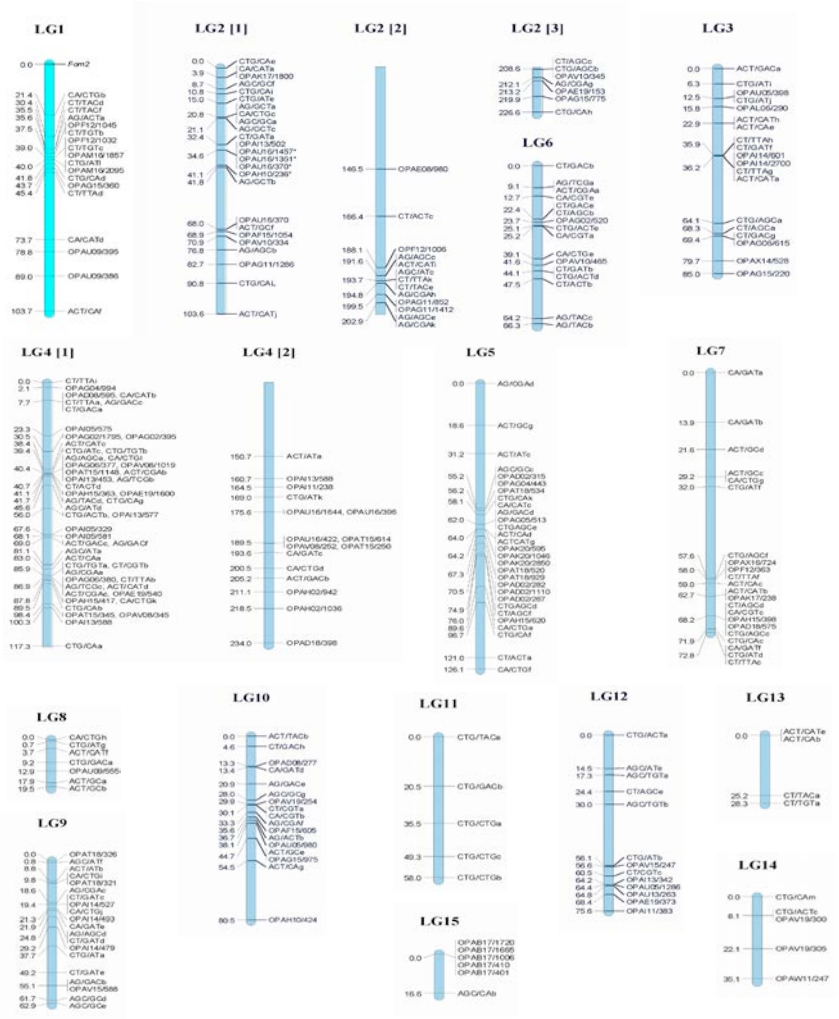

Fig. 1. Linkage groups in *Cucumis melo*, the loci and their size have been listed to the right of bars and the distances between markers have been placed to the left side of bars.

Determination of genetic diversity among Çumra melon genotypes by ISSR markers

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Keywords: Characterization, Çumra, melon, molecular

Abstract

The present study aimed to determine genetic relationships among 32 melon genotypes collected from Çumra-Konya by molecular markers. Thirty-six polymorphic ISSR markers obtained from 10 primers were used to define the genetic similarity among the melon genotypes by dendrograms or two and three dimensional scaling obtained from Simple Matching distance matrix. The computer program POPGENE was also used to calculate the statistical measures of genetic variation (Nei's gene diversity (H), Shannon's information index (I), and percentage of polymorphic loci).

Based on the molecular Simple Matching distance matrix, the most similar genotypes were C15-C22 followed by C23-C26. Of all evaluated genotypes, the most distinct one was C28. The genetic variation estimates for melon genotypes in the present study were high (H = 0.27, I = 0.43 and 100 % polym).

INTRODUCTION

Melon (*Cucumis melo* L.) whose origin is the African continent, but its secondary gene center spans across the world from Turkey to Japan, is a one of the important cucurbit crops in the world and Turkey (Anonymous 2010; Pitrat et al. 1999; Jeffrey 2001; Sensoy et al. 2007a). Its early domestication might have been occurred in the Middle East (Robinson and Decker-Walters 1997; Jeffrey 2001; Luan et al. 2008). It is a diploid plant species with twelve main chromosome number (x=12) and could be categorized into different groups (Robinson and Decker-Walters 1997; Pitrat et al. 1999).

Morphological, isozyme and DNA markers have been employed in genetic similarity and diversity analysis in melon as well as many other crops (Waugh and Powel 1992; Rafalski and Tingey 1993; Lee 1995; Winter and Kahl 1995; Meglic and Staub 1996; Stepansky et al. 1999; Sensoy et al. 2007a; Szamosi et al. 2010). Molecular marker technology is developing rapidly with each passing day, and

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becomes important in plant breeding (Lee 1995; Winter and Kahl 1995). Appropriate strategies have been determined in order to determine the genetic variation in the collections of plant genetic resources. (Gilbert et al. 1999).

In recent years, the use of DNA marker technology in the classification of genetic resources in melon is also common (Sensoy et al. 2007a, b; Luan et al. 2008; Nimmakayala et al. 2008; Sestili et al. 2008; Yi et al. 2009; Chen et al. 2010; Nhi et al. 2010; Soltani et al. 2010; Yildiz et al. 2011).

Konya is one of the major melon producer provinces in Central Anatolia which has a continental climate. The Cumra melon landrace, an *inodorus* type, is produced plenty in the Cumra town of Konya. In the present study, we employed molecular ISSR markers in order to more clearly define genetic similarities among melon genotypes collected in the Cumra melon landrace.

MATERIALS AND METHODS

Plant material: The thirty-two Cumra melon genotypes constituted the plant material.

DNA extraction: The CTAB procedure (Doyle and Doyle 1987) was used to extract genomic DNA; then, DNA was qualified with Biotech UV 1101 photometer and the diluted in water to a final concentration of 25 ng/µl.

ISSR amplification: The used polymorphic ISSR primers (Levi et al. 2005; Djè et al. 2006) are presented in Table 1 and Fig. 1. PCR reaction mixture had 20 ng DNA, 1.5 mM $MgCl_2$, 0.2 µM Primer, 0.2 mM dNTP, 1X PCR buffer, 1 unit of Taq DNA polymerase (Promega, USA) in a total volume of 20 µL. The ISSR PCR reactions were performed as follow: 3 min at 94°C, 60 sec at 94°C, 63 sec at 50-60°C, 2 min at 72°C for 40 cycles and a final extension of 10 min at 72°C DNA Thermal Cycler (Sensoquest Progen Scientific Ltd. Mexborough, South Yorkshire, UK). The gels were prepared by adding 20 µL of ethidium bromide (10 mg/ml) in 500 mL of agarose. The amplified products were electrophoresed on 1.5 % agarose gel in 1X TBE buffer at 115 V using Maxi-Plus Standart Horizontal Gel Unit (SCIE PLAS) for 3 h and visualized by Gel Logic 1500 (Kodak). A 100 bp ladder (Fermentas) was used as molecular weight.

Table 1. The ISSR primers used in the study.

Primers	Nucleotids of primers	Total bands	Polymorphic bands	Tm (°C)
P2	DDC-(CAC)5	5	3	60
889	AGTCGTAGT(AC)5	8	4	55
Sola1	BDB-(ACA)5	4	2	50
Sola2	DD-(CGA)5	5	3	56
Sola4	VHV-(GT)7G	5	4	56
Sola5	DBD(AC)7	5	5	50
Sola6	BDB-(CAC)5	5	3	60
Sola7	(AG)8YT	3	2	52
Sola9	(AC)8G	7	5	52
Sola10	(AC)8YG	7	5	56

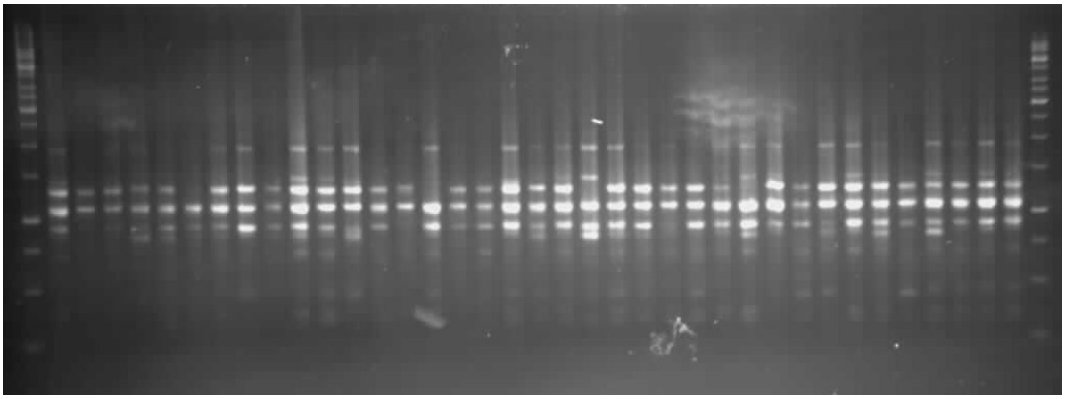

Fig. 1. The bands obtained from Sola 4 ISSR primer.

Data analysis: A binary data matrix (presence (1) /absence (0)) obtained from scoring polymorphic bands was used to determine Simple matching (Sokal and Sneath 1963) similarity coefficient to estimate the molecular genetic diversity among melon genotypes. The unweighted pair-group method using arithmetic average (UPGMA) cluster analysis, the resulting dendrogram and 3-dimentional scaling were performed on the genetic distance matrix using the computer program NTSYpc version 2.02k (Rohlf 1997). The computer program POPGENE (Yeh et al. 1997) was used to estimate the statistical measures of genetic diversity (i.e. Nei's gene diversity (Nei 1973), Shannon's information index (Shannon and Weaver, 1949) and percentage of polymorphic loci) as measured by ISSR markers for Cumra melon genotypes.

RESULTS AND DISCUSSION

Thirty-two melon genotypes were examined and polymorphisms among them detected at thirty-six loci by using ten ISSR primers were employed in the genetic assessment (Table 1). Based on the molecular Simple Matching distance

matrix, the most similar genotypes were C15-C22 (1.00 coefficient) followed by C23-C26 (0.95 coefficient), and C17-23 (0.94 coefficient) (Fig. 2 and 3). Of all evaluated genotypes, the most distinct one was C28 (Fig. 2 and 3). The genetic variation estimates for the studied melon genotypes in the present study were high (H = 0.27, I = 0.43 and 100 % polym.) (Table 2).

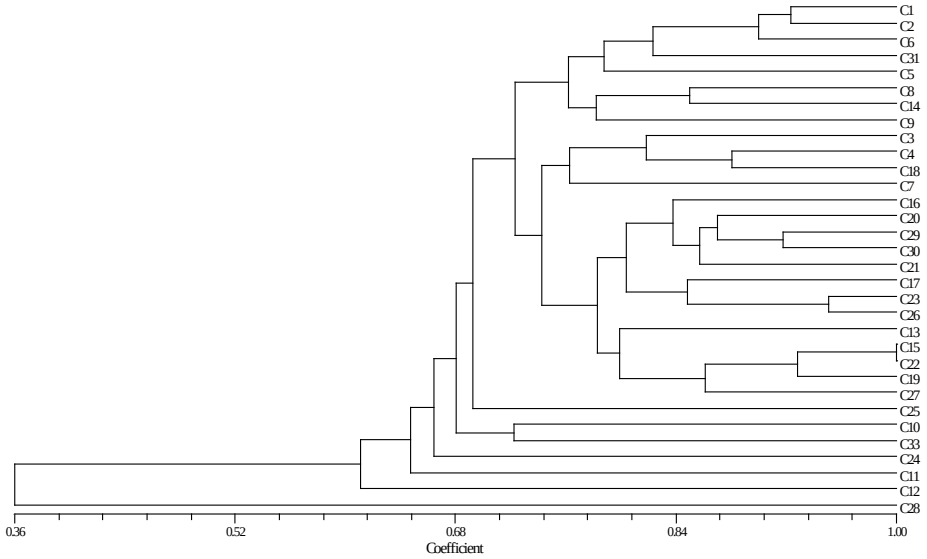

Fig. 2. Clusterings of Cumra melon genotypes revealed by UPGMA analysis on the basis of the molecular SM distance values.

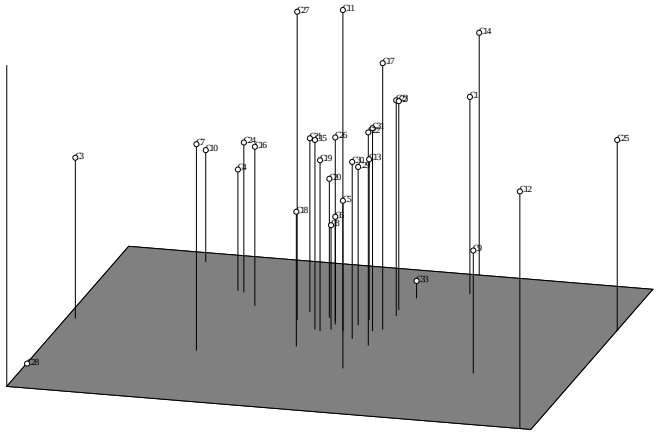

Fig. 3. Insert the legend of figure 3

Table 2. Genetic diversity measurements by ISSR markers.

Genotypes	N[a]	H[b]	I[c]	% Polymorphism[d]
Cumra melon genotypes	32	0.2774	0.4324	100

[a]N= Number of genotypes in each population; [b]H= Nei's gene diversity;
[c]I= Shannon's information index; [d]: Percentage of polymorphic loci.

Dje et al. (2006), showed that ISSR could be used as a powerful molecular tool in the determination of genetic diversity in Africa cucurbit species. It was also observed that ISSR and RAPD markers could be successfully used in determining relationship between the genetic relationships in *Citrullus* and *Cucumis* species (Levi et al. 2005). ISSR was also successfully employed to assess genetic diversity in melon and other cucurbits (Sestili et al. 2008; Dje et al. 2010; Yildiz et al. 2011)

So far melon germplasm in several countries as well as Turkey have been studied by phenotypic and molecular methods (Garcia et al. 1998; Silberstein et al. 1999; Garcia-Mas et al. 2000; Mliki et al. 2001; Staub et al. 2004; Nakata et al. 2005; Sensoy et al. 2007a; Sheng et al. 2007; Luan et al. 2008; Yi et al. 2009; Nhi et al. 2010; Soltani et al. 2010; Yildiz et al. 2011). Sensoy et al. (2007a) found that the genetic variation measurements determined by RAPD markers among Turkish melon genotypes were H = 0.29, I = 0.43 and 90 % polymorphisms. Yildiz et al. (2011) determined the genetic diversity by ISSR, SRAP, and RAPD markers and found high variation among Turkish melon genotypes (H = 0.28, I = 0.43 and 90.7 % polym.). Lopez-Sese et al. (2002) determined the genetic variation of Spanish genotypes as H = 0.17, I = 0.25 and 44 % polymorphism. The genetic variation of African landraces were H = 0.34 and I = 0.50 and 85 % polymorphism (Mliki et al. 2001). Yi et al. (2009) studied the genetic variation in Myanmar melon genotypes and determined the genetic diversity as 0.239. Luan et al. (2008) determined that Chinese melon genotypes had high genetic diversity values (H = 0.33, I = 0.49 and 90.6 % polym.). On the other hand Chen et al. (2010) had relatively lower genetic diversity values ((H = 0.22, I = 0.34) in 61 Chinese melon accessions. In the present study, the genetic diversity among the Cumra melon genotypes were also high (H = 0.27, I = 0.43 and 100 % polym.).

Identical accessions could be discarded after evaluating the collections in the germplasm. Molecular markers have been successfully employed in the determination of genetic relationships (Gilbert et al. 1999). Local melon genotypes might possess valuable characteristics for different biotic and abiotic stress agents (Sensoy et al. 2007b; Ekbic at al. 2010). In the present study, relatively high genetic diversity was observed in Cumra melon genotypes, which might be effectively used in future cultivar improvement programs.

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Genetic transformation in melon

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Keywords: *Agrobacterium*, particule bombardment, pollen tube pathway, marker-free vector, *Cucumis melo* L.

Abstract

Melon is one of the species in *Cucurbitaceae* which has intense transformation researches for two decades. We review here the various approaches reported in the literature. *A. tumefaciens* was used mainly in melon transformation studies. Few studies were reported by using particule bombardment, *A. rhizogenes* and Zucchini yellow mosaic potyvirus-vector. In last years, new methods named ovary injection, pollen tube pathway and new genes called marker-free and vector-free genes were used in melon transformation studies.

MINI REVIEW

Melon (*Cucumis melo* L.), is one of the most economically important and widely cultivated horticultural crops in the world and has been the subject of intense biotechnological researches for two decades. There have been several reports on the genetic transformation of melon with a variety of marker genes as well as genes for disease resistance, biotic and abiotic stress resistance, fruit quality attributes (sweetness and aroma) and long fruit shelf life. In melon transformation, the reporter genes used are GFP (green fluorescent protein) and GUS (beta-glucuronidase), plant selection markers are NPTII (neomycin phosphotransferase II), HPT (hygromycin phosphotransferase) and Bar (phosphinothricin resistance gene) genes (Shiboleth et al. 2001; Galperin et al. 2003; Akasaka-Kennedy et al. 2004; Nunez-Palenius et al. 2007; Castelblanque et al. 2008). Disease resistance has been achieved by the genes coding for the coat protein (CP) gene of various plant viruses such as cucumber mosaic virus (CMV), watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) (Yoshioka et al. 1992; Clough and Ham 1995; Wu et al. 2009; Ren et al. 2012). Transformed melon expressing HAL1 (halotolerance 1) gene is related

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with salt tolerance (Bordas et al. 1997). Some genes coding antisense suppression of alcohol acetyltransferase (antisense AAT2) in ripening melon fruit alters volatile composition (Shan et al. 2012). Antisense ACC oxydase (1-aminocyclopropane-1-carboxylic acid oxidase) gene was used for ethylene inhibition and long shelf life (Ayub et al. 1996; Guis et al. 2000). Recently, a marker free and vector free antisense ACC oxidase gene cassette was used to improve shelf life (Hao et al. 2011). For sex determination studies, dominant negative Arabidopsis ethylene response mutant etr1-1 gene (ethylene receptor) was used to inhibit ethylene action and observe flower structures (Little et al. 2007). Ribozyme and polyribozyme genes confered protection against two potyviruses, WMV and ZYMV (Huttner et al. 2001) (Table 1).

Plant transformation technology is a potential tool for improving new plant cultivars and presents new prospects to conventional melon breeding by allowing the introduction of desirable traits. *Agrobacterium tumefaciens* mediated transformation has been used as the most succesfull method in melon (Fang and Grumet 1990; Dong et al. 1991; Fang and Grumet 1993; Valles and Lasa 1994; Gonsalves et al. 1994; Gray et al. 1995; Ezura et al. 1997; Fuchs et al. 1997; Shetty et al. 1997; Clendennen et al. 1999; Shellie 2001; Huttner et al. 2001; Silva et al. 2004; Taler et al. 2004; Yalcin-Mendi et al. 2004; Curuk et al. 2005; Papadopolou et al. 2005; Nunez-Palenius et al. 2006; Little et al. 2007; Nonaka et al. 2008; Chovelon et al. 2011; Ren et al. 2012). In few reports, particule bombardment (Gaba et al. 1992; Gray et al. 1995), *A. rhizogenes* (Pak et al. 2009) and zucchini yellow mosaic potyvirus-vector (Shiboleth et al. 2001) transformation methods gave good results. In the last two years, a new transformation method, the pollen tube pathway was used by Hao et al. (2011). They transformed a marker-free and vector-free antisense ACC oxidase gene cassete into melon by dripping plasmid DNA solution on to the surface of style. It was mentioned that this method was not tissue culture based which needs technical skills and regeneration capacity by Shan et al. (2012). In addition, it was not genotype dependent. Therefore this method had more advantages than *A. tumefaciens*. Melon ovary-injection transformation method was also performed by injecting plasmid DNA into the ovary 24 h after hand pollination of the melon by Shan et al. (2012) (Table 1). Transformation rates of pollen tube pathway and ovary injection methods were found as approximately 0.7 % and 1.3 % respectively.

Plant transformation efficiency depends on the species and cultivars to a large extent. The method used also determines this frequency. Many researchers mentioned that transformation efficiency in melon was lower than that of other species (Akasaka-Kennedy et al. 2004; Nunez-Palenius et al. 2007; Ren et al. 2012). There are many reports on transformation efficiency in different varieties of melon such as 0.4-1.5 % (Galperin et al. 2003), 2.3 % (Akasaka-Kennedy et al.

2004), 1.6 % (Yalcin-Mendi et al. 2004), 10 % (Nunez-Palenius et al. 2007), 1.8-4.5 % (Chovelon et al. 2011), 0.7 % (Hao et al. 2011), 0.3-0.5 % (Ren et al. 2012), 1.3 % (Shan et al. 2012). Wu et al. (2009) reported that transformation frequency varied widely among cultivars and sources of explants. In addition, many researchers mentioned that high frequency of tetraploid instead of diploid and false positive regenerants were common phenomenon in melon regeneration and transformation (Adelberg et al. 1994; Chovelon et al. 2011). Because high ratio of transgenic melon plants were found to be tetraploid, researchers tried to find new methods to get diploid transgenic lines.

CONCLUSION

Transformation studies in melon has been done for two decades. *A. tumefaciens* transformation method was used in most of the researches. In the last few years, different transformation methods such as ovary injection, pollen tube pathway were used. In addition to these methods, researchers started working with marker-free and vector-free genes. These improvements in transformation will be less time consuming and will not be tissue culture based which needs technical skills and regeneration frequency.

Table 1. Transformation researches in melon.

Melon genotype	Transferred genes	Transformation method	References
Western shipper cantaloupe and Honeydew	NPTII, GUS	*A. tumefaciens*	Ren et al. 2012
*	AAT2 (antisense)	Ovary injection	Shan et al. 2012
Hetao	Marker free and vector-free antisense ACC oxidase	Pollen tube pathway	Hao et al. 2011
Védrantais	GUS	*A. tumefaciens*	Chovelon et al. 2011
Geumssragi-euncheon	GFP-GUS	*A. rhizogenes*	Pak et al. 2009
Oriental melon	ZYMV	*A. tumefaciens*	Wu et al. 2009
Védrantais	Vir	*A. tumefaciens*	Nonaka et al. 2008
Piel de Sapo, Védrantais, Agrestris PI 161375	NPTII, GUS	*A. tumefaciens*	Castelblanque et al. 2008
Hale's Best Jumbo	etr1-1	*A. tumefaciens*	Little et al. 2007
Galia male and female parental lines	GUS-GFP	*A. tumefaciens*	Nunez-Palenius et al. 2007
Galia male parental line	GUS-ACCoxidase (antisense gene from melon)	*A. tumefaciens*	Nunez-Palenius et al. 2006
Kirkagac, Noi Yarok		*A .tumefaciens*	Curuk et al. 2005
Hale's Best Jumbo	ACS synthase (gene from petunia)	*A. tumefaciens*	Papadopoulou et al. 2005
BU21/3 line	At1, At2	*A. tumefaciens*	Taler et al. 2004
Kirkagac	ZYMV, NPTII	*A. tumefaciens*	Yalcin-Mendi et al. 2004
Védrantais, Earl's Favourite Fuyu A	GUS, HPT	*A. tumefaciens*	Akasaka-Kennedy et al. 2004
Védrantais	ACC oxidase (antisense gene from apple)	*A. tumefaciens*	Silva et al. 2004
Hemed, Galia, Ananas Yokneam (local commercial melon cultivars) and Hale's Best Jumbo	GUS-GFP	*A. tumefaciens*	Galperin et al. 2003
*	Polyribozyme, GUS, GFP	*A. tumefaciens*	Huttner et al. 2001
Arava	Bar	Zucchini yellow mosaic potyvirus-vector	Shiboleth et al. 2001
Netted Melon	SAM hydrolase	*A. tumefaciens*	Shellie 2001
Védrantais	ACC oxidase (antisense)	*A. tumefaciens*	Guis et al. 2000
Sunday Aki	GUS	*A. tumefaciens*	Shetty et al. 1997
NR	SAM hydrolase	*A. tumefaciens*	Clendennen et al. 1999
Asgrow western shipper cantaloupe inbreds	CMV, ZYMV, WMV-2 coat protein	*A. tumefaciens*	Fuchs et al. 1997
NR	ACC synthase(antisense)	*A. tumefaciens*	Ezura et al. 1997
Pharo, Amarillo Canario	HAL1, NPTII, GUS	*A. tumefaciens*	Bordas et al. 1997
Védrantais	ACC Oxidase (antisense)	*A. tumefaciens*	Ayub et al. 1996
Eden Gem	NPTII	*A. tumefaciens*, particle bombardment	Gray et al. 1995
Don Luis, Galleon, Hiline, Mission, Parental inbred	ZYMV, WMV, CMV coat protein	*A. tumefaciens*	Clough and Ham 1995
Burpee HYbrid, Hale's Best Jumbo, Harvest Queen, Hearts of Gold, Topmark	CMV-white leaf coat protein	*A. tumefaciens*	Gonsalves et al. 1994
Amarillo Oro	NPTII, GUS	*A. tumefaciens*	Valles and Lasa 1994
Hale's Best Jumbo	ZYMV coat protein	*A. tumefaciens*	Fang and Grumet 1993
Galia	GUS	Particule bombardment	Gaba et al. 1992
Prince and EG360	CMV coat protein	*A. tumefaciens*	Yoshioka et al. 1992
Orient Sweet	DHFR, NPTII, GUS	*A. tumefaciens*	Dong et al. 1991
Hale's Best Jumbo	NPTII	*A. tumefaciens*	Fang and Grumet 1990

*Cultivar not mentioned

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Heterosis effect on plant growth, fruit yield and quality in single, triple and double crosses of melon (*Cucumis melo* var. *cantalupensis*) hybrids

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Keywords: Heterosis, heterobeltiosis, hybrid melon breeding

Abstract

In order to investigate the effect of heterosis, some quantitative characteristics were determined in 53 single, triple and double hybrid melon genotypes compared with the parental lines and Galia F_1 hybrid as control. Within the framework of this research, the heterosis and heterobeltiosis ratios were calculated for hybrids with two, three and four parents for the following characteristics: seedling emergence, lengths of cotyledons and the hypocotyls; length and diameter of the main stem, node number, first female flowering, total and early yields; fruit weight, length and diameter, and total soluble solids (TSS). The highest heterobeltiosis (184.23%) and heterosis (184.55%) rates were produced in earliness by Special Combination (SC)-58 (G22 X C1) genotypes belonging to the single hybrid group. Furthermore, superiority of the single hybrid group was observed both in total yield and in earliness of yield. Plant development criteria were also found better in single hybrid melon group.

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the economically important species of *Cucurbitaceae* family. Turkey is the second major melon producer after China in the world (FAOSTAT 2010). Melon production, generally in open fields, is located mainly in the Aegean, Central Anatolia, Marmara and the Mediterranean regions of Turkey. Nevertheless, production of *Cucumis melo* var. *cantalupensis* varieties under low tunnels and greenhouses by early spring planting is practiced in the Mediterranean region.

Heterosis is expressed as an agricultural phenomenon, in which growth, productivity, earliness, quality and other features of hybrid genotypes are superior

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compared to their parents. While heterosis is defined as the hybrids having higher average values than their parents, heterobeltiosis is the superiority of hybrids over the parents with the highest values. Heterosis may also occur in negative sense (inferiority) depending on the used features which are highlighted (Macit 1972; Yıldırım 1985; Birchler et al. 2003; Virmani et al. 2003; Cho et al. 2004). Heterosis breeding has been investigated for many years and the first hybrid vigor study was on cucumber (*Cucumis sativus* L.) (Rai and Rai 2006). In later years, productivity and earliness heterosis were determined by taking advantage of the genetic male sterility in melon hybrid (Nadpuri et al. 1974). In another study on heterosis in the hybrid individuals, plant heights and stem diameters, compared to parent plants, in terms of thickness and length have been determined (Sari et al. 2003).

A study was conducted on pepper by Geleta et al. (2004) to evaluate single, three-way and double cross hybrids in comparison with a standard control for their yield potential and agronomic performance. Three-way cross hybrid pepper breeding was more effective than other hybrids reported. As expected in melon hybrids, the single hybrids were much more uniform and it was followed by a three-way hybrids. The three-way cross, fruit yield (36.1%) and in terms of fruit length (13.6%) showed heterosis. Two-way hybrids, fruit yield (35.6%), the number of fruits (24.0%) and fruit weight (16.9%) showed good values which can be considered a higher heterosis. This was one of the results of the study, emphasizing the three-way cross could be used in pepper hybrids breeding.

In our study, the hybrid melons variety breeding which was acted on the assumption in single, triple and double crosses melon hybrids were more favorable. For this purpose, the study was to determine the heterosis or hybrid vigor of hybrids with three and four parents in addition to those with only two parents.

MATERIALS AND METHODS

This research was conducted during 2007 and 2008 spring growing season under field conditions at the Department of Horticultural Production and Marketing, Faculty of Agricultural Sciences and Technologies, European University of Lefke in Turkish Republic of North Cyprus. A total of 53 hybrid genotypes (five single hybrids, thirty triple hybrids, nineteen double hybrids with the best combination ability) were obtained by hybridization of two, three and four parents. The hybrid genotypes were produced by using 6 doubled haploid lines (Galia types) obtained by irradiated pollen techniques. Galia F_1 hybrid variety was used as a control. The seeds of 59 genotypes (6 parents and 53 hybrids) and the commercial Galia F_1 hybrid were sown in 2:1 mixture of peat-perlite in a multi-cell tray and placed under small tunnels on April 30 in 2007 and 2008. Prior to sowing 45 seeds of each genotype were divided into 3 groups of 15 seeds for replicates. The seedlings with three true leaves were transplanted to field plots conforming to randomized

complete block design with three replications for each genotype and 10 plants for each genotype per replication. The plant spacing was 1.8 m and 0.5 m between and within rows, respectively. All plots received 250, 80 and 300 kg ha^{-1} respective rates of N, P_2O_5 and K_2O (Zuang 1982). All P_2O_5 was applied before planting while N and K_2O were split into three equal rates: the first two split rates were applied before planting, and the rest at beginning of flowering.

The heterosis and heterobeltiosis ratios were calculated for hybrids with two, three and four parents for the following characteristics: seedling emergence, length apparition of cotyledons and the hypocotyls, main stem length and diameter, node number on the main stem, first female flowering (the number of days), total and early yield; fruit weight, fruit length and diameter, and total soluble solids (TSS) (Sari et al. 2003; Virmani et al. 2003; Cho et al. 2004; Jose et al. 2005). Heterosis and heterobeltiosis were calculated by following equations:

- Heterosis = (F_1-Mean parent value) / Mean parent value × 100
- Heterobeltiosis = (F_1-Better parent value) / Better parent value × 100

The experimental data were analyzed by using MSTATC statistics software (Freed et al. 1989) and comparisons among the average values (at $p<0.01$) were made by the Tukey's test.

RESULTS

With respect to the seedling emergence data, statistically significant differences ($p<0.01$) were observed in the trials of 2007 and 2008. The parent lines had longer emergence times (7.40 days) than single, triple and double hybrids in the trial of 2007. However, the single hybrids had longer emergence times than parent lines, triple and double hybrids in 2008 (Fig. 1a). In both years, the shortest emergence time was from the single hybrids for heterosis and heterobeltiosis values of the seed emergence time. In 2007, the average values of heterosis for the hybrid groups, the effect was negative for single hybrids (-12.2%) (early emergence), but positive (late emergence) in the other groups. However, the effects were positive for all hybrid groups in 2008 (Table 1).

Significant differences ($p<0.01$) were found among cotyledon length in 2007 and 2008. The double hybrids group had higher mean length than the parent lines, single and triple hybrids in 2007 and 2008 (Fig. 1b). Triple hybrids had a positive heterosis for cotyledon length in both years; but positive heterobeltiosis was only observed in 2007 for triple hybrids (Table 1).

Regarding the hypocotyl length data, statistically significant differences ($p<0.01$) were present in 2007 and 2008. Double hybrids had longer hypocotyl than the parent lines, single and triple hybrids in 2007 (Fig. 1c). The average values of heterosis for hypocotyl length were positive for the triple (30.7%) and double (32.7%) hybrids, but were negative for the single hybrids (-0.3%). In contrast,

heterosis was negative (-2.8%) for double hybrids and positive for single and triple hybrids in 2008. The positive heterobeltiosis was found in the triple and double hybrids for hypocotyl length, but the effect was negative (-5.3%) for the single hybrids. However, negative heterobeltiosis was observed in triple and double hybrids and positive heterobeltiosis was observed for single hybrids for hypocotyl length in 2008 (Table 1).

Significant differences ($p<0.01$) were found among main stem length in 2008. The single hybrids had higher length averages than the parent lines, triple and double hybrids in 2008 (Fig. 1d). The heterosis was positive in the single hybrid group (11.6%), and negative for other groups (Table 1). The single hybrid group showed a positive heterobeltiosis, while those of other groups were negative.

Significant differences ($p<0.01$) were observed in main stem diameter in 2008. The single hybrid group had higher average values of main stem diameter than the parent lines, triple and double hybrids (Fig. 1e). The heterosis was positive in single and triple hybrid groups and negative in other group. Only the single hybrid group had positive heterobeltiosis in the last measurement main stem diameter (Table 1).

Significant differences ($p<0.01$) were obtained among average values of main stem node number in the hybrid groups. The single hybrids had higher average values than the parent lines, triple and double hybrids (Fig. 1f). Only the single hybrids had positive heterosis and heterobeltiosis (Table 1).

Significant difference was determined between average values of the first female flower formation in the hybrid groups. The single hybrids had shorter average values than the parent lines, triple and double hybrids both in 2007 and 2008 (Fig. 1g). Only single hybrid group produced negative heterosis (-3.4% in 2007 and -3.3% in 2008) for the first female flower formation. The other two hybrid groups averaged a longer period of the formation of the first female flower with a positive heterosis both in 2007 and 2008 (Table 1).

Significant difference ($p<0.01$) was found between the values of total yield of the hybrid groups in 2007 and 2008. The single hybrid group had significantly higher average values than the parent lines, triple and double hybrids (Fig. 1h). These hybrids had positive heterosis and heterobeltiosis in both years. The highest heterosis were 89.56 – 72.4% and the highest heterobeltiosis were 76.2 – 53.8% (Table 1).

Significant differences ($p<0.01$) were also found for the early production values in 2007 and 2008. In addition, the single hybrid group had higher average values than the parent lines, triple and double hybrids in 2007 (Fig. 1i). In terms of the average values of heterosis for earliness, the effects were positive for the single hybrid group in 2007 with 74.6%, but the effects were negative for the other groups. Additionally, SC-58 (G22 X C1) genotypes provided the highest heterosis (184.55%) and heterobeltiosis (184.23%) rates in earliness among genotypes (Table

1). In 2008, the average values of heterosis for hybrid groups were positive for the single (7.9%) and the triple (5.9%), but negative in the other group. It was determined that there is a positive effect of the single and triple hybrid groups, based on the important average heterosis values from both years (Table 1).

The single hybrid had the highest fruit weight in both years. Single and triple hybrids showed positive heterosis of fruit weight in 2007 and 2008. In 2007, only the single hybrids showed positive heterobeltiosis and the other two groups showed negative heterobeltiosis (Table 1). The single hybrid group had higher average values than the parent lines, triple and double hybrids in both years (Fig. 1j). The single hybrid group had higher average values than the parent lines, triple and double hybrids in both years in fruit diameter (Fig. 1k). The fruit height showed parallel values for the two years and the highest fruit height was recorded for the single hybrid genotypes. The single hybrid group had higher average values than the parent lines, triple and double hybrids in both years (Fig. 1l). In terms of the highest total soluble solids, the single hybrid group showed negative heterobeltiosis, and the triple and double hybrid groups had positive heterobeltiosis (Table1). In addition, the triple hybrid group had higher average values than the single hybrid, double hybrid groups and parent lines in 2007 and the double hybrid group had higher average values than the single hybrid, triple hybrid and parent lines in 2008 (Fig. 1m).

DISCUSSION

This research was conducted to determine the effects of a variety of cantaloupe melon hybrid groups in quantitative breeding parameters and important results were obtained. The longest length of plant was obtained in the single hybrid group. Also the heterosis was positive in the single hybrid group. According to the results of Sari et al. (2003), positive heterosis was determined in the single hybrid melon genotypes. The single hybrid group was affected as a positive due to hybridization and the positive effect of heterosis (El-Shinawy et al. 2004).

The single hybrid group had higher average values than the parent lines, triple and double hybrids in terms of total yields. El-Shinawy et al. (2004) found that some promising F_1 melon hybrids were superior over their parent lines and the commercial varieties in terms of yield.

In this study, the single hybrid group was superior over the other groups in terms of total production. These hybrids had positive heterosis and heterobeltiosis in both years. Sulochanamma (2001) obtained the highest heterobeltiosis value for the total production in single hybrid melons.

The single hybrid had the highest fruit weight in both years. Kitroongruang et al. (1992) found positive heterosis in the hybrids compared to the parents in fruit weight. Kitroongruang et al. (1992) reported that the results from the melon hybrid

individuals had positive heterosis of total soluble solids relative to their parents. In our study, there was positive heterosis of TSS in both years in all three hybrid groups (except single hybrid in first year).

As a result of this study, the single hybrid group was superior over the other groups in both in total and early yield. In future studies, hybridization for different melon types and assessment of hybrid and parents carry significance for determining better heterosis in melons.

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Table 1. Heterosis (HT) and heterobeltiosis (HB) for horticultural traits in single, triple and double hybrid melon genotypes.

Traits	Hybrids	Years			
		2007		2008	
		HT (%)	HB (%)	HT (%)	HB (%)
Seedling emergence					
Range		-45.88 to 48.00	-40.26 to 66.67	-16.21 to 38.89	-14.26 to 59.57
	Single	-12.16	3.27	7.17	14.89
Average	Triple	1.49	23.12	11.68	19.29
	Double	14.12	33.23	7.45	14.84
Cotyledon width					
Range		-24.44 to 48.43	-31.59 to 45.22	-32.64 to 111.72	-37.46 to 102.96
	Single	-0.76	-6.74	5.61	-3.24
Average	Triple	9.16	1.56	7.11	0.64
	Double	9.81	0.50	-1.08	-6.87
Cotyledon length					
Range		-27.99 to 64.18	-37.17 to 48.80	-36.05 to 58.71	-49.54 to 50.87
	Single	-0.56	-8.37	2.35	-8.73
Average	Triple	15.49	5.44	0.33	-8.22
	Double	15.21	3.90	-0.08	-9.83
Hypocotyl length					
Range		-46.78 to 108.24	-55.17 to 61.23	-39.53 to 40.98	-49.94 to 40.69
	Single	-0.28	-5.29	15.05	4.69
Average	Triple	30.72	14.99	0.71	-7.40
	Double	32.68	10.82	-2.80	-11.93
Main stem length					
Range		-*	-*	-22.39 to 39.01	-28.64 to 22.14
	Single	-	-	11.56	4.23
Average	Triple	-	-	-3.49	-10.17
	Double	-	-	-11.40	-15.72
Main stem diameter					
Range		-*	-*	-14.52 to 16.74	-20.05 to 11.75
	Single	-	-	7.50	3.76
Average	Triple	-	-	2.29	-1.80
	Double	-	-	-1.12	-4.77
Main stem node number					
Range		-*	-*	-22.95 to 15.36	-24.22 to 8.56
	Single	-	-	10.57	3.45
Average	Triple	-	-	-5.67	-10.39
	Double	-	-	-13.83	-15.01
First female flower					
Range		-11.83 to 9.03	-7.65 to 12.06	-14.92 to 19.34	-12.11 to 20.31
	Single	-3.36	-1.14	-3.34	4.33
Average	Triple	0.44	3.07	1.16	6.07
	Double	2.61	4,57	2.63	6.28

*No data were collected in 2007.

Table 1 (continued)

Traits	Hybrids	Years			
		2007		2008	
		HT (%)	HB (%)	HT (%)	HB (%)
Total yield					
Range		-47.79 to 89.56	-56.80 to 76.19	-39.95 to 72.40	-46.93 to 53.80
	Single	45.69	32.56	36.99	28.66
Average	Triple	-2.88	-17.91	-4.88	-15.37
	Double	-12.52	-21.36	-15.07	-24.28
Early yield					
Range		-65.79 to 184.55	-69.10 to 184.23	-69.41 to 118.38	-75.73 to 113.26
	Single	74.56	60.94	7.91	-3.43
Average	Triple	-4.81	-20.91	5.94	-6.65
	Double	-32.68	-46.94	-9.39	-22.20
Fruit weight					
Range		-44.31 to 82.98	-59.33 to 50.68	-27.56 to 58.37	-43.79 to 46.13
	Single	16.78	4.56	12.30	2.52
Average	Triple	4.16	-11.57	3.17	-9.21
	Double	-6.36	-23.87	-6.01	-20.19
Fruit diameter					
Range		-13.24 to 99.84	-26.90 to 21.27	-11.92 to 16.45	-13.73 to 16.35
	Single	6.36	0.45	7.10	2.84
Average	Triple	1.64	-5.74	1.68	-4.16
	Double	2.43	-10.78	-2.55	-8.00
Fruit height					
Range		-14.16 to 35.74	-26.86 to 25.81	-13.05 to 14.74	-23.27 to 8.86
	Single	9.73	3.88	4.05	-4.21
Average	Triple	5.04	-3.35	1.83	-5.20
	Double	0.83	-7.37	-0.35	-7.99
Total soluble solid (TSS)					
Range		-15.12 to 26.20	-17.83 to 21.60	-16.89 to 18.46	-21.44 to 14.96
	Single	-5.31	-7.74	3.35	-2.05
Average	Triple	8.39	3.47	3.73	0.05
	Double	8.36	2.86	2.50	0.31

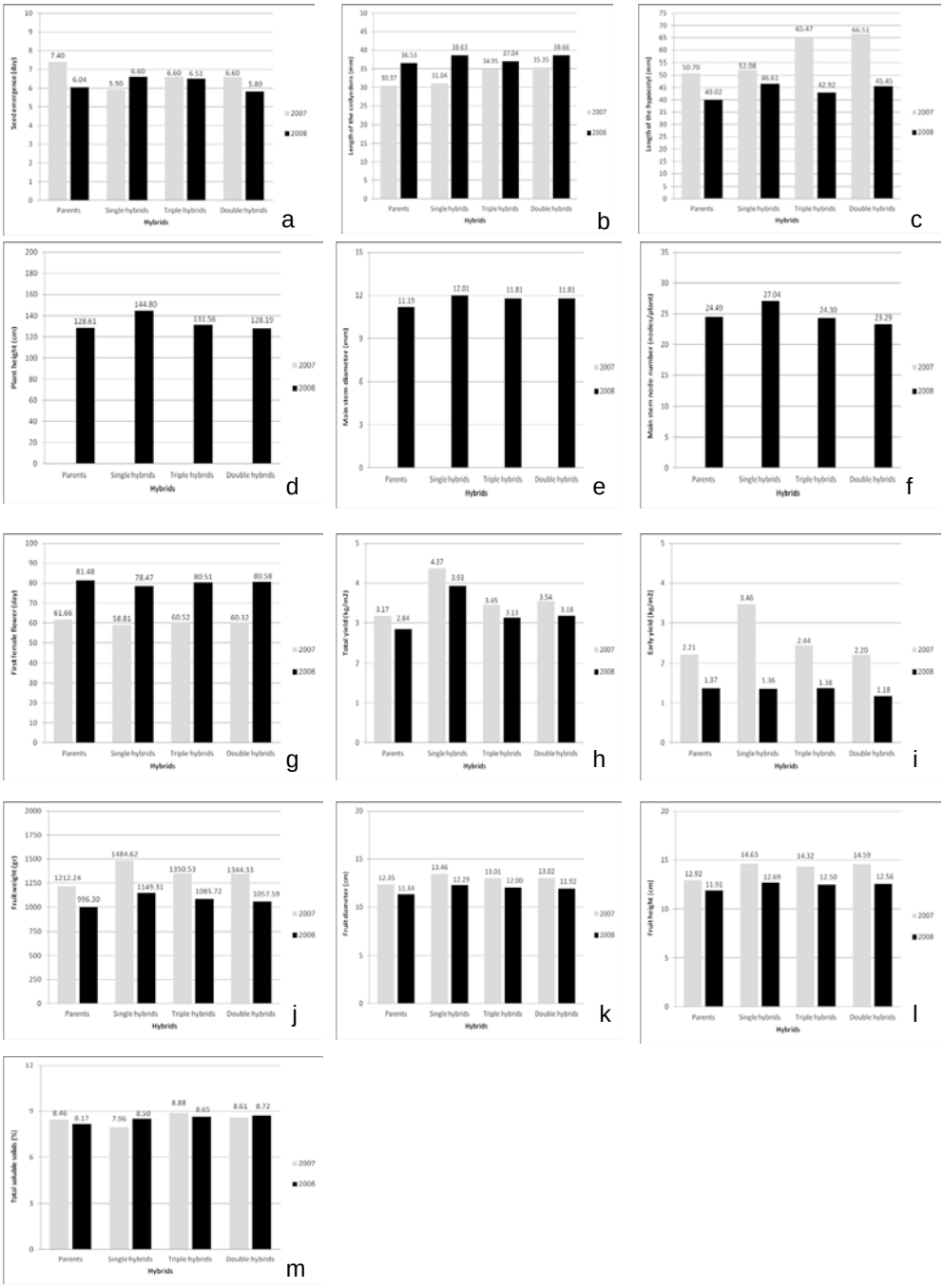

Fig. 1. Average values for two years (2007 and 2008) of seed emergence (a), length of cotyledon (b), length of the hypocotyl (c), plant height (d), main stem diameter (e), main stem node number (f), first female flower's day (g), total yield (h), early yield (i), fruit weight (j), fruit diameter (k), fruit length (l) and total soluble solids (m) in parental lines, single, triple and double hybrid melon genotypes.

Evaluation of genetic relationships on single, triple and double cross melon (*Cucumis melo* var. *cantalupensis*) hybrids by SSR markers

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Keywords: Hybrid melon breeding, genetic relationship, molecular markers, SSR

Abstract

This study was conducted to determine the quantitative characteristics of 53 hybrid melon genotypes which were obtained by single, triple, double hybridization and to compare the genetic relationship between single, triple and double hybrids and their parental pure lines by SSR markers.

In molecular studies, 55 Simple-sequence repeat (SSR) markers were screened and polymorphism among single, double and triple hybrids were compared. Eight of 55 SSR primers used for screening were found polymorphic. Similarity rate was found to be between 0.54-1.00 according to the results of SSR analysis.

INTRODUCTION

In the past two decades, biotechnology studies have concentrated on determining and identifying the genetic structures of the great majority of horticultural crops. Thus, today's important techniques can lead to more reliable conclusions evaluating genetic structures.

DNA markers are powerful tools that provide valuable research opportunities to researchers for investigating genetic variations of plants, as well as defining and identifying close species, varieties (Aka Kacar 2001; Rajapakse 2003). Today's molecular methods that gradually gained wide usage are modern systems that have been developed within the last 20 years.

Markers which are considered the main tools, can be studied in two groups, namely "Methods based on Hybridization and PCR". Among the methods based on PCR, RAPD, AFLP and SSR can be used. SSR markers have various advantages, they have high polymorphism, high heterozygosity and easy to apply (Aka Kacar 2001). The SSR markers are also used for obtaining more reliable results in determining the degrees of relation among genotypes and the degree of resemblance to parents in characters taken from various hybrid groups because of their high

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polymorphism, abundance and co-dominant inheritance. They are well suited for the assessment of genetic diversity within crop species and the study of genetic relationships among species. In 1996, Katzir et al. (1996) developed the first SSR markers in melons. There are several reports that SSR markers have been used for understanding genetic diversity of melon genotypes (Staub et al. 2000; Danin-Poleg et al. 2001; Szabo et al. 2008, Tzitzikas et al. 2009).

MATERIALS AND METHODS

The research was conducted in the Plant Biotechnology Laboratory at the Department of Horticulture, Faculty of Agriculture, University of Çukurova. A total of 60 melon genotypes were studied (Table 1). As control commercial Galia F1 hybrid was used. The seeds were sown in peat : perlite mixture (2:1) in trays.

DNA extraction

Young leaves were collected from each melon genotype and immediately frozen in liquid nitrogen and stored at –80°C. High molecular weight genomic DNA was extracted from the leaf samples following the CTAB miniprep protocol (Edwards et al. 1991). DNA concentration was measured with a NanoDrop ND 100 spectrophotometer (NanoDrop Technologies, Inc.) and gel electrophoresis. DNA was diluted in water to a final concentration of 50 ng/µL and stored at –20°C.

PCR protocol and SSR analysis

Amplification reactions were performed in 10-µL volumes containing 2x PCR Mastermix (Fermentas K0171), 1 U Taq DNA polymerase (Fermentas EP0402), 25 mM $MgCl_2$, 1 µM forward and reverse primers and 25 ng melon genomic DNA. The mixtures were prepared at 0°C and transferred to the thermal cycler. The amplification was performed in a model Master Gradient thermal cycler (Eppendorf) using a program consisting of an initial denaturation step of 2 min at 94°C followed by 35 cycles of 2 min at 94°C, 1 min at 55°C and 2 min at 72°C; the program ended with a 10-min elongation step at 72°C. PCR products were stored at 4°C prior to analysis. After amplification, 1-25 µL of loading buffer containing 95% formamide, 10 mM EDTA (pH 8.0), 0.025% xylene cyanol, and 0.025% bromophenol blue were added to each reaction tube. The samples were heat-denatured for 5 min at 95°C and quickly transferred on ice. After loading 1.0 µL of each sample, PCR products were separated in a 25-cm, 6% denaturing polyacrylamide gel that had been preheated for 25 min. Electrophoresis was conducted at 1500 V, 50 W, 35 mA and 48°C using a Li-Cor DNA Analyzer 4300. A 50-350 bp DNA ladder (MWG Biotech AG, Ebersberg, Germany) was run alongside the amplified PCR products to determine DNA sizes.

SSR Primers

In order to determine the SSR primers used in this study, preliminary screening study was performed Parental lines which used to obtain for single, double and triple hybrids melon genotypes were chosen for screening study.

In this study, fifty-five SSR primers were used based on their polymorphic bands amplification ability for melon hybrid genotypes. Twenty-two SSR primers (CMGA15, CMCT44, CMGA104, CMACC146, CMCTT144, CMTC47, CMAT141, CMCCA145, CMTC168, CMGA172, CMTC123, CMGT108, CMTAA166, CMTAl34a, CMTC160a+b, CSCTTT15a, CSTCC813, CMAT35 CMEFEA, CSAT425 CSMASY, CMTC13, CMAG59, CMGA128); were developed by Danin-Poleg et al. 2001 and Katzir et al. 1996; nine SSR primers (Cgb4765, CLG7996, CLG7992, Cgb4767, ASUW2, ASUW13, ASUW19, Cgb5009, CLG8218) were used in watermelon by Levi et al. 2006; seven SSR primers (CI. 1-06, C.I. 1-12, C.I. 1-20, C.I 1-21, C.I. 2-23, C.I. 2-61, C.I. 2-140); were used by Jarret et al. 1997 in watermelon and seventeen SSR primers (CMGAN92, CMAGN68, TJ24, CSWCT10, CMGAN59, CMAGN73, CMMS2-3, CMTCN9, CMGAN13, CMTCN41, CMTCN30, CMMS31-3, CMTCN1, CMCTN19, CMTCN62, CMTCN14, CMMS35-4) were developed by Gonzalo et al. 2005, Fazio et al. 2002 and Chiba et al. 2003.

Data analysis

Reproducible DNA bands were scored manually in the binary mode with 1 indicating the presence and 0 indicating the absence of a band (Nei and Li 1979). The unweighted pair-group method using the arithmetic average clustering procedure (UPGMA) was employed to construct the clustering dendrogram based on the genetic distance matrix using NTSYS-PC program (version 2.02i) (Rohlf 1998).

RESULTS AND DISCUSSION

In this research, a preliminary study was carried out to determine the polymorphism among single, triple and double hybrid groups and their parents with Galia F1 cultivar by SSR markers. Parental lines of hybrid genotypes were screened with preliminary study with 55 SSR primers. As a result of screening of fifty-five SSR primers, a total of eight SSR primer bands were found to be polymorphic (CMGA128, CMAGN68, CMAGN73, CMTCN41, CMTCN30, CMCTN19, CMTCN62, CMMS35-4) and all eight polymorphic bands were applied to execute the SSR experiment for single, triple and double hybrid groups and Galia F1 cultivar. According to the screening results which were obtained from 55 SSR primers, 22 primer pairs developed by Danin-Poleg et al. (2001) and Katzir et al. (1996) provided eight monomorphic bands, one primer polymorphic (coded CMGA128 Katzir 3) band and the rest of primers had no amplification. In addition, 17 primer

pairs used by Gonzalo et al. (2005), Fazio et al. (2002) and Chiba et al. (2003) were provided seven primer polymorphic (coded CMAGN68, CMAGN73, CMTCN41, CMTCN30, CMCTN19, CMTCN62, CMMS35-4), six monomorphic bands and the rest of the primers had no amplification.

Also, nine SSR primers used by Levi et al. 2006 (Cgb4765, CLG7996, CLG7992, Cgb4767, ASUW2, ASUW13, ASUW19, Cgb5009, CLG8218); and seven SSR primers used by Jarret et al. 1997 (CI. 1-06, C.I. 1-12, C.I. 1-20, C.I 1-21, C.I. 2-23, C.I. 2-61, C.I. 2-140) showed no amplification.

Tzitzikas et al. (2009) used SSR markers to investigate the genetic diversity and population structure of traditional Greek and Cypriot melon cultigens. They reported that all SSR markers were polymorphic with a total number of 81 alleles, averaging 4.7 alleles per locus. In another study, a total of 232 SSR alleles and an average of 10.3 alleles per SSR were obtained for Indian snap melons (*Cucumis melo* var. *momordica*) (Dhillon et al. 2007). Kong et al. (2007) used EST-SSR markers in *Cucumis melo* and found that the number of alleles ranged from two to five with an average of 2.9 alleles per locus. In our study, low polymorphism was detected. It is an expected result because the plant materials used in the present study are obtained by single, triple and double hybridization.

Relationships among hybrid genotypes of melon

Six parental lines (G22, G2, H27, H4, C1 and 8) were used in our molecular research. Similarity rates from SSR analysis were found to be between 0.54-1.00 as shown in the dendrogram (Fig 1). Instead of similarity indicated in the dendrogram, comparing hybrids with their parent would provide a better explanation. As expected, especially H27 parental line with SC-101 (H27 X H4) single hybrid and SC-101 X H27 with SC-109 X H27 triple hybrids took part in the same branch.

In another case, according to SSR analysis results, genetic differences were not determined between SC-58 (G22 X C1) single hybrid, SC-63 X C1 triple hybrid and SC-58 X SC-63, SC-63 X SC-58 double hybrids which have at least one common parent. According to the SSR analysis results, there was no genetic differences (variation) between SC-63 single hybrid and SC-63 X G22 with SC-63 X G2 triple hybrids that were used as a parent of SC-63. Also genetic differences were not found between SC-111 X G22, SC-111 X SC-109, SC-63 X 8, SC-109 X SC-63, SC-111 X SC-63, SC-111 and SC-111 X G2 genotypes. G22 and G2 parental lines clustered as a separate branch like C1 parent in the dendrogram. SC-58 single hybrid was found similar to the parental line G22 by 28%, to C1 by 67 %, whereas SC-63 single hybrid was similar to parental line G22 by 53 %, to G2 by 59 %; also, SC-101 single hybrid was a similar parental line to H27 by 59 %, to H4 by 67 %; whereas SC-109 single hybrid was found similar to parental line 8 by 69 %, to H4 by 61 %; on whereas, SC-111 single hybrid was similar to parental line 8 by

75 %, to G2 by 58 %.

In this research, genetic relationship among single, triple, double hybrids and their parents was determined by SSR markers. A better understanding of the molecular heterosis in subsequent cantaloupe melon studies, it will be important to use hybrid genotypes and parental lines which have broad genetic base.

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Table 1. Parents and Hybrids Used in the Research.

Material Type	**Genotype**
Parents	G22, G2, H27, 8, H4, C1
Single Hybrids	*SC-58 (G22 x C1), SC-63 (G22 x G2), SC-101 (H27 x H4), SC-109 (8 x H4), SC-111 (8 x G2)
Triple Hybrids	SC-58 x H4, SC-63 x H4, SC-101 x H4, SC-109 x H4, SC-111 x H4, SC-58 x G22, SC-63 x G22, SC-101 x G22, SC-109 x G22, SC-111 x G22, SC-58 x G2, SC-63 x G2, SC-101 x G2, SC-109 x G2, SC-111 x G2, SC-58 x H27, SC-63 x H27, SC-101 x H27, SC-109 x H27, SC-111 x H27, SC -58 x 8, SC -63 x 8, SC -101 x 8, SC -109 x 8, SC -111 x 8, SC -58 x C1, SC-63 x C1, SC-101 x C1, SC-109 x C1, SC-111 x C1
Double Hybrids	SC-58 x SC-63, SC-58 x SC-101, SC-58 x SC-109, SC-63 x SC-58, SC-63 x SC-101, SC-63 x SC-109, SC-63 x SC-111, SC-101 x SC-58, SC-101 x SC-63, SC-101 x SC-109, SC-101 x SC-111, SC-109 x SC-58, SC-109 x SC-63, SC-109 x SC-101, SC-109 x SC-111, SC-111 x SC-58, SC-111 x SC-63, SC-111 x SC-101, SC-111 x SC-109
Control	Galia F_1

*SC means Special Combination

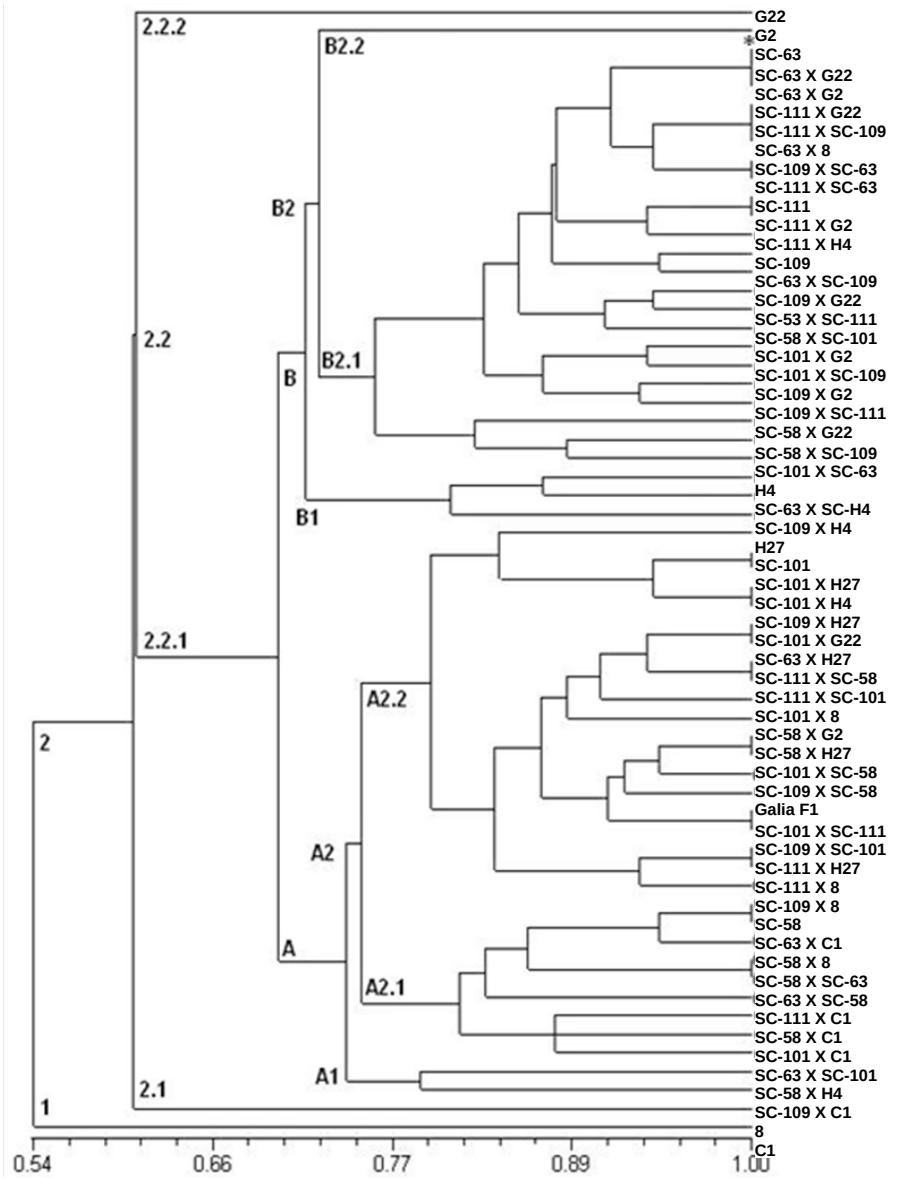

Fig. 1. Dendogram obtained by the analysis results of SSR markers in single, triple and double hybrid melon genotypes (*SC means Special Combination).

Response of grafted cucumber to specific ion (Na+, Cl-) under salt stress

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Keywords: Grafting, NaCl stress, sodium, cucumber, physiological response

Abstract

To test whether the increased salt tolerance of cucumber grafted onto rootstock was mainly associated with sodium toxicity, cucumber (*Cucumis sativus* L.cv. Jinchun No. 2) plants, either self-grafted or grafted onto the Figleaf gourd (*Cucurbita ficifolia* Bouché), were subjected to iso-osmotic Na^+, Cl^- and NaCl stress, and the plant growth and physiological parameters were measured. The results showed that Na^+ salt and NaCl stress significantly decreased the growth of self-grafted and grafted cucumber seedlings. However, plant dry weight, shoot water content, shoot K^+ and Ca^{2+} content, superoxidase dismutase (SOD) activity and chlorophyll content of grafted plants were significantly higher than those of self-grafted plants. Meanwhile, the shoot Na^+ content and Na^+/K^+ ratio of grafted plants were lower than those of self-grafted plants. Compared with the plants under Na^+ salt and NaCl stress conditions, smaller change in plant growth and physiological response were observed in the self-grafted and grafted cucumber plants under Cl^- stress. Therefore, the sensitivity of self-grafted and grafted plants to Na^+ and Cl^- is different, and toxicity to cucumber seedlings caused by Na^+ is much higher than that of Cl^-. Taken together, the improved salt tolerance of cucumber plants grafted onto Figleaf gourd was mainly achieved by reducing the absorption of Na^+ and limiting the translocation of Na^+ to the shoot.

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Variety performance of cucumber (*Cucumis sativus* L.) F_1 hybrid combinations in spring and autumn growing period in greenhouse

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Keywords: Cucumber, heterosis, heterobeltiosis, yield, yield component

Abstract

This research was carried out to improve F_1 hybrids varieties suitable for greenhouse cultivation. Fourteen cucumber F_1 hybrids were used to study some economic traits (yield per plant, early yield per plant, number of fruits per plant) at BATEM (Batı Akdeniz Agricultural Reseach Institute)'s greenhouses during 2005 spring and autumn periods. 10 lines were used as female parents and 4 lines as male parents and single crosses were made to produce F_1 hybrid in 2004. Fourty F_1 hybrids and their parents were grown in randomized complete block design with three replications. Hybrid of A1xB1 exhibited the highest heterosis and heterobeltiosis for yield per plant (46% heterosis and 29% heterobeltiosis), for early yield per plant (65% and 61 %) and for number of fruits per plant (77% and 40%) in spring cultivation. But in autumn period the hybrids of A2xB2 (46%) A8xB4 (87%) and A5xB2 (62%) had the highest positive heterosis values for inspected charactheristics, whereas A2×B2 (29%), A8×B4 (53 %) and A2xB2 (57%) hybrids gaved the highest heterobeltiosis. As a result,B1, B2, and B4 inbred lines which can be used as the male parents with their counterpart female for commercial hybrid production, will be valuable for cucumber breeding and production in both environments.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is the fourth most economically important vegetable crop (Tatlioglu 1993). 57.6 million tons are grown over 1.9 million hectares for pickling or fresh consumption in nearly every country. Turkey was the third highest country for total production (1.74 million tons) and the third largest for growing area (59,000 hectares) in 2010 **(**FAO 2010).

Improved yield has been one of the important objectives in cucumber breeding since the 1990s (Shetty and Wehner 2002). Improvements in yield has been very difficult to achieve because of low heritability for quantitative characters such as fruit yield, earliness and fruit qualityinthe cucumber accessions (Cramer

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and Wehner 2000). To develop new varietiess having these desirable characteristics, selection of parents is the most important stage of the breeding procedure. Heterosis, in other words hybrid vigor, is one of the breeding methods using selection parental lines. Heterosis in cucumber has been credited for total yield and its components; earliness, fruit quality and vegetative characters such as node number at which first female flower appears, days to first female flower, fruit diameter, fruit and vine length, number of nodes per vine and number of branches per vine in plant.

Hayes and Jones (1916) were the first to report heterosis for fruit size and fruit number per plant. They explained that there was a heterosis when the plants having dissimalar fruit size and vine type were hybridized (Cramer and Wehner 1999). Heterosis for yield and quality has also been reported by Cramer and Wehner 1999; Hanchinamani 2006; Hanchinamani and Patil 2009).

The objectives of this study was to examine the amount of mid-parent and better-parent heterosis for some economic traits (yield per plant, early yield per plant, number of fruits per plant) in cucumbers.

MATERIALS AND METHODS

Germplasm

Fourty F_1 hybrids were obtained by crossing 14 diverse parental (10 maternal (P_A) and 4 paternal (P_B)) lines of slicing cucumber through half-diallel method in the 2004 autumn season.

Design and data analysis

Experiments were conducted in unheated greenhouses condition of Department of Vegatables at Bati Akdeniz Research Institute (BATEM). Seeds were sown on 16 March for the spring season and on 25th August 2005 for the autumn season in BATEM. Seedlings were transplanted on 19th April and 8th October 2005 in the greenhouse. The design was a randomized complete block design (RCBD) with 40 F_1 combination and 14 parents (P_A, P_B), two seasons (spring, autumn), and three replications per season. Each plots contained 8 plants of each genotypes.

Total yield was the sum of 12 harvests for spring and 17 harvests for autumn season and early yield was the sum of the first five harvests during spring and autumn 2005. Data were collected as plot means, and consisted of yield and component (total yield, fruit number and early yield per plant). Harvest dates were 17th May for the spring and 12th September for the autumn season. The data were analyzed separately for each family using the GLM procedure of the SAS statistical package (SAS Institute 2001).

Increase or decrease of heterosis of F_1 over mid parent and better parent (heterobeltiosis) for all characters were estimated as formulated by Rai and Rai

(2006).

Heterosis over mid-parental value (relative heterosis: H_{MP}%) = [(F_1-MP)/MP]*100, where MP is mid parent.

Heterosis over beter-parent (heterobeltiosis: H_{BP}%) = [(F_1-BP)/BP]*100, where BP is better (higher) parent.

RESULTS AND DISCUSSION

Total yield per plant (kg/plant)

The hybrids showed a significant difference in producing yield per plant and ranged from 2.63 kg (A6xB4) to 4.15 kg (A1xB1) in spring season and 2.22 kg (A9xB4) to 3.82 kg (A6xB1) in autumn season (Table 1). Our results were supported by Faruk Hossain et al. (2010).

Heterosis over both mid (MP) and better parent (BP) was found to be significantly higher. Whereas, the maximum positive heterosis over mid-and better-parent were obtained in A1xB1 (45.53% and 28.59%) and A2xB2 (43.67% and 33.97%) hybrids in spring and autumn season, respectively (Table 2).The highest negative heterosis over mid-and better-parent was recorded in A4xB2(-16.51 and -24.84) in spring, and A7xB3 (-6.67%) and A6xB4 (-27.64%) in autumn season respectively. This finding was supported by Hanchinamani (2006).

Early yield per plant (kg/plant)

High significant differences in the early yield ($P < 0.01$) was observed a mong the varieties (Table 1) in both seasons. Early yield ranged from 1.32 kg to 2.29 kg in spring and 0.82 kg to 1.62 kg in autumn season. The highest yield was obtained for A1xB1 (2.29 kg) and A3xB1 (1.62kg) hybrids in spring and autumn seasons respectively. Similar results were obtained by Ahmed et al. (2004).

When the hybrids were examined for early yield, cucumber, heterosis over both mid-and better-parent (BP) was found to be highly significant. A1xB1 and A8xB4 hybrids exhibited the maximum positive heterosis over mid-parent (64.59% and 104.21%) and better- parent (60.66% and 77.34%) respectively, the highest negative heterosis over mid-and beter-parent was recorded A4xB2 (-14.64%) and A4xB2 (-27.42%) in spring, A4xB2 (-27.42%) and (-31.62%) in autumn season, respectively.

Number of fruits per plant

The number of fruit per plant varied significantly among the hybrids and ranged from 16 to 35 in spring and 19 to 39 in autumn season (Table 1). Only one hybrid (A8xB1) exhibited a higher number of fruits (35 in spring and 39 in autumn) per plant in both seasons. On the otherhand, the minimum number of fruits per plant was obtained in A6XB4 (16) in spring and A9xB4 (19) in autumn season.

These results are similar to the results of Faruk Hossain et al. (2010)

Significant negative and positive heterosis was observed for mid and better parent for this trait. The hybrids A1xB1 and A4xB3 in spring and A5xB2 and A2xB2 in autumn season noticed maximum positive heterosis over mid-(77.41% and 62.07%) and better-(52.63% and 57.05%) parent, respectively. The highest negative heterosis was recorded in over mid-parents A4xB2 (-9.12% spring) and A6xB4 (-15.15% autumn) and in better-parents A6xB2 (-21.49 spring) and A6xB4 (-31.54% autumn). Similar results were reported by Cramer and Wehner (1999).

As a result, the promising maternallines B1, B2, and B4 for yield and yield components which can be used as the male parents with their counterpart female for commercial hybrid production would be valuable for cucumber breeding and production in both environments.

Table 1. Variation of hybrids in yield and yield component in two season.

Source	Spring			Autumn		
	Yield	Early	No. fruits	Yield	Early	No. fruits
Range	2.63-4.15	1.3-2.29	16-35	2.22-3.82	0.83-2.29	19-39
Means	3.27	1.72	23	3.02	1.23	27
CV (%)	8.59	13.03	10.98	9.27	13.03	10.21
$LDS_{0.05}$	0.453	0.364	4.122	0.454	0.285	4.567
F value	4.72**	3.09**	6.41**	4.65**	2.72**	11.01**

**: Significant differences at $P<0.01$

Yield: total yield per plant (kg/plant);Early: total early yield per plant (kg/plant); number of fruit per plant

Table 2. Range, means, standart deviation and mid-and better-parent heterosis of total fruit yield (yield-kg), early fruit yield (early-kg) and number of fruit per plant (No. fruits) of each generation (P_A, P_B) and F_1 hybrid in two season.

Generation	Spring			Autumn		
	Yield	Early	No. fruits	Yield	Early	No. fruits
P_A						
Range	1.95-3.50	1.26-2.08	11-26	2.48-3.29	0.82-1.53	20-30
Means	2.90	1.61	16.70	2.88	1.15	25
Std. Dev.	0.50	0.27	4.13	0.32	0.21	4
P_B						
Range	2.53-3.51	1.35-1.45	14-23	1.68-2.67	0.63-1.18	17-22
Means	3.05	1.39	19.23	2.27	0.99	20
Std. Dev.	0.42	0.05	4.47	0.44	0.25	3
F_1						
Range	2.63-4.15	1.3-2.29	16-35	2.22-3.82	0.83-2.29	19-39
Means	3.27	1.72	23	3.02	1.23	27
Std. Dev.	0.37	0.23	0.23	0.35	0.16	5.4
MP (%)						
Range	-16.51-+45.53	-14.64-+64.59	-9.12 -+77.41	-6.67-+43.67	-27.42-+104.21	-27.42-+14.21
Means	10.16	14.88	30	17.59	17.07	21
Std. Dev.	14.78	16.58	19.73	13.48	22.15	20.19
BP (%)						
Range	-24.84-+28.59	-23.35-+60.66	-21.49-+52.63	-27.64-+33.94	-23.35-+77.34	-31.5-+57.05
Means	2.78	6.59	15	5.69	4.35	11
Std. Dev.	13.90	18.44	19.71	14.75	19.23	21.81

Std. Dev: Standart deviation, P_A: Maternal line, P_B: Paternal line, F_1: Hybrid; MP: Heterosis over mid parental (%), MP: Mid-parent; BP: Heterosis over better parent (%)

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Generation of new plant populations for exploiting natural variation in melons

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Keywords: *Cucumis melo*, introgression lines, sugar content, aroma, breeding

Abstract

In the past few years a number of genomic resources have become available in melons. A particularly important advance has been the deep sequencing of the transcriptome of several genotypes representing the variation of the species, which has allowed the generation of large SSRs and SNPs collections. The use of SNP arrays in high throughput genotyping platforms has numerous applications to accelerate breeding programs. For example, it facilitates the development of introgression libraries (ILs).

We are constructing new population of ILs using two exotic donors (Ginsen makuwa, subsp. *agrestis* var. *conomon* from Japan, and QPM, subsp. *agrestis* var. *dudaim* from Irak), and two recurrent genetic backgrounds, representing the two groups of most commercial interest (Vedrantais, subsp. *melo* var. *cantalupensis* and Piel de sapo, subsp. *melo* var. *inodorus*).

Parentals and large BC2 and BC3-derived populations were genotyped with a set of SNPs uniformly distributed across melon genome. Two genotyping platforms, Golden Gate and Sequenom technologies, were used to select those plants of each population having 3 donor introgressions or less, and representing the whole donor genome. Fruits of BC1-BC2-BC3 plants were also phenotyped. Interesting traits for improving fruit quality, such as an increased sugar accumulation in the *cantalupensis* background and color and aroma variation in the *inodorus* Piel de sapo were observed. Those plants with interesting genotypes/phenotypes are being selfed to produce sets of 60-80 ILs with homozygous introgressions, useful for the genetic dissection of quantitative traits and for the development of new breeding lines.

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INTRODUCTION

Melon (*Cucumis melo* L.) is a highly diverse species that is cultivated worldwide (Fernandez-Trujillo et al. 2011). Recent advances in massively parallel sequencing have begun to allow the study of nucleotide diversity in this species. Two recent studies have provided the first comprehensive resequencing data for wild, exotic, and cultivated melon transcriptomes, yielding the largest melon SNPs collection available to date (Blanca et al. 2011 and 2012). A set of these SNPs, about 600 markers, was selected to generate an Illumina GoldenGate assay that was used to increase the density of the melon genetic map and to anchor the recently published melon genomic sequence (Garcia-Mas et al. 2012). The use of SNP arrays in high throughput genotyping platforms can also accelerate the development of introgression libraries (ILs) that is collections of nearly isogenic lines. Each IL line carries a delimited portion of a donor source genome into a common recipient genetic background. These types of collections have already shown to be instrumental for the dissection of quantitative traits (Fita et al. 2008).

Traditionally, *C. melo* has been considered to be divided into two subspecies, *melo* and *agrestis* (Esteras et al. 2012). One of the simplest and most accepted classifications describes one single wild variety, var. *agrestis* Naud., and six cultivar groups (*cantalupensis* Naud., cantaloupe or muskmelon, *inodorus* Naud., cassaba and winter melons, *flexuosus* Naud., snake melons, *dudaim* Naud., mango melons, *momordica*, snap melons, and *conomon* Mak., pickling melon). More recently Pitrat et al. (2008) split these varieties into 15 botanical groups. In the framework of a previous project, we established a core germplasm collection, comprising 212 accessions that represent the extant variability in the species (Esteras et al. 2009). This collection was extensively characterized at both the phenotypic (disease resistance, fruit traits and chemical composition, sugars and volatiles) and genotypic levels.

To date only one ILs population is available in melons, derived from the cross Piel de sapo (subsp. *melo* var. *inodorus*) x Songwhan Charmi (subsp. *agrestis* var. *chinensis-conomon* group) (Eduardo et al. 2005). This population has been mainly used to map resistance genes and some fruit traits (Obando-Ulloa et al. 2009). However, the extant genetic diversity in exotic melons is still under exploited.

From our core collection we have selected two new genotypes to be used as donors of interesting traits for melon breeding. With these donors we have started the development of two new ILs collections into two different genetic backgrounds, representing the most commercially important melon market classes. The use of the available genotyping platforms is accelerating this process.

MATERIALS AND METHODS

The strategy was similar to that reported in Eduardo et al. (2005), but taking advantage of the availability of large SNPs collections and high throughput genotyping facilities. The four accessions used for the construction of these two populations were previously genotyped using a Golden Gate genotyping platform with a new set of 600 mapped SNPs, (Garcia-Mas et al. 2012). A subset of those markers that were polymorphic between parentals (99 SNPs), evenly distributed in the genome, were selected and used to design a Sequenom array to genotype 400 BC2 plants of each population. BC2 plants were maintained in a climatic chamber with slow growing conditions till SNPs results were available. A set of BC2 plants with 4 or less introgressions and with high proportions of recurrent genome were selected per cross. Selected plants, representing the whole donor genome, were grown at the greenhouse, backcrossed to produce BC3, and fully phenotyped. Genotyping was again performed in the BC3 population, with the same set of 99 markers, in order to select lines with one, two or three introgressions. Those plants with interesting genotypes/phenotypes have now been selfed. BC3S1 seedlings are being genotyped using high resolution melting (HRM) with the selected SNP markers tagging the corresponding introgressions to finally select plants with single homozygous introgressions.

RESULTS AND DISCUSSION

By means of marker-assisted backcrossing, we have produced two introgression libraries using the Ginsen makuwa accession (subsp. *agrestis* var. *makuwa*, group *conomon*, high sugar content) and the QPM accession (subsp. *melo* var. *dudaim*, medium to high sugar content and strong and complex aroma) as donor genotypes and the Vedrantais (subsp. *melo* var. *cantalupensis*-climacteric) and Piel de sapo (subsp. *melo* var. *inodorus*-non climacteric) as recipients (Fig. 1), and utilized these collections to investigate the genetic basis of quality traits in melon.

The construction of libraries of introgression lines is a difficult, time-consuming task facilitated with the availability of high throughput genotyping tools. Our Sequenom platform allowed us to apply a strong genomic selection at BC2. After genotyping the large BC2 populations, approximately 50 plants having 4 introgressions or less and representing the whole donor genome were preliminarily selected per population, to be transplanted to the greenhouse for further backcrossing. A set of 25 BC3 families derived from the previously selected BC2 plants were selected for further genotyping. All BC3 selected, having from 1 to 3 introgressions, were selfed to produce lines with homozygous introgressions. We will produce 60-80 NILs per cross. The final set of selected genotypes will be verified with the full set of 600 evenly spaced SNPs using the Golden gate platform.

Phenotyping conducted in BC1, BC2 and BC3 indicated that these lines have

traits interesting for breeding melons. For example, different levels of climacteric ripening and variability in fruit shape, color and aroma were found in lines derived from the *dudaim* type (Fig. 1). Sugars content variability was found in the *makuwa* population. Most of the exotic types used to date in melon breeding are non sweet of have low to intermediate sugars levels (Pitrat et al. 2008). The *conomon* group is the one with the highest sugar levels among the varieties belonging to the subsp. *agrestis* (Fig. 2). Ginsen makuwa was selected from our core collection as the accession having the highest level of sugars among the *conomon* types (Fig. 2). This high sugar content is maintained in BC1, BC2 and BC3 plants, with approximatelly a 10% of plants with fruits sweetest than both parents (Fig. 3). It has also other interesting traits as resistance to fungal diseases, a differential pattern of aroma in flesh and rind, and abundant and early flowering.

Sucrose accumulation has been reported to be controlled by a recessive gene, present in sweet melon varieties and absent in non-sweet melons (Burger et al. 2002). Genetic dissection of sugar accumulation has also performed in different crosses of cultivated (sweet) types x exotic (medium to low sweetness, *conomon*, *momordica*, *acidulus*) (Obando-Ulloa et al. 2009; Harel Beja et al. 2010). Results indicate that variation on sugar accumulation is controlled by a large number of QTLs. Our Libraries of introgression lines are a good material to study and breed for sugar levels in melon.

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Fig. 1. Parentals, F1 and some offspring of the new melon ILs. From left to right. row 1:Vedrantais, F1, and Ginsen makuwa; row 2: Piel de sapo, F1 and QPM; row 3: segregation of shapes and colors found in the BC3 derived from Piel de sapo x QPM.

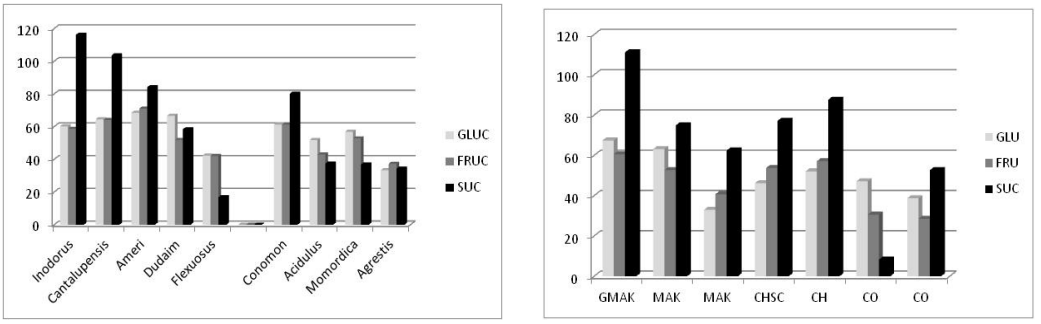
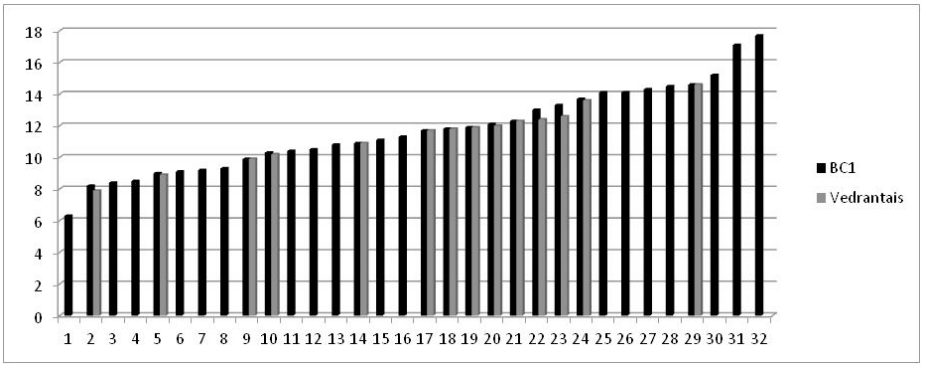
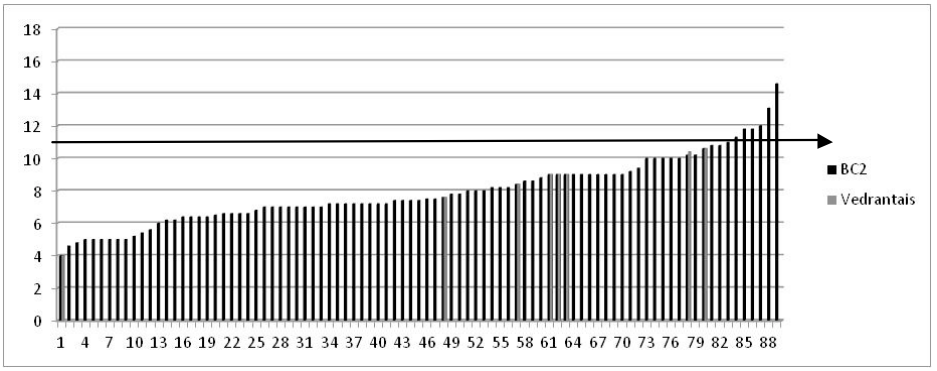


Fig. 2. Left: Average sugar content (µmol g-1FW of glucose, fructose and sucrose) in several accessions of different melon varieties belonging to the two melon subspecies. Sugar content of Ginsen makuwa (GMAK) in comparison with other exotic accessions belonging to the *conomon* group (*makuwa*, *chinensis* and *conomon*), including Songwhan Charmi (CHSC).





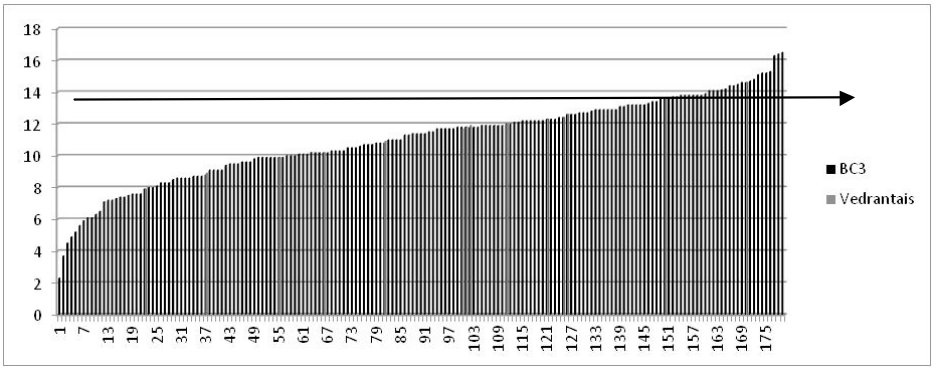


Fig. 3. Brix degree distribution in fruits of BC1, BC2 and BC3 population derived from the cross Ginsen makuwa x Vedrantais. The arrows indicate the maximum Brix degree in fruits of the recurrent parent (grey bars).

Antioxidant enzyme activities and gene expression patterns of grafted cucumber in response to NaCl stress

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Keywords: Grafting, NaCl stress, H_2O_2, cucumber, antioxidant enzyme

Abstract

The objectives of this study were to examine antioxidant enzyme responses to NaCl stress at both enzymatic activity and transcript levels and to determine the major antioxidant processes associated with salt tolerance of grafted cucumber plants. It studied the changes in the time course of H_2O_2 content and main antioxidant enzyme (CAT, APX, GR and MDAR) activities in the leaves of self-grafted and rootstock-grafted cucumber plants under 0 and 75 mM NaCl stress, as well as the expression levels of antioxidant enzyme genes in grafted cucumber plants under NaCl stress for 120 h. The results showed that the H_2O_2 content in self-grafted leaves increased gradually during NaCl stress from 24 h to 74 h and then decreased slowly, whereas the H_2O_2 content in rootstock-grafted increased much slowly and kept low level all the time. This could be associated with its higher antioxidant enzyme activities during NaCl stress from 48 h to 120 h. The changes in the expression levels of *CAT* and *cAPX* in self-grafted leaves and roots, as well as the expression level of *cAPX* in rootstock-grafted leaves were consistent with the changes of their enzyme activity levels under NaCl stress. Taken together, these results suggested that rootstock grafting can decrease the H_2O_2 accumulation of cucumber under 75 mM NaCl stress, due to the enhancement of antioxidant systems at both enzymatic activity and transcript levels.

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The role of ethylene response factors in cucumber (*Cucumis sativus* L.) under waterlogging stress

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Keywords: Cucumber, waterlogging, *ERF*, stagnant solution, DO

Abstract

Plant species display various mechanisms to cope with waterlogging. However, little was known about ethylene response factors (*ERF)* group regulation in waterlogging response in cucumber, which plays an important role in low oxygen tolerance in Arabidopsis (*Arabidopsis thaliana* L.) and rice (*Oryza sativa* L.), associated with waterlogging stress. Three *ERF* group Ⅶ genes in cucumber were identified, named *CsERF1*, *CsERF2* and *CsERF3*, based on the information of differentially expressed genes in waterlogging tolerant line Zaoer-N root under waterlogging stress by digital gene expression profile and Cucurbit Genomics Database (htpp://www.icugi.org). Previous research had shown that *CsERF1* was induced in the root of tolerant line Zaoer-N under waterlogging stress, while *CsERF3* was repressed, and no significant change was found in *CsERF2*. When pretreated with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, *CsERF1* was significantly down-regulated, but *CsERF3* was slightly up-regulated. In the present study, a series of stagnant concentrations (deoxygenated 0, 0.05% and 0.10% Agar) was used to simulate the hypoxic environment and test the expression pattern of *CsERF1* and *CsERF3* at 4h and 12h after stagnant treatment. The results suggested a key role of *CsERF1* in response to low oxygen and waterlogging stress in cucumber.

INTRODUCTION

Cucumber is an agriculturally and economically important crop worldwide, is easily affected by heavy rain and subsequent periods of soil flooding in summer, especially in China（Xiao-Hua Qi 2012）. As the spread rate of oxygen in water is 1000 times lower than in air, oxygen deprivation is the main cause of damage for plants under waterlogging (R.K. SAIRAM, 2008). Tolerance to low oxygen availability has been considered to be influenced by different members of subgroup

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VII of the *ERF* transcription factor family in Arabidopsis (*RAP2.12*, *RAP2.2*; *HRE1* and *HRE2*) and rice (*SUB1*, *SK1* and *SK2*) (Francesco Licausi 2011; Francesco Licausi 2010).

Transgenic Arabidopsis plants, which over-expressed *HRE1*, showed an improved tolerance of anoxia, whereas a double-knockout mutant hre1hre2 was more susceptible than the wild type. *HRE1* over-expressing plants showed an increased activity in the fermentative enzymes pyruvate decarboxylase and alcohol dehydrogenase together with increased ethanol production under hypoxia, but not in normoxia. Whole-genome microarray analyses suggested that an over-expression of HRE1 increased the induction of most anaerobic genes under hypoxia.

The objective of this study was to make clear which member of *ERF* group Ⅶ regulates waterlogging response in cucumber.

MATERIALS AND METHODS

Plant Materials and Waterlogging Stress

Seedlings of cucumber (waterlogging tolerant line 'Zaoer-N') were grown in 25 cm in diameter pots containing peat, vermiculite, and perlite (3:1:1, v/v) in a greenhouse at 28/18 °C (12/12 h) day/night temperature, and relative humidity ranging 70%-85% at the experimental farm of the Department of Horticulture in Yangzhou University. Treatment was begun at three- leaf stage. The control plants were watered regularly as required to maintain vigorous growth.

RNA Extraction

Total RNAs of Zaoer-N's roots were isolated using RNAiso plus (TAKARA).

Real-Time PCR

The roots were harvested directly into liquid nitrogen and stored at -80℃ at 0h, 2h, 4h, 8h, 12h, 24h, 48h after waterlogging treatment. Three separate samples of waterlogging stress treatment and control were used for Real-Time PCR.

The pairs of specific primers used to amplify the *Actin* in cucumber were TCGTGCTGGATTCTGGTG (Forward primer) and GGCAGTGGTGGTGA ACAT (Reverse primer). The pairs of specific primers used to measure the transcript levels of *CsERF1* were AGGTTAGCGTTATTGCTGC (Forward primer) and CTGACCGTCAAGGTATGG (Reverse primer). The pairs of specific primers used to measure the transcript levels of *CsERF3* were TCAGACGAGGGAAGTAAC (Forward primer) and AGTCTTAGCACTAGCACCT (Reverse primer).

RT-PCR was performed according to the TaKaRa manufacturer specifications (TaKaRa SYBR® PrimeScript™ RT-PCR Kit, Dalian, China). SYBR Green PCR cycling was denatured using a program of 95 °C for 10 s, and 40 cycles of 95 °C for 5 s and 52 °C for 30 s and per- formed on an iQ™ 5 Multicolor real-time PCR detection system (Bio- RAD, USA).

Stagnant Treatment

A series of stagnant treatment containing 0, 0.05% and 0.10% Agar (SIGMA) were prepared, after cooling, dissolved oxygen content were detected by Dissolved Oxygen Meter (HANNA). Soil attached to the roots of the seedlings was carefully washed off, and put into the holes of bubble board, then were placed liquid level (Fig. 3).

RESULTS AND DISCUSSION

A series of stagnant solution were taken advantage of to simulate hypoxia stress, which contained different concentration of dissolved oxygen (Fig. 2). The expression of *CsERF1* in Zaoer-N was gradually increased as the decrease of dissolved oxygen concentration at 4h and 12h after treatment. However, the expression of *CsERF3* was low and no significant change was found (Fig. 4.).

The results showed that there was no necessary link between *CsERF3* and concentration of oxygen, but *CsERF1* might play a key role in hypoxia stress.

In this paper, we verified two ERF group Ⅶ genes expression patterns in different DO content, suggesting that *CsERF1* could take part in hypoxia stress caused by waterlogging. It was reported that when the oxygen concentration was low—as during flooding—RAP2.12 was released from the plasma membrane and accumulated in the nucleus to activate gene expression. The putative amino acid sequences of *RAP2.12* and *CsERF1* also had the same conserved amino-terminal amino acid MCGGAII (Fig.1.), which had been proven to closely related to hypoxia acclimation. It could be inferred that *CsERF1* had an important role during hypoxia and waterlogging stress in cucumber, but this need to be further explored by functional analyses.

ACKNOWLEDGEMENTS

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Fig.1. *ERF* group Ⅶ genes amino acid sequence alignment between cucumber and arabidopsis.

	OD_1	OD_2	OD_3	Average
Water	7.04	7.23	7.53	7.17
0.05%Agar	6.44	6.20	6.10	6.25
0.1%Agar	3.68	3.35	3.48	3.50

Fig. 2. DO content in stagnant solution (ppm, 24).

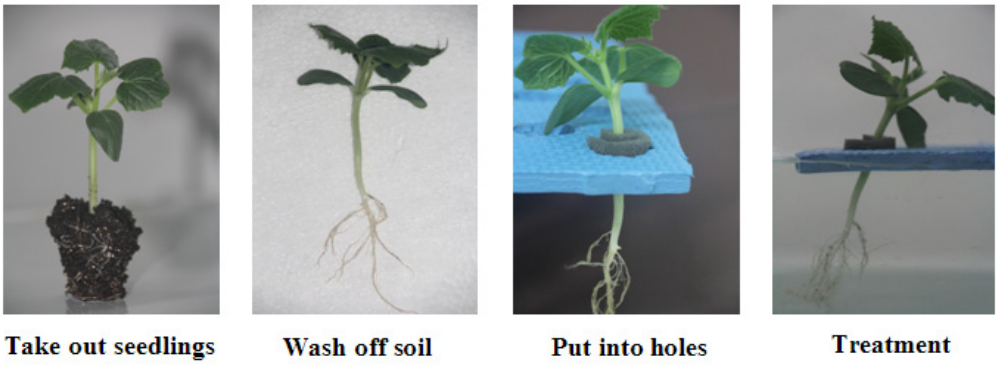

Fig. 3. Pretreatment of cucumber seedlings before hypoxia stress.

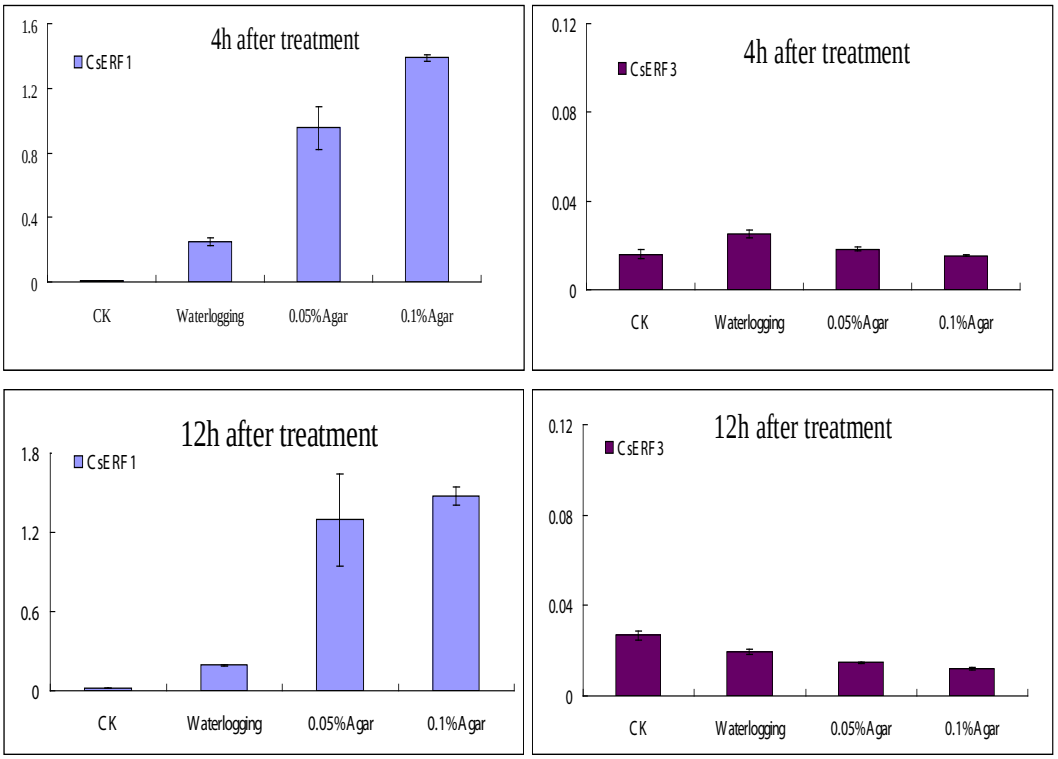

Fig. 4. Transcript levels of *CsERF1* and *CsERF3* under waterlogging and stagnant solution treatment.

Fruit texture variation among Beit Alpha, European greenhouse and Japanese cucumbers (*Cucumis sativus*)

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Keywords: Crispness, firmness, flesh, skin, placenta

Abstract

Fruit texture traits, such as firmness and crispness, of cucumber (*Cucumis sativus* L.) are of great importance, because they are related to the fruit's commercial value directly. Cucumber cultivars are classified into several types, including Beit Alpha, European greenhouse, and Japanese, and the fruit texture of each type has been genetically improved to meet different consumer's demands for a long time. To clarify the differences in fruit texture traits among the Beit Alpha, European greenhouse, and Japanese types, we evaluated the fruit firmness and crispness at three different stages (immature, intermediate, and mature) through analyses of the force–deformation curve obtained by mechanical measurements. Firmness and crispness clearly differed among the three types and among the three stages. The flesh crispness was significantly higher in Japanese cucumbers at all fruit stages, and they maintained relatively high crispness even at maturity. The crispness of the other types remained low at all stages. These results suggested that Japanese genotype could be used as genetic resources to breed new cultivars with high crispness.

INTRODUCTION

A wide variety of cucumber (*Cucumis sativus* L.) types, including Beit Alpha (a Middle-Eastern slicer), European greenhouse (a Dutch greenhouse slicer), pickling (an American pickling), slicer (an American slicer), and an oriental trellis slicer type, are cultivated around the world (Shetty and Wehner 1998). The oriental cucumbers comprise at least three types: the North-Chinese, South-Chinese, and Japanese types (Sakata and Sugiyama 2002). The primary differences among the cucumber types are the appearance of the fruit, such as the shape and color (Shetty and Wehner 1998), and fruit texture, which differs greatly among types (Sakata et al. 2011).

Fruit texture, which can be assessed using characteristics such as firmness and crispness, are the most important traits in cucumber, because they are related to the product's commercial value directly. Fruit texture and appearance traits have been

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genetically improved by breeders to meet local consumer's demand for a long time. In addition, the marketable fruit size differs greatly among cucumber types, and this results in a wide variation of fruit texture of cucumber in the market, because size is related to the relative degree of maturity when a given cultivar is typically harvested. However, the differences in fruit texture among cucumber types and growth stages have not yet been clearly defined, because textural traits other than firmness have been difficult to quantify objectively.

The objective of this study was to determine the fruit texture characteristics of Beit Alpha, European greenhouse, and Japanese cucumber types using a new texture measurement method (Horie et al. 2004, Yoshioka et al. 2009). Time-dependent changes among stages (immature (ca. 130g), intermediate (ca. 300-800 g), and mature) in the physical properties of each cultivar type were analyzed, and the prospects for breeding and fruit texture research in cucumber were discussed.

MATERIALS AND METHODS

Plant materials and cultivation procedures

Twenty-nine cultivars (6 Beit Alpha, 8 European greenhouse, and 15 Japanese) were tested. Plants were grown during spring and summer of 2011 at the NARO Institute of Vegetable and Tea Science (Tsu, Mie, Japan). To evaluate the texture of immature fruits, seeds were sown in a greenhouse in 9-cm-diameter plastic pots filled with 1:1 mixed soil of Rakusaku (Mikado Kyowa Seed Co., Tokyo, Japan) and Engei Baido (Kureha Chemical Industry Co., Tokyo, Japan) on 17 March 2011, and seedlings were transplanted in the greenhouse on 6 April. The immature fruits were harvested from 4 June to 15 July. To evaluate the texture of intermediate and mature fruits, seeds were sown and transplanted on 11 April and 2 May, respectively, under the same conditions used for the immature fruits. The intermediate and mature fruit were harvested from 6 June to 27 July. Four plants of each cultivar were transplanted.

Evaluating the fruit physical properties

A 15 mm thick transverse slice was cut from the middle of each fruit to measure flesh and placental firmness. In addition, 5-mm-thick sliced skin samples were prepared to examine the skin firmness. Both samples were punctured with a Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) fitted with a 3.0-mm ø plunger. The speed of advance was 150 $mm \cdot min^{-1}$. Transverse samples were punctured in the direction of the long axis of the fruit for a distance of 8 mm; the placenta was punctured once, and the fleshy part at three separate locations. The middle 2/3 of each force–deformation curve was analyzed. Skin samples were penetrated in the direction from within outward for a distance of 12 mm, three times per sample. The max peak force in each force–deformation curve was read from the graph.

We calculated the flesh, placental, and skin firmness and a flesh crispness

index (CI), which were commonly regarded as basic elements of cucumber fruit texture. CI, which is strongly correlated with sensory crispness evaluation results (Yoshioka et al. 2009), is defined as the total of the absolute values of the second derivatives of the force at arbitrary intervals in the force–deformation curve (Horie et al. 2004).

RESULTS AND DISCUSSION

There was no clear difference in flesh firmness at the immature stage (Fig. 1a). As the fruits matured, each type exhibited distinctive characteristics. The firmness of the Japanese type remained relatively high even at maturity, whereas that of the European greenhouse type decreased. In the Beit Alpha type, the firmness of some cultivars remained high as they matured, but that of others decreased (Fig. 1a). The placental firmness of the European greenhouse type was higher than that of the other types at the immature stage, but there was no clear difference among types at the intermediate and mature stages (Fig. 1b). We believe that the placental firmness of the immature fruit was affected by fruit diameter, because the European greenhouse type was thinner than the other types. The skin firmness of the Beit Alpha and Japanese types tended to be higher than that of the European greenhouse type, but there were no clear differences among the types (Fig. 1c).

The flesh crispness of the Japanese type was higher than that of the other types at all fruit stages, and remained relatively high even at maturity (Fig. 1d). In contrast, that of the other types remained low at all stages (Fig. 1d). Sakata et al. (2011) also reported that the flesh crispness of Japanese type cucumber cultivars were higher than those of the Beit Alpha and European greenhouse types at 17 days after anthesis.

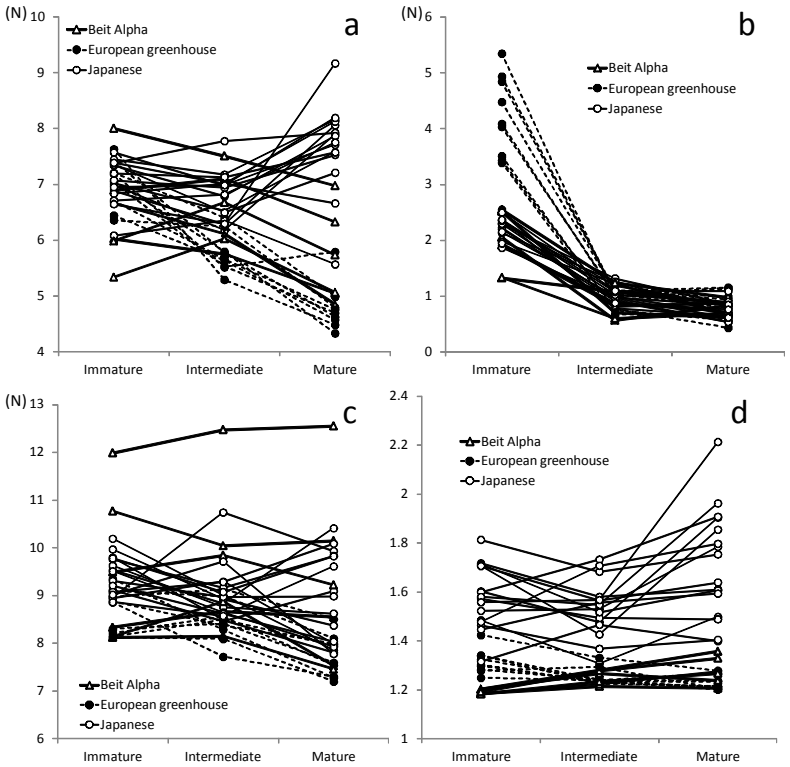

Fig. 1. Time-dependent changes in fruit texture traits among cucumber types: (a) flesh firmness, (b) placental firmness, (c) skin firmness, (d) crispness index.

In conclusion, the force–deformation curve method was successfully used to distinguish the cucumber fruit texture of three important cucumber types, and it revealed that fruit texture (especially flesh crispness) differed distinctly among types. The evaluation method provided an objective and quantitative indicator of cucumber fruit texture, and showed considerable promise for use in identifying suitable cultivars for use in breeding programs and cucumber research. In addition, the high crispness index of the Japanese cucumber type indicated that breeders had focused on improving fruit texture and flesh crispness. To meet consumer demand for high crispness in the other types, the Japanese genotypes could be used as breeding material.

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Inheritance of low temperature seed germination ability in cucumber

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Keywords: *Cucumis sativus*, epistasis, chilling, resistance

Abstract

The objective of this study was to determine the type of gene action controlling low temperature germination ability in cucumber accession PI 390953. Two separate tests were run for genetic analysis. 'Chipper' (P_1) was used as the susceptible parent in crosses with PI 390953 (P_2) to produce seven generations for this study: P_1, P_2, F_1, $F_{1\ reciprocal}$, F_2, BC_{1P1} and BC_{1P2}. D Detection of Bacterial Fruit ifferences in low temperature germination ability at 13°C in the seven generations were observed using two germination test methods, days to germinate and percentage of germination. PI 390953 (P_2) germinated at 94.3% and 96.3% after 14 and 21 days, respectively. 'Chipper' and BC_{1P1} failed to germinate at the same temperature. The F_1 and $F_{1\ reciprocal}$ did not differ in cold germination ability, indicating no cytoplasmic or maternal effects. Additionally, these populations showed high DTG index (17.7 and 17.8, respectively), indicating recessive inheritance of low temperature germination ability in PI 390953. The F_2 indicated non-allelic gene interactions for inheritance of low temperature germination ability. Chi-square estimates suggested double dominant epistasis with an expected ratio of 15:1 (susceptible : resistant). This interpretation was largely confirmed by the segregation ratios in the backcross populations.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a chilling susceptible crop. Early planting of cucumber is important to growers interested in the early market. In Poland, cucumbers are often seeded in the middle of May when the occurrence of low-temperature or cold stress affects seed germination and seedling establishment, causing partial or complete stand reduction.

Our previous studies indicated that low temperature seed germination ability and chilling resistance at the seedling stage were inherited as separate traits (Kozik et al. 2007; 2010). Having evaluated the cucumber germplasm collection the PI

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390953 was identified as chilling resistant cultigen, which exhibited high seed germination rate under low temperatures (11 and 13°C).

The objective of this study was to determine the type of gene action controlling cold germination ability in the resistant accession PI 390953.

MATERIALS AND METHODS

The parental monoecious lines used in this study were resistant PI 390953 and susceptible 'Chipper' (Kozik et al. 2007; 2010). 'Chipper' (P_1) was hybridized with PI 390953 (P_2) to develop five generations (F_1, $F_{1reciprocal}$, F_2, BC_{1P1} and BC_{1P2}).

Non-imbibed seeds of all these populations were tested in 150 mm diameter Petri dishes (50 seeds/Petri dish) with two layers of filter paper and 3 ml of distilled water. Sseeds did not soak beforehand. The experiment had three replications for both parents, four replications for both F_1 populations, six replications for BC_{1P1} and BC_{1P2} and eight replications for F_2 (one Petri plate/one replication).

Seed germination (radicles $\geq$ 3mm long) was recorded daily during three weeks of incubation in growth chambers in the dark at 13°C and 25°C (control temperature). Data were expressed as germination percentage and mean number of days for seeds to germinate (DTG) for each population using the formula of Smith and Millet (1964). The results of the test were summarized using analysis of variance (ANOVA) for DTG. Differences between populations were tested using Newman-Keul's test at 5% of probability.

RESULTS AND DISCUSSION

Differences in germinability at low temperature (13°C) were observed among both parents and their hybrid populations (Table 1). The fastest germination and the highest germination percentage were observed in PI 390953 (P_2) with germination of 94.3 and 96.3% after 14 and 21 days, respectively. 'Chipper' and BC_{1P1} were not able to germinate at 13°C. The F_1 and reciprocal F_1 populations showed no difference with that regard, indicating no maternal effects. The high DTG index (17.7 and 17.8, respectively) indicated recessive inheritance of cold germination ability in PI 390953.

For genetic analyses, populations segregating for cold germinability populations were grouped into three classes: 1) resistant – R (seeds germinated within first 14 days of treatment, which is as rapidly as the resistant parent PI 390953); 2) moderate – M (seeds germinated between 15th and 21st day of incubation period); 3) sensitive – S (seeds not germinated within 21 days, as sensitive parent Chipper) (Table 2). We tried to fit our data of F_2 segregating population to Mendelian inheritance models. Seeds of F_2 population germinating at 13°C as rapidly as the resistant parent PI 390953 were classified as homozygous recessive with 2 or 3 recessive genes for low temperature germinability. In this case, F_2 population

showed 10.75% of seed germinability, indicating no fit with a two – gene (6.25% homozygous recessive) nor a three – gene (1.56% homozygous recessive) model. It suggests that the inheritance of the germinability at low temperature is a quantitative trait.

Data analysis of F_2 population showed non-allelic gene interactions for the inheritance of resistance to low temperature germination. Chi-square estimates of five types of epistasis (Table 2) showed that double dominant epistasis with theoretical segregation 15:1 (15/16 sensitive - germinating after 14 days or not germinating at all; 1/16 resistant – germinating within 14 days). The result was also largely confirmed by the segregation ratios in the backcross population. In the BC_{1P2} a segregation ratio of 3:1 (3/4 sensitive and 1/4 resistant was obtained for chi square=0.16 at P=0.69.

Previous reports regarding the mode of inheritance of cold germination ability in cucumber were very scarce. Low heritability of this trait was showed by Nienhuis and Lower (1981) and Wehner (1982; 1984). Taking into account the results of this study and other studies, it may be concluded that appropriate breeding strategy for low–temperature germination ability should be based upon family selection only, instead of single plant selection or backcross method which are an ineffective practice in this case.

ACKNOWLEDGEMENTS

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Table 1. Seed germination ability at low temperature (13°C) displayed by seven generations of resistant x sensitive cucumber

Generation	Percentage germination		DTG[z]
	14 days	21 days	
P1 (Chipper)	0	0	21 e
P2 (PI 390953)	94,3	96,3	7,3 a
F1	1,3	15	17,7 d
F1 Reciprocal	1,3	10,7	17,8 d
F2	20,0	29,0	12 b
BC1P1	0	0	21 e
BC1P2	11,3	21	14,8 c

[z]DTG = mean number of days to germination
Means were compared using Newman-Keuls' test at $\alpha = 0.05$.

Table 2. Genetic analysis of low temperature seed germination ability at 13°C

Type of epistasis	F2 segregation		χ^2	df	P
	theoretical	Observed			
Recessive	9 : 3 : 4 S : M : R	346 : 11 : 43	161,06	2	0,00
Double recessive	9 : 7 S + M :R	357 : 43	177,04	1	0,00
Dominant	12 : 3 : 1 S : M :R	346 : 11 : 43	76,61	2	0,00
Dominant and recessive	13 : 3 S + M :R	357 : 43	16,75	1	0,00
Double dominant	15 : 1 S + M :R	357 : 43	1,62	1	0,20

S – sensitive – not germinating within 21 days at 13°C
M – moderate – germinating within 14 and 21 days at 13°C
R – resistant – germinating within first 14 days 13°C

A new approach of investigating low temperature stress in cucumber (*Cucumis sativus* L.) lines by using SPAD-502 in unheated greenhouse conditions

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Keywords: Cucumber, low temperature stress, SPAD-502, cold tolerance, yield component

Abstract

The objective of this study was to establish relationship between SPAD-502 value in cucumber leaves at vegetative stage and generative growth traits of cucumber under the unheated greenhouse conditions. For the vegetative test, seedlings were placed in greenhouse with an average photoperiod of 14 h at 16.2/6.6°C day/night for 14 days using randomized complete block design with 4 replications in early spring of 2010. Chilling tolerance for the seedlings was evaluated by the severity of chilling injury (SCI) for cotyledon, the first and second true leaf. The scale values were rated 0 to 9. The SPAD values (chlorophyll contents) ranged from 22.13 to 61.25. The chlorophyll contents of Ch37, Ch45, Ch48, Ch50 and Ch56 lines were measured higher and their values were not decreased due to low temperatures. After the chilling, they were moved to unheated greenhouse conditions for the generative test. The result showed that only five lines Ch45, Ch48, Ch 55, Ch8 and Ch56 from 68 were found to be the best combiner parental lines for yield component. As a result, the study indicated that Ch45, Ch48 and Ch56 lines were promising in unheated greenhouse condition.

INTRODUCTION

Cucumber is one of the most susceptible vegetable crops to chilling injury (CI), especially at temperatures below 6°C (Kozik and Wehner 2008). The physiological symptoms such as stunted growth, reduced photosynthetic capacity, necrosis and discoloration, wilting, acceleration of senescence and death at seedling stage are referred to " chilling injury (CI)" or "low temperature injury (LTI)". In the field condition, low temperature affects fruit setting and development and causes the decrease of yield and fruit quality for cold sensitive varieties (Wang 1982; Chung et al. 2003; Kozik and Wehner 2008).

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The Mediterranean region has typically warm and mild winters. Low temperature is an important factor limiting the productivity in early spring and single crop growing seasons in cucumber crop. In Antalya, heating for vegetable production is used during cold periods in the greenhouses by taking some precautions against frost. This makes it possible to grow vegetables such as cucumber at any time of the year under very simple protection during the coldest period.

Measuring chlorophyll loss, which is associated with environmental stress, using SPAD meter provides useful information as variation in total chlorophylls is an important indicator of stress in plants (Hendry and Price 1993; AT Netto et al. 2005). Moreover, there is no study about SPAD meter reading variation induced by low temperature stress levels in cucumber seedling.

The objectives of this study were to determine 1) SPAD meter readings variation of different seedling stage under the low temperature; 2) yield and yield components of cucumber lines suitable for early spring growing in the unheated greenhouse condition.

MATERIALS AND METHODS

Plant material

Sixty-eight lines and three cultivars were used for vegetative test screening under the low-temperature. The cultivars were İberia F_1 (Tolya Seed Co.), Termessos F_1 (Seminis Seed Co.) and Halley F_1 (Seminis Vegetable Seeds Inc.).

Chilling treatments

Experiments were conducted in unheated greenhouses conditions of the Department of Vegetables at Bati Akdeniz Agricultural Research Institute (BATEM). Seeds of 68 lines and three cultigens (control) were sown on December 30, 2010. 23 day-old seedlings were transplanted in plastic pots with dimensions of 15×15×15 cm on January 19, 2011. The design was a randomized complete block design (RCBD) with four replications and each plot contained three plants of each line.

At the seedling stage, the genotypes were grown in screening greenhouses at an average day length of 14 h with 16.8°C day /7.8°C night temperatures for 14 days. Plants were watered with the standard phytotron nutrient solution (Thomas et al. 2009).

Seedlings were rated after the 14 days under the low temperature condition, rating the damage (chlorosis and necrosis) on the first true leaf. The scale was 0 to 9: 0 = no damage, 1 to 2 = trace of damage, 3 to 4 = slight damage, 5 to 6 = moderate damage, 7 to 8 = advanced damage, and 9 = plant dead. Data were collected as means over all leaves on the plants within each line (Kozik and Wehner 2008).

Data analysis

In this experiment, seedlings rated 0 to 6 were considered to be resistant, and those rated 7 to 9 were considered susceptible. Seedling rated 7 to 9 would not recover from CI, but those rated 0 to 6 would recover and grow out of their injury. Data were collected as means over all leaves on the seedlings within each line (Kozik and Wehner 2008).

SPAD chlorophyll meter readings (SCMR)

The means of SCMR values were obtained from the cotyledon, the first and the second true leaf stage within each genotype by using the portable chlorophyll meter (SPAD-502, Minolta, Japan). The experiment was a RCBD with 4 replications and 3 plants each replication.

Greenhouses performance

The genotypes were evaluated in a greenhouse in early spring 2011. Seeds of 68 lines were sown on February 1th and transplanted on February 23th in 750 m^2 plastic houses. 23 day-old seedlings with 2-3 true leaves were planted in 2 double rows in each plot. The spacing was 60 cm between the rows and 50 cm between plants on each row. Irrigation system was micro-tube with 25 cm dripper distance.

Total yield was the sum of six harvests (twice each week for 3 weeks), and early yield was the sum of the first two harvests during spring 2011. Data were collected for yield and yield component (average total yield and fruit number, average early yield and fruit number per plant) and other horticultural characters. The design was a RCBD with two replications and 10 plants each replication. Data were analyzed using GLM the procedure in SAS (SAS Institute Inc. 2001).

RESULTS AND DISCUSSION

Vegetative test

A direct usage of SCMR values was first reported in cucumber under low temperature stress by our studies. Table 1 indicated that SCMR under low temperature stress was different between lines and leaves of different stages for a given line. The coefficient of variation (CV) values were 3.69%, 9.45% and 6.04% for cotyledon, the first and the second true leaf stage, respectively. The means of SCMR values were 50.34, 34.41 and 42.61 for three stages, respectively. SCMR values were higher in the cotyledon stages than the first and second true leaf stage.

When the seedlings were exposed to low temperature for 14 days, SCMR values of second true leaf stage were lower than cotyledon leaves. But highly tolerant and moderately resistant lines did not show any differences in their SCMR values (Table 1). Smeets and Wehner (1997) reported that seedlings at cotyledon stage were less sensitive to CI than seedlings at the first true fully expended leaf.

These results were consistent with the results in our research.

Mean chilling damage increased with increasing the duration of chilling, which resulted in decreasing SCMR values. However, values of SCMR for Ch45, Ch48, Ch37 and Ch50 lines had higher SCMR values at the second true leaf stage than the other stages. There were no large changes in Ch3, Ch43, Ch15 and Ch47 lines for SCMR values (10-25% difference) than the others.

The chilling damage rating ranged from 0 to 9 on the first true leaf stage in cucumber seedling. The highest scores were obtained for Ch65 (8) and 40 (6) lines which showed curling, stunted growth, necrosis and discoloration, as well as wilting and death at the seedling stage. In comparisons Ch1, Ch11, Ch20, Ch37, Ch38, Ch45, Ch48, Ch56, Ch57, Ch58, Ch59, Ch61, Ch62 and Ch71 exhibited no damage within the 14 days; consequently, they were identified as cold tolerant-resistant lines.

Generative Test

The obtained yields of varieties in cold season were analyzed and results have been shown in Table 2. Results showed that there was a high significant difference in the yield ($P < 0.01$) (Table 3). Total yield ranged from 288 to 1409 g/plant. The highest yield (1409, 1378 and 1368 g/plant) was obtained for Ch48, Ch8 and Ch27 line, which was significantly different from other lines. The total average yield of the lines was 933.24 g/plant.

High significant differences in the early yield ($P < 0.01$) were observed among the lines (Table 3). Soleimani et al. (2009) explained that average yield in cold season showed significant difference between years and cultigens. These results were confirmed in our experiments. Ch15 had the highest (459 g/plant) and Ch68 had the lowest yield (101 g/plant) in cold season.

The high significant differences in the fruit number ($\alpha = 0.01$) were observed between varieties in a year (Table 3). Therefore, the selection of lines with high fruit number in the first step and with high fruit mass in the second step could help in successful cucumber production. In total yield (per plant), selection of Ch27, Ch48 and Ch8 lines for commercial production at this season were beneficial because the highest number of fruit (13.12 and 11 fruits per plant) and the highest yield were obtained from these lines. On the other hand, in early yield, fruits number ranged from 1 to 4 per plant. Ch45, Ch23 and Ch61 had the highest number per plant in the first two harvests during spring 2011.

Table 1. Variation of SCMR at the different leaf stage in cucumber lines.

Source	Seedling Stage		
	Cotyledon	First true leaf	Second true leaf
Range	41.28-66.60	22.13-45.23	35.53-54.18
Means	50.34	34.41	42.61
CV (%)	3.69	9.45	6.04
LSD (5%)	2.47	10.55	6.62
F Test	21.94**	5.43**	4.80**

**: Significant differences at $P<0.01$

Table 2. Different growth stage for SPAD Chlorophyll Meter Readings (SCMR) values in cucumber seedlings.

Seedling Stage	SPAD Chlorophyll Meter Readings (SCMR)										
	Lines								Control		
	Ch45	Ch48	Ch37	Ch50	Ch3	Ch43	Ch15	Ch47	C1*	C2*	C3*
Cotyledon	41.83	41.28	53.88	44.50	49.85	44.08	49.78	48.10	49.18	45.60	57.68
First true leaf	35.63	33.88	38.98	30.95	34.50	34.53	36.03	35.20	37.18	37.13	41.50
Second true leaf	46.55	41.75	54.18	44.58	48.50	42.68	46.30	44.73	43.95	43.38	44.05

*: C1: İberia F_1; C2:Termessos F_1; C3:Halley F_1

Table 3. Variation of lines in yield and yield component.

Source	Yield (per plant)		Early yield (per plant)	
	Fruit wt (g)	No. Fruit	Fruit wt. (g)	No. Fruit
Range	288-1409	3-13	101-459	1-4
Means	933.24	8	280.77	2
CV (%)	6.95	13.21	26.99	32.02
LSD (5%)	129.48	2.09	151.27	1.62
F Test	31.89**	8.02**	2.75**	1.73**

**: Significant differences at $P<0.01$

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Factors influencing fruit size and shape differences in cucumber

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Keywords: Ovary, fruit development, ovule number, cell division, cell number

Abstract

Size and shape are key commercial fruit qualities in cucumber (*Cucumis sativus*). To examine factors influencing variation in fruit growth, cultivars representing two extreme types, American pickling (Gy14) and 'Chinese long' (CL) were chosen for study. Ovaries and fruits of the two parents and their F_1 and F_2 progeny were analyzed from 7 days pre-anthesis (dpa) to 20 days post-pollination (dpp) for: ovule number, ovary length and diameter, fruit length and diameter, length/diameter ratio (L/D), rate and period of cell division and expansion, number of cells, and cell size. Differences in length and diameter were already apparent by 7 dpa. CL had approximately twice as many ovules as Gy14. Cell number and rate of cell division pre-anthesis was higher for CL than Gy14 in the longitudinal direction. CL also had a greater number of cells than Gy14 in cross section at 7 dpa but subsequent rate of cell division was higher in Gy14. The relative rates of cell division in the longitudinal and transverse directions continued post-pollination. Fruit growth was largely completed 10-12 dpp for Gy14 but continued for approximately 20 dpp for CL. Cell size was comparable for CL and Gy14 throughout fruit development. F_2 progeny showed a high correlation of ovule number, ovary length, and ovary L/D at anthesis, with fruit length. In contrast, ovule number, ovary diameter, and ovary L/D were not correlated with fruit diameter. Fruit length showed high correlation with L/D but not fruit diameter. These results suggest that variation in fruit size and shape in CL and Gy14 is associated with growth-related factors pre- and post-anthesis, including ovule number, ovary length, rate of cell division, plane of cell division, length of period of cell division, and cell number. Factors controlling length were largely determined pre-anthesis while factors regulating diameter were largely determined post anthesis.

INTRODUCTION

Fruit size and shape are important quality traits in cucumber which are influenced by ovary and fruit growth. Cucurbit ovaries exhibit distinctive sizes and

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shapes, and studies of cucumber and melon showed a correlation between size and shape of ovaries and mature fruits, indicating that factors controlling these traits exist during early ovary development (Goffinet 1990; Perin et al. 2002). Ovary factors that can influence fruit size and shape include carpel number, ovule number, and cell number (Fernandez-Silva et al. 2010; Varga and Bruinsma, 1990; Rosati et al. 2011).

Following fertilization, fruit growth is driven by a period of cell division followed by increase in cell size (Gillaspy et al. 1993). In cucumber, rapid cell division occurs 0-4 days post pollination (dpp) then slows down until 8 dpp; increase in cell size and fruit size in pickling cucumber primarily occurred from 4-12 dpp (Boonkorkaew et al. 2007; Ando and Grumet 2010). Cell number has been shown to be a predominant influence on fruit size in several species including melon where difference in pericarp cell number resulting from variation in period of cell division was associated with difference in fruit size (Higashi et al. 1999). Importance of cell number or size has been observed in studies of strawberry, apple olive, and blueberry (Cheng and Breen 1992; Malladi and Hirst 2010; Rosati et al. 2011; Johnson and Malladi 2011).

In this study we determined if cucumber fruit size and shape is a function of: ovule number, ovary size, cell number, cell size, and period and rate of cell division and expansion pre- and post-anthesis. The role of these factors was examined using two sequenced cucumber genotypes with extreme differences in fruit size and shape, 'Gy14' (pickling) and '9930' (North Chinese type, Chinese Long) along with their F_1 and F_2 progenies.

MATERIALS AND METHODS

Two experiments were performed in the greenhouse including 60 plants each of Gy14, Chinese Long (CL), and their F_1 progeny (Exp.1), or Gy14, CL, F_1 and F_2 progeny (Exp. 2) (parental seeds were provided by Dr. Y. Weng, Univ. of Wisconsin). Growth conditions were as described by Ando and Grumet (2010). Developing floral buds from ten plants of Gy14, CL, and F_1 were measured daily for length and diameter from 7 days pre-anthesis (dpa) to anthesis. At anthesis, ten flowers from each genotype were dissected to count ovules. To minimize environmental effects and inter-fruit competition, 1-2 flowers were hand-pollinated on the same day on 60 plants for each genotype; only one fruit was allowed to develop per plant. Ten fruits/genotype were measured daily for length and diameter until 20 dpp.

To determine cell number and cell size during ovary growth, five developing floral buds from 7-0 dpa were collected from each genotype. Floral buds were fixed, stained and embedded as described by Jackson (1991). Longitudinal and transverse sections (10um) were prepared using Leica rotary microtome (2125RT; Leica Microsystems, Buffalo Grove, IL). Tissue sections were viewed using light

microscope with Spot RT3 digital camera system (SPOT Imaging Solutions, Diagnostic Instr., Inc., MI). To measure cell size, three boxes (300um x 300um) were drawn on each of the images and number of cells determined. To examine cell number and cell size post-pollination, five fruits of each age were harvested at 2 day intervals from 0-16 dpp for microscopy as per Ando and Grumet (2010).

RESULTS AND DISCUSSION

The difference in ovary length between CL and Gy14 was observed at 7 dpa (1.05±0.05cm vs 0.37±0.04cm) and continued to increase until anthesis (5.2±0.19cm vs 1.9±0.05cm). CL also had a larger diameter than Gy14 at 7 dpa (0.23±0.01cm vs 0.14±0.01cm); however, at anthesis their diameter was equivalent (0.60±0.02cm vs 0.58±0.02cm).The ovule number was two times greater for CL (67±2.9) than for Gy 14 (32±1.3); F_1 was intermediate between the two parents (46±1.02).

Ovary cell size pre-anthesis was comparable for CL and Gy14 (data not shown), however, cell number and rate of cell division pre-anthesis was higher for CL in the longitudinal direction with an accelerated increase beginning 2 dpa (Fig. 1A). CL also had a greater number of cells than Gy14 in cross section at 7 dpa, however, the rate of cell division was higher in Gy14, such that at anthesis the cell number in both genotypes was equivalent (Fig. 1B). In blueberry and olive, cell division pre-anthesis was also found to influence fruit size (Johnson and Malladi 2011; Rosati et al. 2011). In mutant "Grand Gala" apple, higher cell number also was associated with enhanced carpel size however, larger cell size and increased ploidy through endoreduplication were also considered as a contributing factor to the increase in fruit size (Malladi and Hirst 2010).

Following pollination, Gy14 and CL both exhibited a typical sigmoidal pattern of growth (data not shown). CL continued to increase in length and diameter until about 20 dpp but, Gy14 largely completed its growth by 10-12 dpp. However, the rate of increase in diameter of Gy14 was 2-fold greater than for CL. Similar to ovaries pre-anthesis, cell size remained comparable for both CL and Gy14 throughout fruit growth (data not shown). Cell number in the longitudinal section increased for several more days for CL than Gy14 and F_1 (Fig. 1C), however Gy14 showed a longer period of rapid cell division in the transverse direction (Fig. 1D). Difference in cell number, not cell size, was also shown to influence fruit size in melon, olive, blueberry and strawberry (Cheng and Breen 1992; Higashi et al. 1999; Rosati et al. 2011; Johnson and Malladi 2011).

Analysis of F_2 progeny showed that ovule number (ON), ovary length (OL) and ovary L/D at anthesis were all highly correlated with each other and with fruit length (FL) and fruit L/D at 20dpp indicating that ovule number and ovary length are good predictors of fruit length (Table 1). In contrast, ovule number, ovary diameter, and ovary L/D were not correlated with fruit diameter. Fruit length and L/D were

highly correlated but neither was strongly correlated with fruit diameter. These findings were consistent with the QTL study by Yuan et al. (2008) wherein they also observed low correlation between fruit length and diameter, and no correlation between fruit L/D and diameter.

These results indicate that differences in fruit size and shape are apparent in CL and Gy14 from pre-anthesis through exponential growth. Variation is associated with growth-related factors pre- and post-anthesis, including: differential rate and period of cell division in the longitudinal and transverse directions in ovary and fruit, cell number, ovary length, and ovule number. Factors controlling length are largely determined pre-anthesis however, factors regulating diameter occur primarily post anthesis.

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Table 1. Correlation coefficient between ovary traits at anthesis and fruit traits at 20 days post pollination of GY14 X CL F_2 progeny.

Trait	ON	OL	OD	OL/D	FL	FD
Ovule number (ON)	-					
Ovary length (OL)	0.5***	-				
Ovary diameter (OD)	-0.23ns	0.24ns	-			
Ovary L/D (OL/D)	0.65****	0.76****	-0.43*	-		
Fruit length (FL)	0.60***	0.80****	-0.14ns	0.83****	-	
Fruit diameter (FD)	-0.03ns	0.44*	0.35ns	0.18ns	0.39*	-
Fruit L/D (FL/D)	0.63***	0.59***	-0.36ns	0.79****	0.84****	-0.16ns

ns, *, **, ***, **** R value not significant, or significant at $p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$, respectively

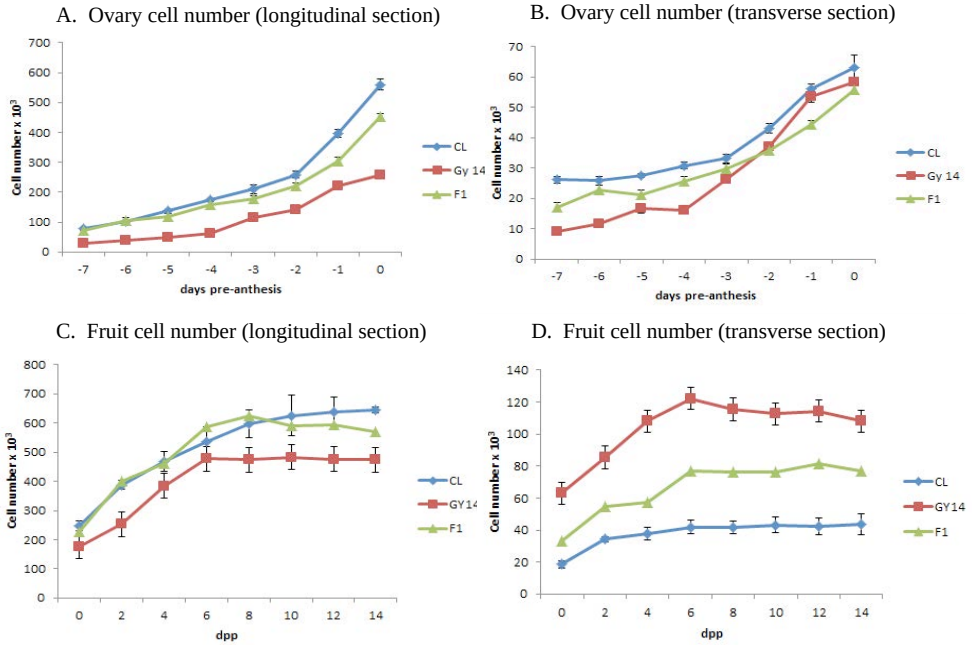

Fig. 1. Ovary and fruit cell number of Chinese long, Gy14 and F_1 progeny.

Developmental changes in inhibited, senescent and fruit-setting ovaries of cucumber

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Keywords: *Cucumis sativus*, transcript profiling, cytokinin, ARR5 reporter gene

Abstract

The dynamics of fruit set in cucumber is not fully understood, despite its importance for yield determination. A fertilized ovary must integrate signals from distant plant parts (leaves, previous fruits) and "decide" whether to set fruit, or to remain inhibited, and later senesce. We set out to characterize First Fruit Inhibition (FFI), *i.e.*, the inhibitory effect of the first fruits that set on subsequent development of younger ovaries. After the first fruits have set, younger fertilized ovaries remain in a temporary state of inhibition that may be reversed upon fruit removal. Under our conditions, the reversible stage lasted one week. Microarray and candidate gene expression analyses were undertaken to target genes that mark three different ovary fates: fruit set, inhibition and senescence. In inhibited ovaries, lower expression of genes involved in cell growth and proliferation was identified, compared to fruit-setting ovaries, along with stronger expression of stress and senescence related genes. Their expression patterns suggest that inhibition is probably an intermediate state between developing and senescing ovaries. Another approach addressed the role of phytohormones in fruit set. Hormone-reporter constructs, where a reporter gene is controlled by a hormone responsive promoter, have been introduced to cucumbers. Such transgenic plants report hormonal changes in the ovary in response to different stimuli, such as fertilization and previous-fruit inhibition.

INTRODUCTION

Non-parthenocarpic fruit set requires fertilization, however many fertilized ovaries will not set fruit. When assimilates are limiting, competition influences fruit size and quality (Baldet et al. 2006). In cucurbits, the first developing fruits inhibit flower maturation and fruit set of younger female flowers, a phenomenon known

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as FFI (first fruit inhibition; Baniel et al. 2008). In the literature, two (non-mutually exclusive) hypotheses were raised to explain FFI. One claims that phytohormones actively inhibit the younger ovaries, in what appears as a pre-set developmental program (O'Neill 1997). The second emphasizes the importance of sink-source relations (Bangerth 2000): fruit removal experiments (Marcelis 1993) showed that cucumber plants partitioned assimilates between as many as seven small fruits in an "equalitarian" manner. Recently, Baldet showed that a fertilized tomato ovary in a single-fruit truss expresses more cyclins than a fertilized one in a five fruit-truss (Baldet et al. 2006), suggesting that differences in assimilate distribution influence early fruit development by regulating cell cycle genes.

We have studied FFI in a gynoecious cucumber line. Fruit removal was applied to assess reversibility of inhibition, and preliminary data on differential gene expression in inhibited *vs*. developing and senescent ovaries were gathered. Finally, we monitored active cytokinin during fruit set in transgenic cucumbers that express a reporter gene under the control of a hormone-sensitive promoter.

MATERIALS AND METHODS

Gynoecious cucumbers 'Elem Female' (Zeraim Gedera) were sown in 100 L planters (4-6 plants per planter). Androecious 'Erez' was used as pollenizer. Total RNA from 100 mg ovary tissue was extracted, single stranded cDNA synthesized using SuperScript® First-Strand kit (Invitrogen), labeled with cy5 or cy3 and hybridized with microarray chips containing 3,070 melon cDNAs from different melon tissues (Fei et al. 2007). Statistical analysis of signals was carried out at the Microarray Unit in Bar-Ilan University. Individual gene expression was examined using real time quantitative PCR with SYBR Green detection (KAPA SYBR, KAPA Biosystems) and the StepOnePlus™ cycler (Applied Biosystems). The genes assayed were: *β-tubulin* (Csa009074), *ACC oxidase* (Csa007981), *Hsp90* (Csa001697), *Cpn60* (Csa016939), *Histone H2A* (Csa018348). The ARR5:GUS reporter plasmid was provided by J. Kieber, North Carolina. Cucumber transformation followed Gal-On et al. (2005) and Leibman et al. (2011) starting from cotyledon explants of cucumber cv. 'Ilan', with kanamycin selection. Stable transformation was verified by PCR, GUS staining (Jefferson et al. 1986), and T_2 seed germination on kanamycin medium.

RESULTS and DISCUSSION

Reversibility of the fist-fruit-inhibited state

In gynoecious cucumber 'Elem female', at each node along the stem, a single female flower reaches anthesis daily. The ovary's fate is determined by its position and by the presence of previous fruits: the first fertilized ovaries set fruit, whereas fertilized ones above them do not develop, and remain in a temporary

state of inhibition (Baniel et al. 2008). We asked whether the putative inhibitory stimulus is transient, and could be reversed by fruit removal. Following a series of sequential pollinations, we removed developing fruits at increasing time intervals. The inhibited ovaries set, suggesting that the putative inhibitory signal disappears quickly upon removal. The period in which ovaries can still recover must depends on the nutritional status of the plant and the number of previous fruits. Under growing conditions of 20 L soil per plant, in the presence of 2 to 3 developing fruits, the reversible stage lasted one week (Fig. 1); with increasing inhibition periods, a smaller fraction of the ovaries recovered.

Transcript profiles of cucumber ovaries

We performed microarray experiments to compare gene expression between developing cucumber ovaries, senescing ones, and ovaries inhibited by the presence of previous developing fruits, two days post anthesis. For the fruit-setting sample, nine ovaries were pooled, to provide a "basal" sample; senescing and inhibited ovaries, that could be subject to more extensive physiological variation were sampled individually, each representing a biological replicate (3 senescent and 4 inhibited samples were analyzed). Following hybridization to melon cDNA chips, "Fold Change" values were calculated as the ratio between hybridization signals of the senesced or inhibited ovary and the signal of developing ovaries. Genes with Fold Change values >1.5 (in at least 3 of 4 "inhibited" replicates, or 2 of 3 "senescent" replicates) were marked as "up regulated", and those below 0.67 were "down regulated". The analysis revealed 707 potentially differential genes between the three developmental states; 40% were common to the inhibited and senescent states compared to the developing ovaries, but 60% had different expression in the inhibited *vs.* senescing samples. This could suggest that senescence and inhibition are two different physiological processes having unique gene expression patterns. We noted, however, that for most potentially-differential genes, Fold-Change values were modest, mostly smaller than 3. This could reflect the physiological "lability" of transient states, affected by the environment and whole-plant status; for many genes, we were unable to replicate the differential expression patterns in real-time PCR experiments. The putative differential genes were divided into 14 functional categories (Table 1). Inhibited and senescing ovaries had lower expression of genes involved in growth and proliferation, such as histones, ribosomal proteins, photosynthesis and metabolic genes, and higher levels of transcripts related to stress, senescence, ethylene synthesis and chaperones.

Expression pattern of selected genes

A few genes showing differential expression patterns, and genes related to fruit set and senescence from other studies, were chosen for more detailed analysis using

RT-qPCR. Four ovary states were compared: fruit set, inhibition and senescence (at 2 days post anthesis), and non-inhibited, non-fertilized ovaries at anthesis. Expression level was normalized using *β tubulin*. Figure 2 depicts the results with four genes. *ACC oxidase* encodes the last enzymatic step in ethylene production. Its expression was up-regulated in senescent and inhibited ovaries, compared to low transcript levels at anthesis and fruit set; levels in senescing ovaries were 3-fold higher than in the inhibited ones. *Heat shock protein 90* encodes a chaperone that could be involved in signal transduction, cell cycle regulation, folding and degradation of proteins and also in stress resistance (Chen et al. 2006). *Chaperone 60* encodes a mitochondrial GroEL homologue that assists in protein folding (Hartl, 1996). Their expression was down-regulated in inhibited ovaries, and more so in senescent ones. *Histone H2A* is involved in chromatin structure, DNA repair and gene expression (Li and Liu 2006). Its expression paralleled that of the previous two genes.

Transgenic cucumbers that report hormonal status of the ovary

To examine the possible effect of cytokinins on fruit set, transgenic plants that express the GUS reporter gene under the ARR5 promoter, that responds to endogenous changes in active cytokinin (D`Agostino et al. 2000), were produced. Cytokinin (along with auxin and gibberellins) plays a role in fruit set of different plants (Ozga and Reinecke 2003). Cucumber transformation efficiency with the ARR5:GUS construct was 3.5%. Endogenous cytokinin activity (GUS expression) was observed in the root cap, where most of the hormone is synthesized (Fig. 3a), and in the vascular bundles of the ovary (Fig. 3b). The possible effect of cytokinin during fruit set was examined in ovary tissues, at different developmental stages. Several expression patterns were observable in the cucumber ovule, and we were able to monitor frequency of such patterns among developmental stages. For example, the frequency of cytokinin-induced GUS in the middle of the ovule (marking the embryo sac) increased after fertilization (Fig. 3c). We also examined the effect of first fruit inhibition (FFI), by comparing GUS expression in developing *vs*. inhibited ovaries. In many cases, the frequency of cytokinin sensitive expression patterns was higher in inhibited ovaries (not shown). Such unexpected increase could result from cytokinin interaction with other hormones, such as ethylene.

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Table 1. Functional categories of differentially expressed transcripts. The numbers of genes matching every pattern are given. "up" – upregulated expression (>1.5 fold change) in most replicates, compared to fruit set. "down" – downregulated (<0.67 foldchange in most replicates), compared to fruit set. "inh." – inhibited ovary, "sen."- senescent ovary.

Expression pattern	histones	translation process	metabolism	chparones	stress related	proteases & nucleases	Ethylene signaling
up inh	1	6	13	7	16	5	10
up sen	3	2	13	3	10	6	9
down inh	12	38	27	8	6	5	0
down sen	12	36	32	17	8	5	0
Expression pattern	mitochondria	senescence	photosynthesis & respiration	transcription factors	ubiquitin	regulators	cytoskelton & cell wall
up inh	2	4	2	15	5	18	7
up sen	2	4	7	13	5	26	7
down inh	1	0	21	11	2	39	11
down sen	2	2	14	11	1	26	13

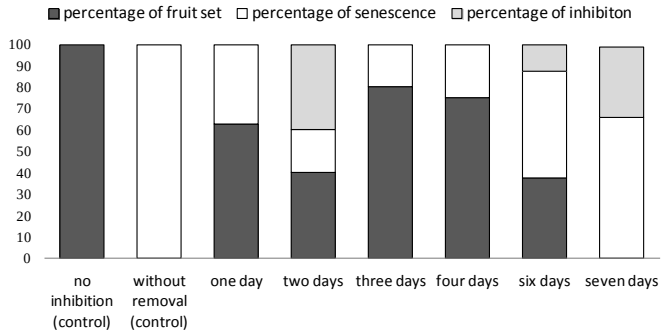

Fig. 1. Estimation of the period of reversible First Fruit Inhibition. Columns represent the percentage of younger ovaries that set fruit, senesced or remained inhibited after removal of older fruit, at different time points after pollination of the younger ovaries. Each group included 5-8 plants. When no older fruits were present, 100% fruit set occurred, whereas without older fruit removal, all subsequent ovaries were irreversibly eventually senesced.

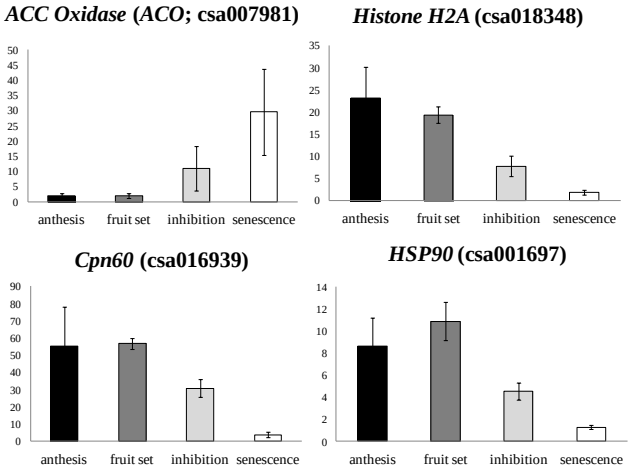

Fig. 2. Relative expression levels of four selected genes, assayed by RT-qPCR. Error bars represent the standard error of three biological replicates (each prepared by mixing 3 ovaries). Samples included anthesis-day ovaries and 2 d post-anthesis ovaries that either set fruit, senesced in the absence of pollination, or were inhibited by previous fruits.

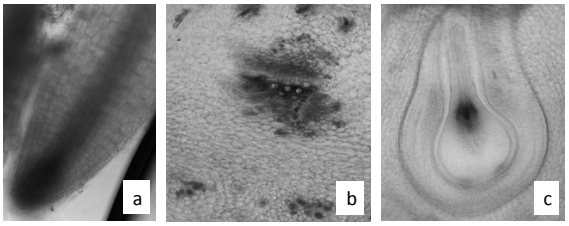

Fig. 3. GUS activity regulated by the ARR5 promoter. (a) in Root cap. (b) Vascular bundles of the ovary. (c) Embryo sac.

QTL mapping of subgynoecy in cucumber (*Cucumis sativus* L.)

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Keywords: Cucumber, sex determination, ethylene, QTL, SSR

Abstract

Cucumber (*Cucumis sativus* L.; 2*n*=2*x*=14) belonging to the family Cucurbitaceae is a very important vegetable crop worldwide, and now it is becoming a model plant for sex determination research. It has three types of flowers: male, female, and bisexual. The distribution of these three flower types on the plant results in seven sex types of cucumber plants. Sex expression is regulated by gene expression, and is also influenced by environmental conditions and plant hormones. Ethylene is highly correlated with femaleness in cucumber. In our research, we studied a backcross population (BC_1) and F_2 population derived from a cross of S-2-98 (subgynoecious) × 95 (monoecious) and mapped QTLs for accumulating female on the cucumber genome based on a high-density SSR map. By combining the bulked segregant analysis (BSA) and the simple sequence repeat (SSR) technology, we identified three quantitative trait locus (QTLs) for subgynoecy in our plant population. We named them *Mod-F1a*, *Mod-F1b* and *Mod-F1c*. One major QTL, *Mod-F1a*, with positive effects located on chromosome 3 explained a large part of the phenotypic variance. Two minor QTLs, *Mod-F1b* and *Mod-F1c*, were located on chromosome 6. *Mod-F1b* is a QTL with positive effect, whereas *Mod-F1c* is a QTL with negative effect. The three QTLs together explained 62.9% of the phenotypic variance in the BC_1 population, and 51.2% in the F_2 population.

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A TILLING population for cucumber forward and reverse genetics

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Keywords: *Cucumis sativus*, EMS mutagenesis, mutant screen

Abstract

Ordered collections of mutants serve as invaluable tools in biology, and in functional genomics in particular. TILLING (targeting induced local lesions in genomes) provides an efficient method to study the possible phenotype controlled by gene sequences whose function is unknown. This method can replace transgenic techniques for the functional validation of cloned genes, especially in the case of transformation-recalcitrant plants such as cucumber. We report the development of a TILLING cucumber population, prepared by EMS mutagenesis in the Poinsett-76 genetic background. The population was evaluated by screening morphological mutations, and its suitability for detecting single nucleotide polymorphism in selected genes has been tested. Such previously-unavailable asset in cucumber can be exploited for diverse breeding and research purposes.

INTRODUCTION

In the genomic era, full complements of coding sequences are available for many organisms, but our ability to attribute a function, or phenotype, to a given sequence relies on reverse genetics methods. TILLING is a high efficiency reverse genetics strategy to detect mutations in specific genes, by screening a saturated collection of mutants. Seeds are treated with EMS (ethyl methanesulfonate), a mutagen that saturates the genome with point mutations, mostly G/C to A/T substitutions (Anderson 1995). The treated seeds are germinated and the resulting M_1 plants self pollinated to collect M_2 seeds. DNA samples are screened by PCR amplification of the gene of interest, followed by digestion with an endonuclease that cleaves at mismatched sites (Comai and Henikoff 2006). If a particular M_2 family carries a point mutation in the amplified fragment, heteroduplex DNA will form during replication of mutant and wild-type alleles present in the particular family. Genetic analysis of the segregating family allows the discovery of mutant phenotypes and sheds light on the gene's function. The method has been refined

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by Bendahmane and co-workers for different crops; the use of Arabidopsis endonuclease, ENDO-1, rendered the screening particularly efficient (Nieto et al. 2007; Triques et al. 2008).

TILLING is a relatively inexpensive non-transgenic technique, suitable for any species. Mutations are spread randomly in the genome, and a spectrum of mutated alleles, including weak ones that are desirable for essential genes, can be obtained (Perry et al. 2003; McCallum et al. 2000). Here we provide a first report on a TILLING population raised for *Cucumis sativus*, the cucumber.

MATERIALS AND METHODS

Preparation of the TILLING population

Seeds were incubated at room temperature for 15 hrs, in 3 volumes of freshly-prepared EMS solution, in 0.2 M phosphate buffer (pH=7), with gentle stirring. After extensive washing, seeds were sown in Speedlings trays and moved to the greenhouse at the 2-3 leaf stage. Liquid EMS waste and contaminated lab-ware were neutralized in 1 M NaOH. Plants were grown under standard agronomic conditions, self-pollinated, and M_2 seeds were harvested (50-400 per plant). From each family, six seeds were sown, and after two weeks, 4 young leaf discs were sampled from four individuals of the family and pooled for DNA extraction by the CTAB method (Rogers and Bendich 1985). Uniform DNA concentrations were obtained in 200 µl water and kept at -80°C. Individual family-samples of the first 768 families were arrayed in eight 96 well plates, and DNA aliquots were diluted 10 times (to obtain a 400 µl volume, ~50 ng DNA/µl) and kept at -20°C. DNA pools were prepared (8 families/ pool) and arrayed in a single 96-well pooling-plate representing the 768 families.

Target gene amplification and screening

Amplification included two steps of nested PCR. The first involved a pair of external unlabeled primers, reacted with genomic DNA for 30 PCR cycles. The second round was performed with two pairs of internal primers present together in the same reaction. The external primer pair in the second round comprised a 3' region that specifically binds the PCR products of the first round, while the 5' region represents a universal M13 sequence "tail". The second primer pair is fluorescently marked (IRDye™ 700 and IRDye™800) and matches the M13 sequences. The reaction included 35 cycles, the first 10 performed at the specific annealing temperature of the specific primer pair, then 25 cycles at a lower annealing temperature, 50°C, required for the universal primers (Colbert et al. 2001; Triques et al. 2008). The products were digested for 20 min at 45°C with EndoI and separated on a polyacrylamide sequencing gel in a capillary machine (LICOR 4300). The pooled 96 DNA samples were screened, and positive pools displaying

amplicon digestion were deconvoluted to identify the positive M_2 family within the pool. The family's DNA sample (prepared by mixing four individual progeny) was sequenced, to look for a mixed peak, indicating a mutation at a position predicted by the endonuclease digestion pattern. A few progeny of the family were sown and their amplicons sequenced, to confirm the mutation and correlate it with a possible phenotype.

RESULTS AND DISCUSSION

Mutagenesis and multiplication

We performed two calibration experiments in which seeds of cucumber 'Poinsett76', 100-200 seeds per treatment, were exposed to 0, 0.5, 1, 1.5, 2 and 2.5% EMS. Germination rates were recorded and ranged between 95% (untreated seeds) and 73% (2-2.5% EMS). We also recorded the rate of seedlings exhibiting somatic mutations, *i.e.*, leaf distortion, mosaicism or reduced growth. Rates increased gradually from 3% total ratio of apparent abnormalities in untreated seedlings, to 78% in the 2.5% treatment. We selected 1.5%-2% EMS as an optimal concentration that mildly affects germination and has a substantial proportion of visible somatic damage. Aliquots of these plants were grown further and we noted that in most plants the new leaves that developed were normal, while only a small proportion displayed persistent mutations at the M_1 generation. Thirty seedlings from the 1%, 1.5% and 2% treatments were transferred to the greenhouse for further growth and fertility assessment. We concluded that fertility was similarly good in all three EMS concentrations.

A total of 1,200 M_1 plants (treated with 1.5-2% EMS in three different batches) were grown to full maturity at a farmer's plot, or at Bar-Ilan University, and self-pollinated to collect M_2 seeds. This resulted in ~1,000 M2 families with adequate seed samples. Six seedlings/ M_2 family were germinated and a mixed DNA sample from four of them was prepared. The first 768 families were arranged in 96 well plate wells (see Methods) and used for a pilot screen.

Phenotypic evaluation of the population

We utilized the M_2 seedlings grown for DNA extraction to record mutant phenotypes that are observable at the cotyledon and first leaf stages. In many cases, the phenotype was apparent in more than one individual of the same family, demonstrating the heritability of the putative mutation. The phenotypes included post-germination death (19 families of 768), dwarfism (18/768), spontaneous lesions in cotyledons (25/768), mosaic or yellow leaf (9/768), albino (3/768), leaf distortion (8/768), narrow, dark cotyledon or leaf (4/768), tall seedling (5/768), with 12% of the families displaying a seedling mutant phenotype. Seedlings with prominent phenotypes were grown to maturity and their phenotypes described

further. Examples of seedlings and mature plant phenotypes are shown in Fig. 1. Since each M_1 plant and the descendant M_2 family harbors multiple point mutations, genetic analysis is required to discern them and correlate a specific mutation with a single molecular event.

Molecular evaluation using the PDS gene

We asked whether we could interrogate our population with a query gene and isolate point mutations in its sequence. We chose cucumber *phytoene desaturase-3* (*Pds-3*), accession Csa002881, that encodes a carotene biosynthetic enzyme. When inactive, it could give rise to an albino phenotype. We constructed three amplicons, focusing on conserved regions (Fig. 2). Four mutated families were found, two in amplicon B and two in D. We sequenced and verified the mutations in families 53 and 254 and each carried a different G-to-A substitution. In family 254, three plants were heterozygous and two had the wild type allele; in family 53, two were wild-type, two homozygous for the mutation and four were heterozygous (Fig. 3). All four mutations mapped to introns present in the amplicons and no mutant phenotypes were observed, suggesting that more care should be taken to include as little intronic sequences as possible in the screen. Nevertheless, the experiment confirmed that the mutation ratio in the population is good: 2-4 mutations out of 768 plants were found by screening three amplicons, that, together, measured ~2,700 bp. Analysis of additional genes is under way.

CONCLUSION

Our study provided a valuable tool for functional genomics in cucumber. With the entire cucumber genome sequence available, TILLING provides a means to link genes and phenotypes. The present population, in the Poinsett 76 background, is a rich source of morphological mutations and was successfully screened to recover point mutations in a given gene.

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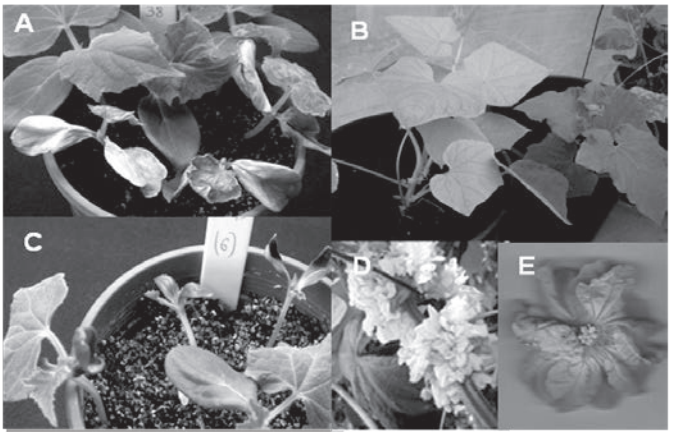

Fig. 1. Mutant phenotypes segregating in M_2 families at the seedling stage (A, C), and among mature plants (B, D, E). A – spontaneous necrotic lesions, B – virescent (light green) plant, C – dark narrow cotyledons, D – fasciated sessile inflorescence, E – multiple petals.

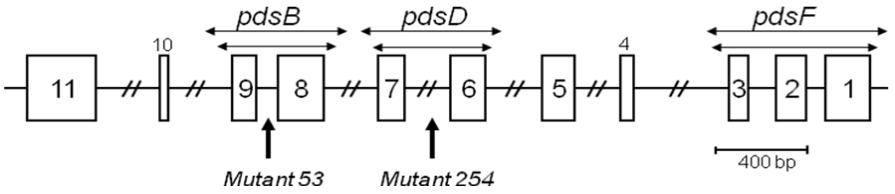

Fig. 2. Gene model and amplification scheme of the cucumber *phytoene desaturase-3* gene. The approximate position of three amplicons (B, D and F) screened by TILLING are indicated, each delimited by two pairs of nested primers. The two mutations that were verified by sequencing in families 53 and 254 are indicated.

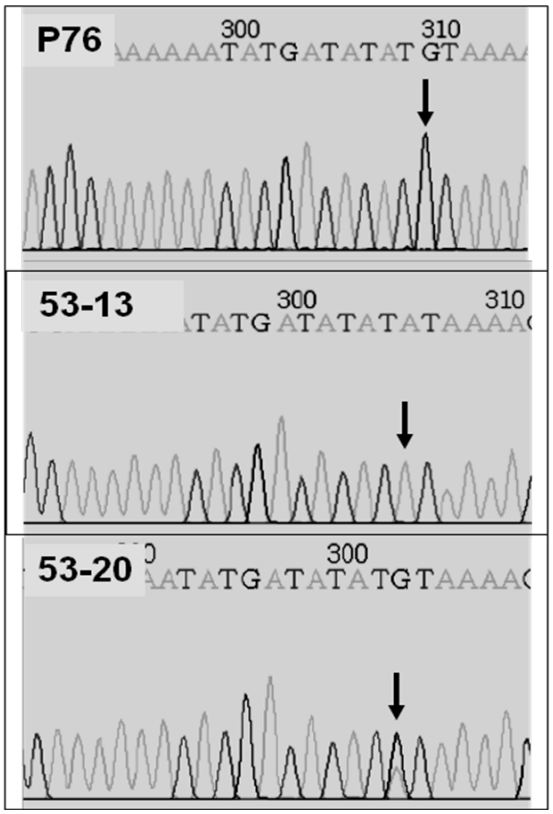

Fig. 3. Sequence chromatograms of a G-to-A mutation in family 53. Top: the wild type sequence in cultivar Poinsett-76. Below: plant 53-13 is homozygous for the mutation, plant 53-20 is heterozygous.

Physical Mapping of Highly Repetitive DNA Sequences in *Cucumis sativus* and *C. melo*

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Keywords: *Cucumis,* centric fusion, fluorescence *in situ* hybridization

Abstract

Chromosomes serve as one of the most important molecular aspects in the studies of the evolution of species. Most of crucial mutations that led to differentiation of species during the evolution have occured at the chromosomal level. Furthermore, the analysis of pachytene chromosomes is the invaluable tool for the study of evolution due to its effectiveness in chromosome identification and precise physical gene mapping. To find out the evidence that cucumber chromosomes 1 and 2 may have evolved from fusions of ancestral karyotype with chromosome number n=12, 45S rDNA and CsCent1 probes are applied to cucumber pachytene chromosomes using the fluorescence *in situ* hybridization technique. These results are further supported by the centromeric sequence similarity between cucumber and melon, which suggests that these sequences evolved from a common ancestor. It may be after or during speciation that these sequences were specifically amplified, after which they diverged and specific sequence variants were homogenized. Additionally, a structural change on the centromeric region of cucumber chromosome 4 was revealed by fiber-FISH using the mitochondrial-related repetitive sequences, BAC-E38 and CsCent1. These showed the former sequences being integrated into the latter in multiple regions. These data are useful resources for comparative genomics and cytogenetics of *Cucumis* and for the genome sequencing project of cucumber.

INTRODUCTION

The genus *Cucumis* consists of about 30 species including *C. sativus* L. (cucumber, 2n=2x=14) and *C. melo* L. (melon, 2n=2x=24). *C. sativus* and *C. melo* are widely cultivated throughout the world. Cucumber and melon diverged from a common ancestor approximately 9 million years ago (Schaefer et al. 2009). Recent comparative genetic analysis clearly showed that five of the cucumber's

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seven chromosomes arose from fusions of ten ancestral chromosomes after divergence from *C. melo* (Huang et al. 2009). Comparative fluorescence *in situ* hybridization (FISH) mapping using the same sets of fosmid clones revealed different centromeric positions between two pairs of chromosomes from these two species (Han et al. 2009). Thus, chromosomal fusion and centromere repositioning occurred during the evolution of cucumber and melon chromosomes. However, the reduction of total cucumber chromosome number from 24 to the current count of 14 chromosomes remains to be examined in a further study. There is still uncertainty toward potential karyotypic evolution between the two species and it will likely require more extensive evaluation and careful scrutiny before a complete understanding of chromosome evolution between these species can take place. In this study we isolated and sequenced several repetitive DNA clones derived from the pericentromeric regions of cucumber and melon chromosomes. We found that the CsCent1 satellite and CsCent2 retrotransposon in cucumber and the CmCent satellite in melon are the primary DNA components of cucumber and melon centromeres, respectively. Moreover, by localizing CsCent1 and 45S rDNA on cucumber pachytene chromosomes, we found an unusual distribution of the FISH signal which appears to have evolved through a chromosomal fusion in the ancestors of cucumber.

MATERIALS AND METHODS

Plant materials

Cucumber (*Cucumis sativus* L. cv. Winter Long) and melon (*Cucumis melo* L. cv. Romance) seeds were obtained from Dongbu Hannong Seeds Co., Korea. Plants were grown under greenhouse conditions. Young leaves were collected for DNA isolation, and young fast growing root tips and anthers at meiotic prophase I were selected for chromosome preparations.

Cloning of repetitive DNA elements and FISH

An overview of all repeats used in this study is presented in Table 1. The CsCent1 and CsPc pericentromere repeats from the repeat-rich B68 and B17 BAC clones were selected. Sequencing the ends of BAC-B68 revealed a retrotransposon-like DNA, which was used to design the oligonucleotide primer 5'-TAGAGGATCCATTAACCTAT-3' for amplifying the 2,107 bp CsCent2 fragment; this fragment was then subcloned in the pGEM-T-Easy vector (Promega). A second pericentromere repeat, CmCent, was isolated by PCR amplification of *C. melo* genomic DNA using oligonucleotide primer pair 5'-AGCTTCGGCCATCTTTTGGA-3' and 5'-TCCAACGAGTGGCGAACGCC-3'. These primers were designed on the basis of the DNA satellite sequence from NCBI database (Acc. no.: X97847). The resulting 354 bp PCR product was subcloned in pGEM-T-Easy. The FISH

procedure applied to both mitotic and meiotic chromosomes was essentially the same as reported in Koo et al. (2005).

RESULTS AND DISCUSSION

This paper presents the first detailed study on the structure and evolution of repetitive DNA sequences in cucumber and melon chromosomes. Major classes represent the centromeric satellite repeat (CsCent1), a putative retrotranspon (CsCent2), mitochondrial DNA related sequence (BAC-E38) and dispersed repeat (CsPc), which were all found in the pericentromere heterochromatin blocks (Fig. 1). Using high resolution pachytene-FISH with 45S rDNA as a probe, we report unusual chromosome morphology in cucumber pachytene chromosomes. This is seen in chromosomes 1 and 2, which appear to have evolved by chromosomal fusion of the progenitor of cucumber (Fig. 2). FISH analysis supported this idea of chromosomal fusion by showing that 45S rDNA was interposed between the CsCent1 regions of chromosomes 1 and 2. The most intriguing finding is the existence of 45S rDNA sequences at both ends of the centromeric sequences, as revealed by the presence of CsCent1 in cucumber chromosome 2 (Fig. 2a). In the present findings, FISH using CsCent1 and 45S rDNA supports the possibility of chromosomal fusion of the progenitor of cucumber, which indicates that the genome of cucumber is highly likely to have originated from fusions of ancestral chromosomes after divergence from *C. melo* (Huang et al. 2009). This conclusion was further demonstrated by the sequence comparison between the two centromeric satellite repeats (data not shown). Significant sequence similarity observed between these centromeric satellite repeats implies that both types of satellite sequences were present in a common progenitor. It may be that after or during speciation, these sequences were specifically amplified, after which they diverged and specific sequence variants were homogenized.

ACKNOWLEDGEMENTS

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Table 1. Summary of repetitive DNA sequences used in this study.

Name	Type	Length (bp)	Source
CsCent1	Satellite DNA	166-167	BAC-B68
CsCent2	Retrotransposon	2,107	BAC-B68
BAC-E38	Mitochondrial DNA	85,887	BAC-E38
CsPc	Dispersed repeat	429	BAC-B17
CmCent	Satellite DNA	354	Brennicke and Hemleben (1983)
45S rDNA	Satellite DNA	9,100	Gerlach and Bedrock (1979)

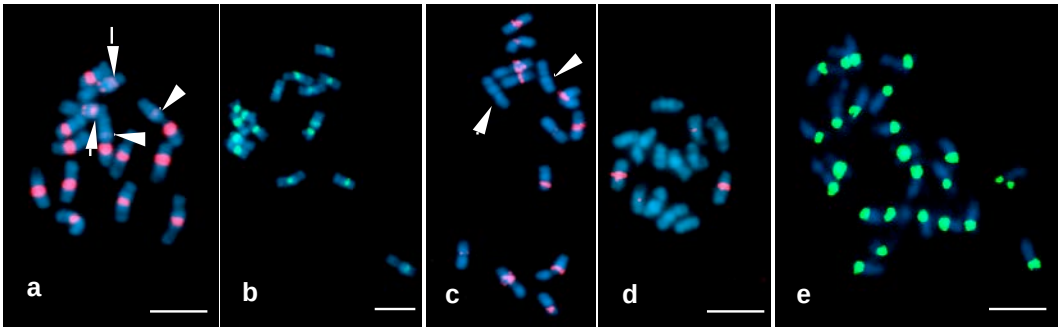

Fig. 1. Physical mapping of repetitive DNAs on cucumber and melon mitotic metaphase chromosomes. **a,** CsCent1; **b,** CsCent2; **c,** CsPc; **d,** BAC-E38; **e,** CmCent. Bars, 5 μm.

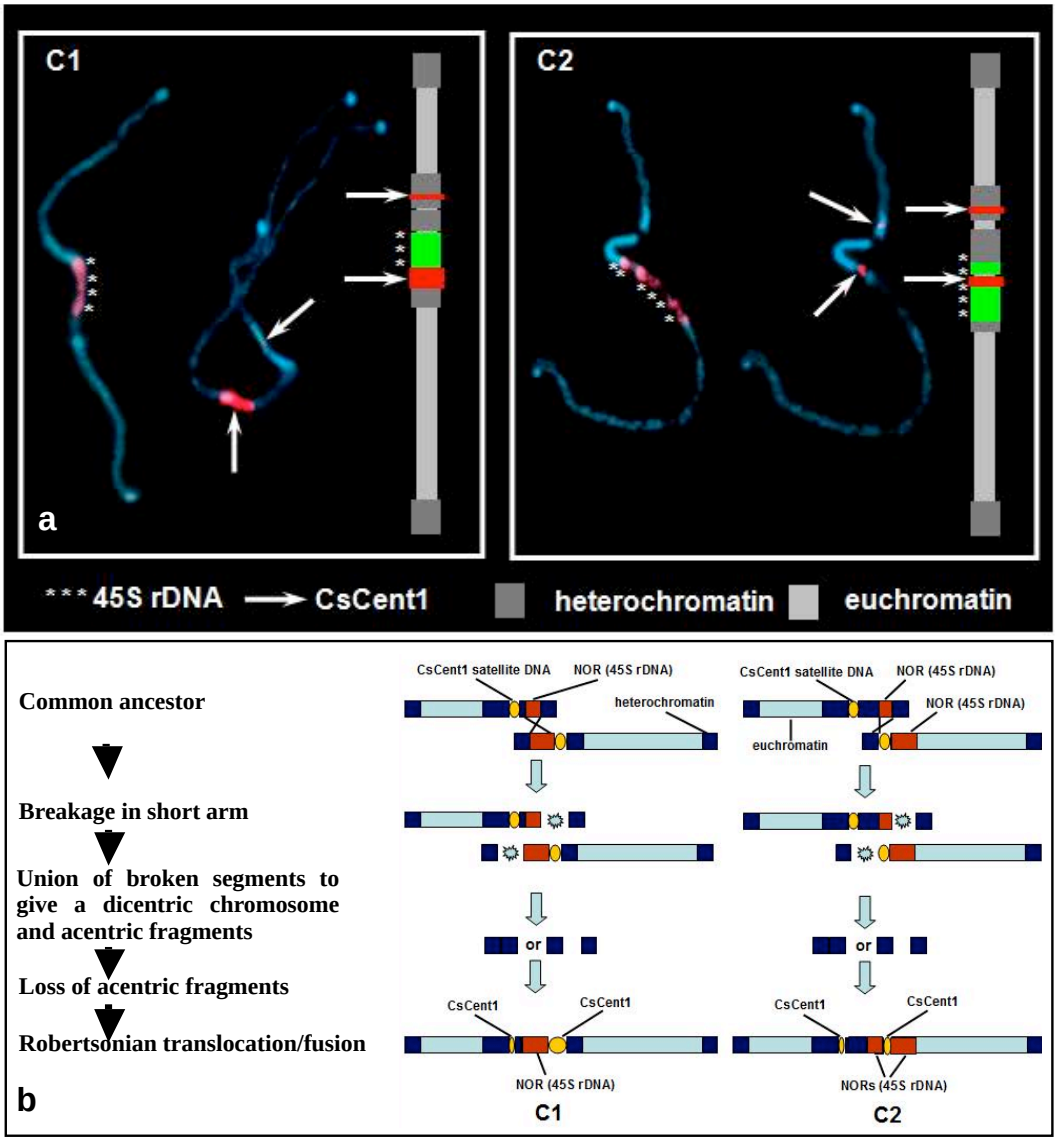

Fig. 2. An ideogram of the cucumber karyotype (**a**); Locations of rDNA are indicated by asterisks and CsCent1 (centromeric satellite DNA) by arrows. The dark gray regions represent heterochromatin (DAPI-bright regions). A possible model for formation of Robertsonian translocations in cucumber chromosomes 1 and 2 (**b**); Robertsonian translocations are formed from a break in the short arms of two ancestor acrocentric chromosomes each of which have a nucleolus organizer region (NOR). The resultant chromosome contains the long arms of each chromosome, both centromeres, and a fragment of each short arm. The remainder of both short arms is lost. C1 and C2 indicate cucumber chromosomes 1 and 2, respectively.

Rationalization of a genebank cucumber collection with SSR markers

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Keywords: Cucumber, *Cucumis sativus*, *ex situ* collection, SSR markers, rationalization

Abstract

The CGN cucumber *(Cucumis sativus*) collection consists of 937 accessions. The majority of accessions originated from the working collection of the former Institute for Horticultural Plant Breeding (IVT), where they were used for breeding. The collection mainly includes old cultivars received from Dutch and foreign seed companies, and genebanks, but also contains landraces and accessions of the crop wild relative *C. hardwickii*.

Passport data were updated in 2002, and used to rationalize the collection.

In 2009, CGN participated in a project initiated by The Institute of Vegetables and Flowers of the Chinese Academy of Agricultural Sciences in Beijing (CAAS), China. The project aimed at developing a structured core collection of cucumber germplasm using well-distributed and highly polymorphic SSR markers. For this purpose, the collections of CAAS, of the USDA (United States) and of the CGN were used. A total of 3,318 accessions were analyzed with 23 highly polymorphic microsatellite (SSR) markers, resulting in a core set of 109 accessions (in preparation).

In addition to the collaborative project, the SSR data of 752 CGN accessions were analyzed separately in order to find further options to rationalize the collection based on these markers. A phylogenetic tree was constructed based on Jaccard similarity values. Four distinct groups could be recognized, i.e. *C. hardwickii*, landraces from South Asia, varieties from Asia, and varieties predominantly from Europe and USA. The SSR data were also used to verify passport data, such as origin country, and to improve accession documentation.

Twenty-seven groups of accessions with identical microsatellite profile were identified. Trials are planned to compare these potential duplicate accessions morphologically.

In the past, a "cucumber" group and a "gherkin" group were recognized within the collection, based on morphological traits. However, this division was not supported by the SSR data and therefore abandoned.

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Development and application of SSR markers linked to *Zucchini Yellow Mosaic Virus* resistance in Japanese cucumber

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Keywords: *Cucumis sativus*, potyvirus, recessive resistance gene, SSR markers, marker assisted selection

Abstract

Resistance to *Zucchini yellow mosaic virus* (ZYMV) was evaluated in 128 F2:3 cucumber families produced by crossing the resistant line Japanese type 'S93-18' with the susceptible line Indian type 'CS-PMR1.' Progeny analysis revealed that resistance to ZYMV was conferred by a single recessive gene (*zymS93-18*). Codominant PCR-based SSR markers were used to develop *zymS93-18*-linked molecular markers. We mapped the *zymS93-18*-linked SSR markers to cucumber Chr. 6 and found that the genetic distances of the two closely linked SSR markers were 0.9 and 1.3 cM, respectively. Additionally, one SSR marker cosegregated with *zymS93-18*. The phenotypes of 32 ZYMV-inoculated inbred lines were compared with their *zymS93-18* genotype. The coincidence rate was 93.8%, suggesting that these SSR markers can be used to screen for ZYMV-resistant Japanese cucumber plants.

INTRODUCTION

Zucchini yellow mosaic virus (ZYMV) is an economically important potyvirus that infects cucumber plants. From summer to early autumn, cucumber plantings are particularly vulnerable to ZYMV owing to increased vector populations, and some plants are suddenly wilted by superinfection with ZYMV and other viral species, including *Cucumber mosaic virus* (Gal-On 2007). Recent reports have shown that resistance to ZYMV in cucumber is inherited as a recessive allele at a single locus (*zym*), as has been observed for other potyvirus resistance-related plant genes.

Marker-assisted selection (MAS) has been shown to be a powerful tool in the development of disease-resistant cultivars, especially for recessive resistance genes. Several DNA markers linked to the recessive ZYMV resistance gene *zym*

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have been developed as amplified fragment length polymorphisms (AFLP) and sequence-characterized amplified region (SCAR) markers (Park et al. 2000, 2004). However, molecular markers are sometimes monomorphic in a breeding population, and thus these markers may not be polymorphic between some ZYMV-resistant and -susceptible plants. In this study, we developed *zym*-linked molecular markers and explored whether these markers could be used for ZYMV resistance breeding in the field.

MATERIALS AND METHODS

Plant materials

A ZYMV-resistant inbred line of Japanese cucumber (S93-18) and a susceptible line (CS-PMR1) were used as parents. To determine the inheritance pattern of ZMVY resistance, F1, F2, and backcrossed populations from the cross between S93-18 and CS-PMR1 were produced in a greenhouse, and 128 F3 families were obtained by self-pollination of each F2 line (F2:3 population) for ZYMV inoculation testing and genetic mapping.

Virus inoculation test

A viral inoculum was prepared by grinding virus-infected leaves (1:10 w/v) in 0.05 M phosphate buffer (pH 7.2) with a mortar and pestle. Carborundum-dusted cotyledons of 7- to 8-day-old seedlings were mechanically inoculated by rubbing with cotton swabs dipped in the inoculum. Two weeks later, the plants were scored as follows: 0=no symptoms, 1=a slight mosaic pattern limited to the lower leaves, 2=a clear mosaic pattern on the lower leaves and a slight mosaic pattern on the upper leaves, 3=a moderate mosaic pattern on the upper leaves, and 4=a severe mosaic pattern on all leaves. A total of 20 plants from each F2:3 family were evaluated to identify the resulting phenotype. A plant with a disease severity index (DSI) of $\leq$1.0 was considered resistant; plants with a DSI >3.0 were considered susceptible.

DNA markers

A set of 388 SSR markers distributed throughout the seven linkage groups or chromosomes (Ren et al. 2009, Fukino et al.2010, Miao et al. 2011) was used to screen the *zym*-linked SSR markers. Next, a bulk segregant analysis (BSA) was conducted using SSR markers around the *zym* locus to identify SSR markers more tightly linked to the *zym* gene. The PCR-amplified products were separated by electrophoresis through a 5% (stacking) and 13% (running) non-denaturing polyacrylamide gel in Tris-Glycine buffer as described previously (Xu at al. 2009).

RESULTS AND DISCUSSION

Approximately 14 days post-inoculation, the susceptible line CS-PMR1

and F1 plants showed mosaic symptoms, whereas the resistant line S93-18 was symptom-free (Fig. 1). The results of our genetic analysis are shown in Table 1. The segregation of resistance in the F2:3 population was nearly 1:2:1 [resistance (R) : heterozygous (H) : susceptible (S)], suggesting that ZYMV resistance in line S93-18 was conferred by a single recessive gene (named *zymS93-18*) as reported previously (Kabelka et al. 1997).

In our initial screen of SSR markers for linkage map construction, 153 SSR markers were clearly polymorphic between S93-18 and CS-PMR1. These markers were segregated into seven linkage groups. *zymS93-18* was mapped to two flanking DNA markers on linkage group 6 and cosegregated with one of the linkage markers. By combining these results with those of our bulk segregant analysis, the genetic distances of the two closely linked SSR markers were found to be 0.9 and 1.3 cM.

Two of the 16 susceptible inbred lines (*Zym*/*Zym*) were shown to possess the allele of the resistant line (S93-18; *zym*/*zym*) using the *zymS93-18* cosegregating SSR marker. On the other hand, the genotype coincided with the disease phenotype in 16 inbred ZYMV-resistant lines (*zym*/*zym*) (Table 2). These results show that SSR markers can be used for ZYMV resistance-assisted selection in cucumber since the coincidence rate between the genotype of each marker and the disease phenotype in 32 inbred cucumber lines was 93.8%.

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Table 1. Segregation analysis of the resistance to ZYMV in the $F_{2:3}$ and BC_1 populations derived from cucumber lines S93-18 and CS-PMR1.

Population	Observed No. of plants					
	Plants tested	R	H	S	Expected ratio	χ^2
$F_{2:3}$	128	32	62	34	1:2:1	0.91
BC_1 (F_1 x S93-18)	106	58	-	48	1:1	0.33
BC_1 (F_1 x CS-PMR1)	112	0	-	112	0:1	-

R resistant, H Heterozygous, S susceptible

Table 2. Cucumber lines evaluated with the zymS93-18 gene linked SSR marker.

	Lines	Phenotype[a]	Marker[b]		Lines	Phenotype[a]	Marker[b]
1	A122-1	R	A	17	HF221	S	B
2	A122-5	R	A	18	ST245-b	S	A[c]
3	16AS	R	A	19	H129	S	B
4	S19	R	A	20	28KGT	S	B
5	T126	R	A	21	GH195	S	B
6	202K	R	A	22	MV276	S	B
7	RJ2	R	A	23	ER23	S	B
8	KY23	R	A	24	86A	S	B
9	DZ2	R	A	25	HO1	S	B
10	JG12	R	A	26	234K	S	B
11	AS53	R	A	27	56B	S	B
12	KS1	R	A	28	S001	S	B
13	HE1	R	A	29	KA23	S	B
14	KMY90	R	A	30	DSA21	S	B
15	LKJU23	R	A	31	QW36	S	A[c]
16	GR291	R	A	32	EDD231	S	B

[a]Inoculation test phenotype designation : R resistance, S susceptible
[b]Marker genotype designation : A S93-18 allele, B CS-PMR1 allele
[c]Disagreement between genotype and phenotype

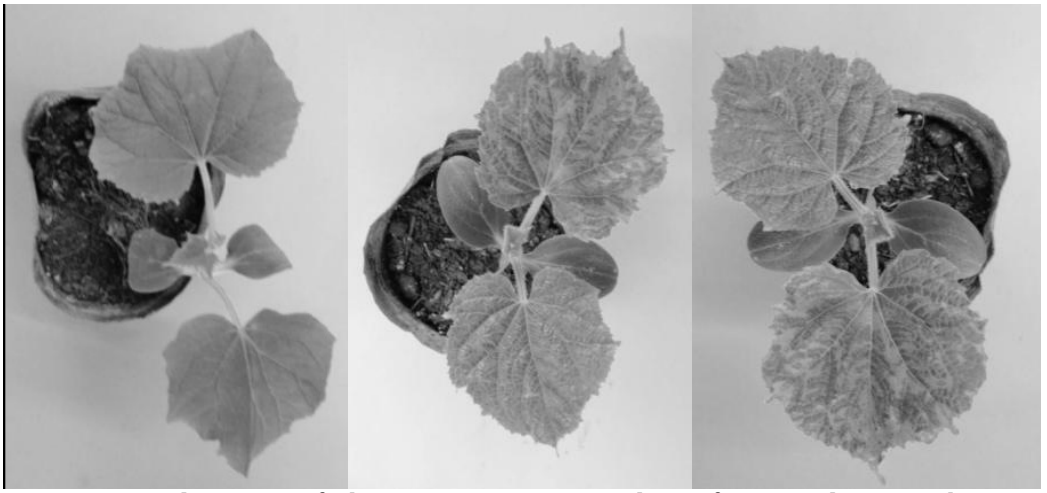

Fig. 1. Typical images of showing symptoms 14 days after inoculation with ZYMV (Left:S93-18, Middle:F_1 and Right:CS-PMR1).

Using pumpkin as a model to study stress-induced somatic embryogenesis

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Keywords: *Cucurbita pepo* L., nitrogen, DNA methylation, peroxidase, esterase

Abstract

Somatic embryogenesis in pumpkin can be induced on auxin-containing medium but also on hormone-free medium containing 1 mM ammonium (NH_4^+) as a sole source of nitrogen. NH_4Cl as the sole source of nitrogen permitted the establishment of highly uniform cultures in which preglobular and globular embryos comprised more than 70% of tissue mass. Proembryogenic cell clusters consisted of the small spherical cells with dense cytoplasm and callose-rich cell walls. The domination of preglobular and globular embryos correlated with high level of global DNA methylation. Globular embryos proceeded to maturity only after the re-supply of nitrogen in form of nitrate or L-glutamine and development was followed with a significant increase of global DNA methylation. The activities of antioxidative enzymes superoxide dismutase (SOD) and soluble peroxidase (POD) were high in tissues growing into NH_4^+ medium, and decreased after re-supply of nitrogen what indicated oxidative stress in the tissue assimilating only NH_4^+. In addition, NH_4^+-induced embryogenic tissue was characterized by elevated activity of esterase, the early marker of somatic embryogenesis, what was in accordance with highly proembryogenic state of the culture. Together, results show that the induction and development of embryogenic competence in pumpkin tissue is associated with the oxidative stress induced by nitrogen limitation.

INTRODUCTION

Plant cells are able to form adventitious or somatic embryos by a process similar to zygotic embryogenesis. This process demonstrates a true totipotent nature of plant cells. It begins with the initiation phase, when cells dedifferentiate and become competent for embryogenesis. During the induction phase cells become determined to form embryos. In the last phase the embryogenic potential is expressed by development of embryos (De Klerk et al. 1997). Somatic embryogenesis has been studied *in vitro* on numerous plant species. The only reliable conclusion is that the

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culture needs exogenous auxin supply in order to become embryogenic. The young embryos are able to develop to mature stages after removal of auxin or decreasing its concentration. Somatic embryogenesis can also be triggered by non-hormonal factors like different stress conditions and these could be the following: electric field, non-optimal salt concentration, heavy metals, ethanol or gamma irradiation (Leljak-Levanic et al. 2004a, and references cited therein). In addition, inorganic nitrogen in nitrate and ammonium forms improves embryo development (Burza and Malepszy 1995; Leljak-Levanic et al. 2004a). Lack of nitrogen affects protein biosynthesis negatively and generates oxidative stress in plant tissues. One of the aspects by which plants tolerate lack of nutrition or other types of stress conditions is the modification of cell wall properties. The cell wall represents a mechanical barrier around cells but also a place of dynamic synthesis of bioactive compounds related to stress. It was also shown that cell wall participates in the control of growth and differentiation of cells during embryogenesis in plants by involvement in signal transduction and the formation of tensions influencing the cell shape and division plane (Malinowski and Filipecki 2002). For example, callose deposition in the cell wall typically observed in response to biotic and abiotic stresses also have a role in the first step of dedifferentiation and initiation of embryogenesis (Dubois et al. 1990). Peroxidases and esterases are enzymes that participate in physiological processes of cell wall modification, auxin methabolism and stress response (Gershater and Edwards 2007). In plants, soluble peroxidases (POD; EC 1.11.1.7) have been shown to be parameters of metabolic activity during growth alternations and cell differentiation and suggested as early markers of somatic embryogenesis (Joersbo et al. 1989). The same is with esterases, a group of enzymes that hydrolyze ester bounds in a broad range of substrates. Detection of changes in expression and activity of esterases has been used as a marker system of biochemical modifications that occurred during somatic embryogenesis and organogenesis (Tchorbadieva and Odjakova 2001). In addition, Phillips et al. (1990) emphasized that certain stressful conditions in tissue culture provokes alterations in DNA methylation. Lo Schiavo et al. (1989) found a positive correlation between exogenously added auxin and cytosine methylation in carrot. The aim of the presented work was to investigate the involvement of stress responses during somatic embryogenesis in pumpkin and to show whether mentioned biochemical markers can be useful in predicting developmental events in tissue cultures, and in optimizing somatic embryogenesis protocols.

MATERIALS AND METHODS

Tissue culture, media and conditions

Excised and mechanically wounded mature embryos of pumpkin (*Cucurbita pepo* L.) detached of their cotyledons were used as initial explant material.

With respect to regeneration and growth conditions three types of embryogenic callus cultures were established (Leljak-Levanic et al. 2004): (1) a PEDC (pro-embryogenicdetermined cells) line of proembryogenic-determined cells, preglobular and globular embryos that was established and subcultured in a modified, hormone-free MS medium supplemented with 1.0 mM NH_4Cl as the only source of nitrogen and 3.37 mM thiamine-HCl (MSNH4); (2) a DEC (2,4-D-induced embryogenic cells) line induced and established in MSC medium with 4.5 mM 2,4-D (MSC2,4D) that contained mostly embryos in the early developmental stages; (3) a HEC (habituated embryogenic cells) line derived from cells initially induced in MSC medium with 2,4-D, then transferred and maintained in hormone-free MSC medium (MSC0); this line contained equal ratios of all stages of embryo development. All three lines have been maintained on the induction media without any loss of embryogenic capacity for more than 12 years. For the cell culture experiments described in this study, beside on induction media described above, embryogenic tissue was grown on several modified MS media compositions. Different types of nitrogen sources were supplied into the MSNH4 medium: nitrate (2 and 20 mM KNO_3) and Gln (1 and 10 mM). We used different concentrations of KNO_3 and Gln because of the difference in the number of nitrate groups. The media are later designated always according organic compounds or nitrogen sources added, as for example MSKNO3, MSGln, MSCKNO3 etc. In parallel, embryogenic tissue was transferred from MSNH4 into hormone-free MS medium with conventional 18 mM KNO_3 and 20 mM NH_4NO_3 (designated as MS0 medium), known to enable development of later embryo stages in pumpkin. Prior to the re-supply of nitrogen, proembryogenic tissue was maintained on MSNH4 medium for at least three 30-day subcultures. For testing the effect of 5-azaC on DNA methylation, we first dissolved 5-azaC, then sterilized it by filter-sterilization and added it into the autoclaved liquid medium (final concentration: 12.3 mM) immediately before tissue inoculation. DNA was isolated from the tissue after 4 days of culture. For the phenotypic characterisation of cultures treated with 5-azaC, tissue was cultured on medium solidified with 0.7% agar for 2 months. During that period the 5-azaC was added in the form of drops every 2 days onto a piece of filter paper placed between the medium and tissue. All tested media were supplemented with 250 mM glucose, adjusted at pH 5.8 with 0.2 M KOH, and sterilized. Cultures were incubated in a growth room at 24 ± 1ºC and with a 16 h light period (light intensity of 17 Wm^{-2}). Long-term tissue cultures were subcultured to a fresh medium solidified with 0.8% (w/v) washed agar (Sigma®) over a four-week interval.

Histological observation

To determine cell viability, fresh tissue samples were stained with 2% acetocarmine and slightly squashed onto the slide. For aniline blue labeling of

callose, embryogenic culture samples were fixed in 6% glutaraldehyde in 0.07 M phosphate buffer (pH 6.8) for 1 h under vacuum, and for 24 h at room temperature. The samples were subsequently dehydrated in an ethanol series, embedded in metacrylate resin (Leica Historesin Embedding Kit) for 1 h under vacuum, 24 h at 4 ºC, and then polymerized at room temperature for three days. The semi thin sections (0.4 µm) were made with Leica RM 2155 ultramicrotome and stained with aniline blue (0.05%, w/v, in 0.015 M Hepes buffer pH 9.25) for 20 min. Specimens were examined in Zeiss Axio Scope FL epifluorescent microscope (Carl Zeiss, Germany), fitted with the UV filter set 02 (excitation at 365 nm, emission at 420 nm).

Soluble protein content determination and enzymes activity assays

Lyophilized pumpkin samples were ground at 4 ºC using a mortar and pestle, and tissue extracts were prepared in an ice-cold 50 mM potassium phosphate (K_2HPO_4/KH_2PO_4) buffer pH 7.0 containing 0.1 mM EDTA, with addition of insoluble polyvinylpyrrolidone (PVPP, Sigma). The homogenates were centrifuged at 25 000 × g for 30 min at 4 ºC, and supernatants were used for soluble protein content, and GS, SOD, POD and esterase activity analysis. Sephadex G-25 desalting of the extract did not improve the sensitivity of enzymes activity assays and was omitted from the purification procedure. Soluble protein content was determined by the method of Bradford (1976) using bovine serum albumin (BSA, Sigma) as the standard. The activity of GS biosynthetic enzyme was assayed according to Márquez et al. (2005). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich, 1971). The activity of POD was measured using pyrogallol as the substrate according to Chance and Maehly (1955). Esterase activity was determined using 2-naphthylacetate as a broad spectrum substrate for arylesterase (EC 3.1.1.2) and carboxilesterase (EC 3.1.1.1) (Burlina and Galzigna, 1972). The activity of GS, SOD, POD, and EST was expressed as mol of a product per min per mg protein (mol min−1 mg protein−1).

Genomic DNA digestion and random PCR amplification

The technique used to identify DNA methylation is based upon the coupled restriction enzyme digestion and random amplification (CRED-RA) published by Cai et al. (1996). Isolated genomic DNA was restricted with isoschizomeric pair *Hpa*II and *Msp*I endonucleases Following total digestion, the nascent fragments were amplified by the PCR using six random 10-base primers: OPL-04, OPL-O7, OPL-11, OPL-13, OPBB-09 and OPBB-18 (Operon Technologies, Alameda, Calif.). Reactions consisted of 25-ml volumes of 50 ng plant DNA, 0.2 mM dNTP, 2.0 mM MgCl2, 1 U Taq polymerase and 0.4 mM primer. PCR conditions were: 40 cycles of 1 min at 94 ºC, 1 min at 35 ºC and 2 min at 72 ºC. Amplified samples

were electrophoresed on 1.5% agarose gel in TBE and the DNA visualised with ethidium bromide. Differences in the electrophoretic patterns that resulted from total digestion with the pair of enzymes and amplification of the digested DNA fragments with each individual primer represented the criteria for evaluating the level of DNA methylation. To insure that any differences in electrophoretic pattern would result from the specificity of amplification for each tissue/primer combination, the experiment was repeated five to six times. Consistently reproducible bands were analysed using the computer programme rapddistance (ver. 1.04).

Statistical analysis

For each analysis, data were compared by analysis of variance (ANOVA), using the STATISTICA 7.1 (StatSoft, Inc., USA) software package, and differences between corresponding controls and exposure treatment were considered as statistically significant at $P < 0.05$. Each data point is the average of three replicates obtained from three independent experiments. Same software package was also used for the statistical significance obtained by comparing numerical values of DNA methylation levels.

RESULTS AND DISCUSSION

Embryogenic cultures and callose deposition

Embryogenic tissue grown in the presence of 2,4-D had nodular structure with most embryos in globular stage, and a few embryos continued to develop into later embryo stages. Globular embryos of about 300–1000 µm in diameter had disordered surfaces, and were able to produce secondary embryos from a single cell and aggregates detached from existing embryos (Fig. 1A). Replacement of 2,4-D-containing MS medium with hormone-free MS medium was favorable for the development of later embryo stages. Medium with 1 mM NH_4Cl as the sole source of nitrogen blocked pumpkin somatic embryogenesis in the early proembryogenic stage, as previously reported. The culture consisted of small cell aggregates and single cells that continued to multiply (Fig. 1B). Cells were nearly spherical, with dense cytoplasm, and intensively stained with acetocarmine or FDA (Fig. 1C). Subcultivation of NH_4^+-induced embryogenic tissue to MS0 medium with nitrogen enabled the development of later embryo stages. Fluorescence microscopic analysis revealed massive callose accumulation in tissue grown on MS medium with 1 mM NH_4Cl, more prominent than that observed on MS with 4.5µM 2,4-D. High callose content was disposed uniformly throughout the cell walls of cells grown on MSNH4 (Fig. 1E). In embryogenic tissue grown in the presence of 2,4-D, callose was present as small bodies scattered over the cell walls (Fig. 1D). In MS medium, which enabled the development of mature stage embryos, callose content decreased further, but was still present in some cells (Fig. 1F). Callose deposition in plants

can be considered as the most prominent physiological response to various stress conditions, but also the first visible manifestations of metabolic changes leading to somatic embryogenesis (Dubois et al. 1990). In the proembryogenic pumpkin culture induced and maintained in the presence of ammonium ions as a sole source of nitrogen, a massive callose deposition surrounding single cells or small cell aggregates was observed while in auxin-induced embryogenic tissue, callose is deposited as spots through the cell walls. The observed variations in pattern of callose deposition were reversible as medium composition changes (Mihaljevic et al. 2011). The proposed function of the callose deposition is to isolate the embryogenic cells from the influence of surrounding cells while they followed reprogramming and a new developmental pathway (Dubois et al. 1990). The callose deposition disappears as embryos grow and products of it degradation, together with other cell-wall components, represents a source of different carbohydrates and proteins with signalling information for plant growth and development (Malinowski and Filipecki 2002).

Nitrogen effects on the development of embryogenic tissue

We show for the first time that somatic embryogenesis of pumpkin can be reliably accomplished on hormone-free medium supplemented with NH_4Cl as the sole nitrogen source. These data support the results published by Smith and Krikorian (1991) on preglobular cultures of carrot and suggest that $NH4^+$ allows the division of proembryogenic cells, such as the cells of zygotic embryos or embryogenically-determined cells induced by 2,4-D in a species other than carrot. Cultures of three embryogenic lines responded similarly to the same composition of nitrogen salts and exogenous 2,4-D in medium buffered at pH 5.8 (Fig. 2). The lines induced on medium MSC2,4-D (DEC and HEC) and cultured on the same medium had a highly nodular structure with most embryos in the globular stage. Some of the globules continued their development into the heart and torpedo stages. MS medium without 2,4-D supported embryo development to mature stages and some of those were able to develop plantlets. After transferring the cultures into the media MSNH4 or MSCNH4, proembryogenic masses, embryogenic globules and rare heart and torpedo stage embryos survived. Successive cultivation on the same medium (three or more subcultures) kept growing only preglobular cells and globular embryos, and cultures gained the characteristic PEDC phenotype. After transferring the globules to the MS or MSC medium supplemented with conventional 20 $mmolL^{-1}$ NH_4NO_3 and 20 $mmolL^{-1}$ KNO_3, mature stages of embryos developed. The modified media supplemented with 60 $mmolL^{-1}$ KNO_3 ($MSCKNO_3$), or 60 $mmolL^{-1}$ $NaNO_3$ ($MSCNaNO_3$) or both 1 $mmolL^{-1}$ NH_4Cl and 55 $mmolL^{-1}$ KNO_3 ($MSCNH_4/KNO_3$), supported the continuity of embryo development. The PEDC line cultured on MSNH4 medium had the consistence of friable nodules, and most embryos

settled in preglobular and globular stages. The PEDC line on the medium with conventional MS nitrogen sources (MS0 and MSC0) accomplished characteristic changes in the developmental process. Although some cell masses remained at the preglobular stage, many of the embryos continued their development to heart and torpedo stages but never to the cotyledonary stage. After transferring the PEDC line as well as DEC line to the modified MSC medium with 60 mmolL^{-1} KNO_3 ($MSCKNO_3$) or 60 mmolL^{-1} $NaNO_3$ ($MSCNaNO_3$) alone, or supplemented with 1 mmolL^{-1} NH_4Cl and 55 mmolL^{-1} KNO_3 ($MSCNH_4/KNO_3$), further development of embryos occurred but tissue became brown and dry at the end of subculture. Smith and Krikorian (1992) show that the low pH with a reduced nitrogen source can replace 2,4-D in maintaining and multiplying the embryonic tissue of carrot. The nitrogen uptake and pH could be related to the connection between ammonium transport and electrochemical gradient. The plants maintain a fairly constant electrochemical gradient across the membrane (despite fluctuations of the major nutrients – ammonium and nitrate) by the regulation of a primary active transporter, such as the plasma membrane H^+ATPase that pumps protons out of the cell and into the apoplastic space (Howitt and Udvardi 2000), or into the medium.

SOD, POD and esterase activity

Although is not a classical stressor, nitrogen supply as the most limiting nutritive factor for plant growth and development, if insufficient, affects protein biosynthesis and secretion (Leljak-Levanić et al. 2011), and generates oxidative stress (Mihaljevic et al. 2011). Moreover, suboptimal level of ammonium as a sole nitrogen source lead to increased lipid peroxidation and activities of antioxidative enzymes superoxide dismutase (SOD) and soluble peroxidase (POD) what indicates oxidative stress in the tissue (Mihaljevic et al. 2011). All this results show that the induction and development of embryogenic competence is associated with the oxidative stress induced by nitrogen limitation. The antioxidative status of pumpkin embryogenic tissue cultures in relation to different culture media was analyzed by measuring SOD; POD and esterase activity. Activity of gluthamine synthetase (GS) was the measure of ammonium assimilation. SOD activity was high in embryogenic tissue grown in MS2,4D, and even higher in MSNH4 (Fig. 3B).

After the transfer of both types of tissues into MS0 medium, SOD activity decreased. Buffering of NH_4^+-medium did not affect SOD activity. Re-supply of nitrogen in form of 1 or 10 mM Gln, or 2 mM NO3− in MSNH4 transiently decreased SOD activity (day 4th). After that, SOD activity increased, becoming about equal to the value measured in MSNH4 (Fig. 3G). The most significant reduction in SOD activity was observed after addition of 10 mM Gln, whereas the equimolar concentration of KNO3 (20 mM) did not have such an effect. In tissue maintained in MSNH4 medium, POD activity was three times higher than in other tested media (Fig. 3D).

Buffering MSNH4 medium with MES transiently decreased POD activity during the first four days of culture, but after that it reached a value similar to that in non-buffered MSNH4 medium. The most remarkable drop in POD activity was obtained after the transfer from MSNH4 into MS0. Addition of Gln or $NO3^-$ had less prominent effects on POD activity (Fig. 3I). As shown in Fig. 3E and J, variations in specific esterase activity provoked by different culture media were similar to changes in POD activity (Fig. 3D and I). The highest EST activity was obtained in tissue grown on MSNH4, lower on MSNH4 medium buffered with MES, and the lowest in embryogenic tissue grown in the presence of 2,4-D or after the transfer from MS2,4D into MS0. Supply of Gln or $NO3^-$ nitrogen in all examined concentrations led to declining esterase activity. In general, proembryogenic tissue induction and proliferation were characterizes by an increased POD activity, while a decrease in POD activity preceded late-stage embryo development. The same is with esterases, a group of enzymes that hydrolyze ester bounds in a broad range of substrates. Detection of changes in expression and activity of esterases has been used in predicting developmental events that occurred during somatic embryogenesis (Tchorbadjieva and Odjakova 2001).

DNA methylation

Differences in the electrophoretic pattern following digestion and amplification were the criteria used for evaluating the levels of DNA methylation. The values obtained and the average values for each line and medium are presented in Table 1. The highest levels of DNA methylation were observed in PEDC and DEC tissue cultivated on MSNH4 medium and in the HEC line on MSC medium supplemented with 2,4-D. The PEDC and DEC lines were also highly methylated on MSC medium with 2,4-D. The differences in DNA methylation levels of all three lines were not significant during cultivation either on MSNH4 or on MSC with 2,4-D. Both media were favourable for development of embryogenic cultures with preglobular- and globular-stage embryos. The addition of 5-azaC to MSC medium containing 2,4-D caused a certain decrease in DNA methylation in the PEDC line and a significant decrease in the DEC line, but in both cases the phenotype of the embryogenic tissue was not changed. The removal of 2,4-D from MSC medium containing 2,4-D enabled embryo maturation and at the same time, DNA methylation was twofold lower in the PEDC and DEC lines and significantly (threefold) lower in the HEC line. Following the replacement of 2,4-D with 5-azaC in the MSC medium, DNA methylation was significantly lower in the DEC and HEC lines and twofold lower in the PEDC line. Upon the addition of IAA to the MSC medium, which had a favourable effect on plantlet regeneration, the level of DNA methylation in the PEDC and HEC lines increased slightly, while in the DEC line it increased twofold in comparison with the level obtained on MSC medium. In our experiments, the medium containing

2,4-D and that containing NH_4Cl maintained the embryogenic status of the cultures and early embryo development. The DNA of early embryogenic tissue cultivated on these media was at least twofold more methylated than that in tissue containing all stages of embryo development cultivated on 2,4-D free, unmodified MSC medium. Kaeppler and Phillips (1993) proposed that the stress provoked by tissue culture is related to the alteration in DNA methylation, which leads us to hypothesise that the presence of 2,4-D as well as a low concentration of NH4Cl as the sole source of nitrogen in hormone-free MS medium used in our culture system might cause stress in embryogenic culture Based on our results and the observations of others we can speculate that somatic embryogenesis could be induced by stressful conditions manifested through methylation changes.

CONCLUSION

The molecular understanding of somatic embryogenesis induction is based on experiments with different model systems which together provide a background about developmental pathways involved in somatic embryogenesis.

Presented data on stress related enzymes and extracellular components that have key roles during onset of somatic embryogenesis clearly show the relation of stress-related responses and somatic embryogenesis.

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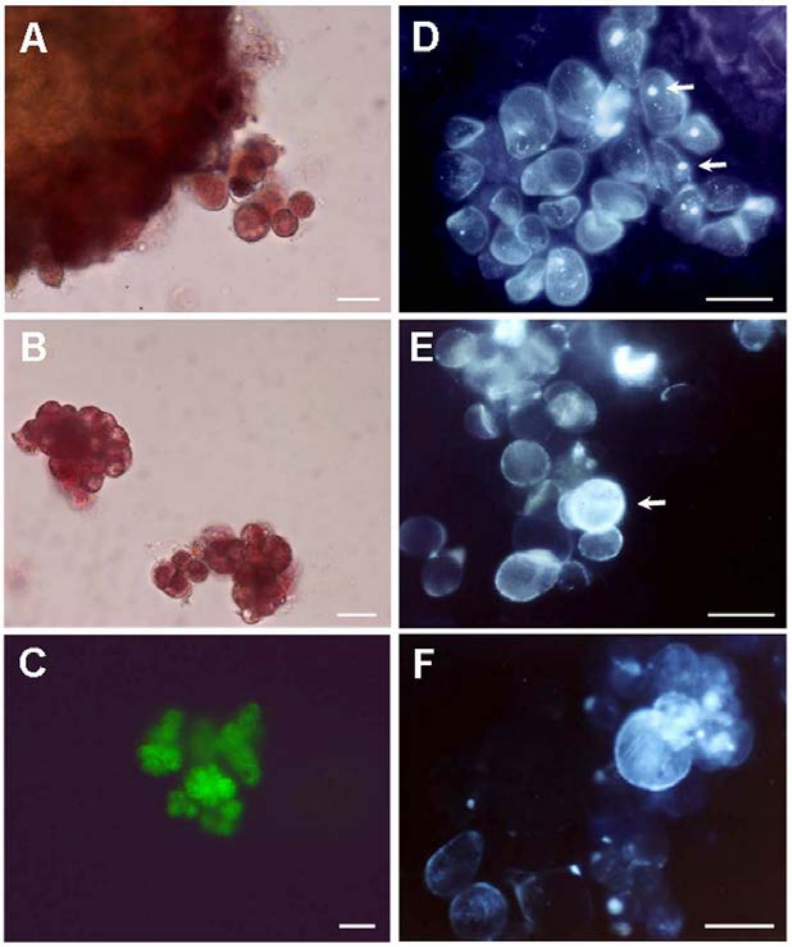

Fig. 1. Morphology (A–C) and callose deposition in the cell walls (D–F) of pumpkin embryogenic cultures: (A) globular embryo in MS2,4D and (B) proembryogenic masses in MSNH4 stained with acetocarmine; (C) tissue in MSNH4 stained with FDA. Callose deposition visualized with decolorized aniline blue in semi-thin sections of tissue grown in (D) MS2,4D; (E) MSNH4; (F) MS0. Arrows point to callose depositions. Scale bars = 50 μm. A and B - bright field images and C, D, E, F - epifluorescent images.

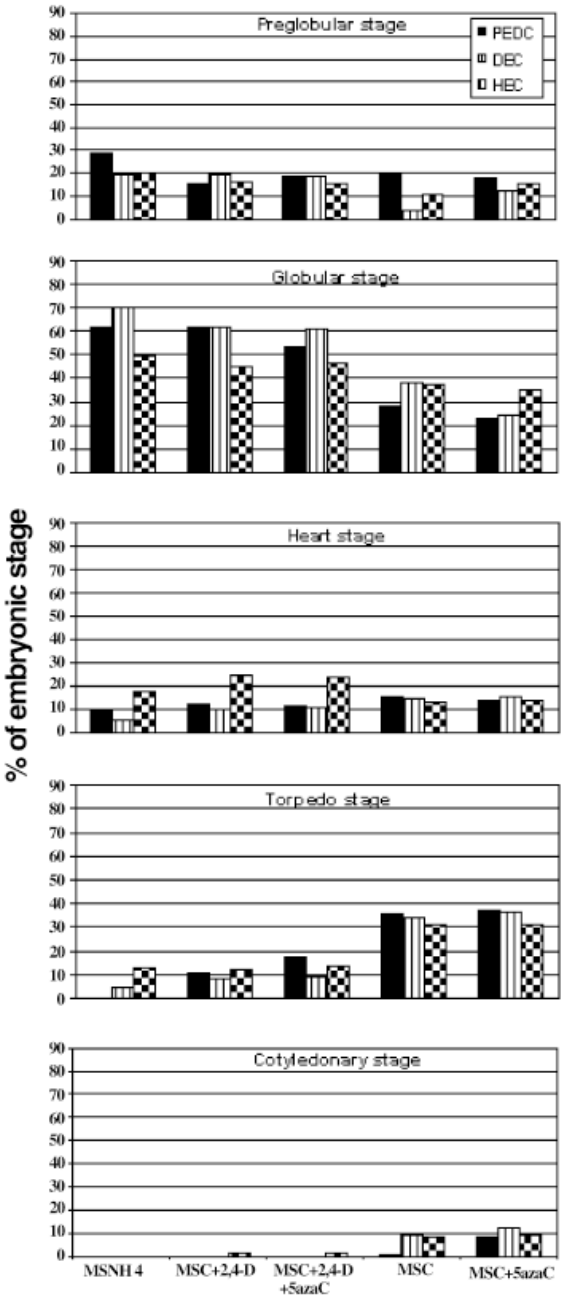

Fig. 2. Frequency of developmental embryo stages in pumpkin PEDC, DEC and HEC lines cultivated on MSNH4, MSC+2,4-D, MSC+2,4-D+5azaC, MSC, and MSC+5azaC media. The frequency of a single embryo stage is expressed as a percentage of the total number of embryos counted under that specific experimental condition (12 tubes per treatment and three replicates).

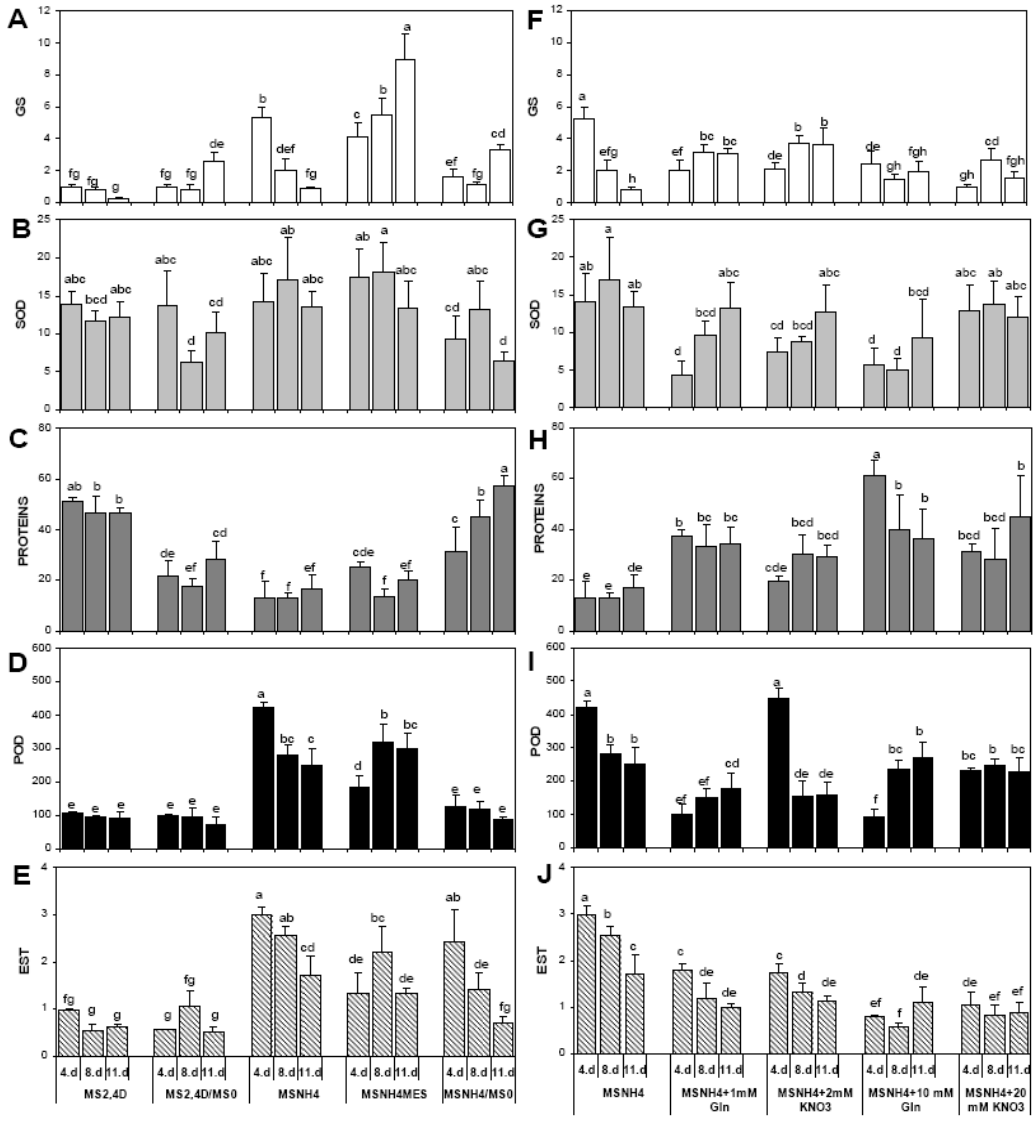

Fig. 3. A time course study of specific activity of: (A and F) glutamine synthetase, (B and G) superoxide dismutase, (C and H) soluble proteins (mg), (D and I) soluble peroxidase, and (E and J) esterase activity in C. pepo embryogenic tissue lines grown for 11 days in different culture media. GS, SOD, POD, and EST activity was expressed as μmol of a product per min per mg protein (μmol min−1 mg protein−1). Tissue transferred from MS2,4D into MS0 (MS2,4D/MS0), and from MSNH4 into MS0 (MSNH4/MS0). Bars represent ± STDEV (n = 3). Means followed by the same letters are not significantly different at $P < 0.05$.

Table 1. Analyses of methylation in embryogenic callus lines PEDC, DEC and HEC on different media. DNA methylation level is presented in form of numerical values obtained by the rapddistance programme (Armstrong et al. 1994). Statistical analysis of data was evaluated using the DNMR test at the 5% level of probability. Average values denoted with the same letter are not statistically different.

Line	Primer	Medium					
		MS+NH_4Cl	MSC+2,4-D	MSC+2,4-D+5-azaC	MSC+5-azaC	MSC	MSC+IAA
PEDC	OPL-04	0.385	0.462	0.070	0.070	0.070	0.308
	OPL-07	0.600	0.400	0.133	0.067	0.267	–
	OPL-11	0.526	0.158	0.474	0.158	0.000	0.000
	OPL-13	0.462	0.231	0.231	0.231	0.077	0.154
	OPBB-09	0.200	0.267	0.000	0.067	0.067	0.333
	OPBB-18	0.500	0.556	0.330	0.330	0.556	–
Average		0.445a	0.346a,b,c	0.206b,c,d,e	0.154c,d,e,f	0.173b,c,d,e	0.199b,c,d,e
DEC	OPL-04	0.083	0.250	0.167	0.000	0.000	0.000
	OPL-07	0.200	0.400	0.067	0.067	0.067	–
	OPL-11	0.438	0.000	0.188	0.000	0.188	0.188
	OPL-13	0.235	0.353	0.118	0.059	0.059	0.353
	OPBB-09	0.278	0.222	0.222	0.333	0.389	0.444
	OPBB-18	0.556	0.500	0.000	0.220	0.110	–
Average		0.310a,b,c,d	0.287a,b,c,d	0.127e,f	0.113e,f	0.135c,d,e,f	0.246a,b,c,d
HEC	OPL-04	0.000	0.300	0.400	0.300	0.100	0.000
	OPL-07	0.417	0.500	0.500	0.167	0.417	–
	OPL-11	0.500	0.143	0.429	0.071	0.071	0.214
	OPL-13	0.000	0.533	0.067	0.133	0.133	0.200
	OPBB-09	0.154	0.615	0.231	0.000	0.000	0.231
	OPBB-18	0.500	0.167	0.440	0.330	0.000	–
Average		0.262a,b,c,d	0.376a,b	0.345a,b,c,d	0.167c,d,e,f	0.120e,f	0.161c,d,e,f

Production of haploids in hull-less seed pumpkin genotypes through parthenogenesis induced by irradiated pollen

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Keywords: *Cucurbita pepo*, soft x-ray irradiation, in vitro pollen germination, embryo rescue, flow cytometry, SSR markers.

Abstract

Haploids are the first step towards homozygous doubled haploid (DH) lines which show great value in breeding programmes and genetic studies. Our research focuses on the development of DH lines of styrian oil pumpkin (*Cucurbita pepo* subsp. *pepo* var. *styriaca* Grebenšč.), used to produce seed oil with high nutritional value. The aim of this study was the development of methods applicable to a wide range of different genotypes and yielding a sufficient percentage of haploids. Preliminary we optimized in vitro pollen germination conditions for a possible pollinator line to study the effect of soft x-ray irradiation on pollen germination and estimate the approximate dose suitable for haploid induction. Exposure to irradiation resulted in a decrease of in vitro germination as well as a lower embryo formation rate in fruits harvested 4 weeks after pollination. The lowest germination rate was observed after irradiation with 300 Gy, which also gave best results for some genotypes in haploid induction. Seven different hull-less land races and commercial cultivars were used to evaluate the genotype dependence of haploid induction. Female flowers were isolated one day prior to anthesis and pollinated the following day in the morning by freshly collected and irradiated (50, 100, 150, 200, 300 and 350 Gy) anthers. Fruits were harvested 4 weeks after pollination and surface sterilized, followed by embryo rescue and embryo culture on the E20A medium. After ploidy was determined by flow cytometry, the possibility of spontaneous polyploidization was tested using available SSR markers on regenerants showing diploid or higher ploidy level.

INTRODUCTION

Oil seed pumpkin (*C. pepo* subsp. *pepo* var. *styriaca* Grebenšč.) is gradually gaining importance in the agriculture of Central Europe, therefore a lot of effort has been put into developing new high yield hybrid cultivars with other desired characteristics like disease resistance, bush growth habit etc. Haploids are the first step towards homozygous doubled haploid (DH) lines, which are used as parental lines for developing new hybrid cultivars in open pollinated species. Gynogenesis

seems to be the method of choice for haploid induction in *Cucurbita* spp., since there are only few reports on androgenesis reporting limited success (Metwally et al. 1998a; Mohamed and Refaei 2004; Rakha et al. 2012), whereas reports on successful haploid induction using in vitro culture of unfertilized ovules (Dumas de Vaulx and Chambonnet 1986; Metwally et al. 1998b; Shalaby 2007; Rakha et al. 2012) and obtention of haploid embryos and plants via pseudofertilization with gamma ray irradiated pollen followed by in vitro culture of immature embryos (Kurtar et al. 2002, Kurtar and Balkaya 2010) are more frequent. Beside gamma rays also UV and X-rays are being used for in situ haploid induction. The pollen remains physiologically active after irradiation, but is not able to fertilize the egg cell and polar nuclei. Embryo development is stimulated by pollen germination and pollen tube growth within the style. Beside a number of factors (genotype, environmental conditions etc.) the irradiation dose is one of the main factors affecting haploid induction. At lower doses, the generative nucleus is partially damaged and maintains capacity to fertilize the egg cell resulting in a number of embryos all of hybrid origin, whereas an increase in irradiation dose causes a decrease in the number of developed embryos, but obtained regenerants are mostly of haploid origin (Murovec and Bohanec 2012).

The aim of the present study was the development of a haploid induction protocol which is applicable to a wide range of different genotypes interesting for oil pumpkin breeding. To reduce the time and costs needed for developing a haploid induction protocol we evaluated the most suitable dose range for haploid induction by in vitro germination tests.

MATERIALS AND METHODS

Plant material, in vitro pollen germination and pollination with irradiated pollen

Seven hull-less genotypes (‘Gleisdorfer Diamant’, HSC 151 (‘Beppo’), ‘Gleisdorfer Ölkürbis’, ‘Slovenska golica’ ‘Rumena golica’, PI 615102, PI 420329) were used as female donor plants and ‘Gl Opal’ as pollen donor in vitro germination studies as well as pollination of female flowers.

Pollen germination studies were carried out with pollen collected early in the morning at the day of anthesis and irradiated (Faxitron RX-650; Faxitron bioptics LLC, Tucson, USA) with different doses (0, 100, 200, 300 and 400 Gy) to determine the in vitro germination rate and hereby estimate the approximate dose suitable for haploid induction. A modified Brewbaker and Kwack (1963) medium was used in hanging drop culture and pollen was allowed to germinate for approx. 2 hours in

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darkness at 25 °C. Germination rate was determined by light microscopy (Nikon Eclipse 80i microscope; Nikon Corporation, Tokyo, Japan).

Female flowers were isolated one day prior to anthesis and pollinated the following day in the morning by freshly collected and x-ray irradiated pollen. Female flowers of 'Gleisdorfer Ölkürbis' were used to compare the embryo formation rate and efficiency of different irradiation doses (50, 100, 150, 200, 300 and 350 Gy) for haploid induction, while all seven genotypes, pollinated with 300 Gy irradiated pollen, were used to evaluate whether the genetic constitution of the female parent affects the success of haploid induction.

In vitro embryo culture

Fruits were harvested 4 weeks after pollination, cleaned under tap water, followed by flame sterilization using 70 % ethanol. Seeds were extracted individually in a laminar flow and rescued embryos were cultured on solid E20A medium (Sauton and Dumax de Vaulx 1987) in petri dishes with 25 compartments at 23°C with a 16-hour photoperiod.

Determination of ploidy level and homozygosity testing

A total of 1,898 embryos (of which 831 for genotype and 1,067 for dose comparisons) were subjected to ploidy determination by flow cytometry (CyFlow space; Partec GmbH, Görlitz, Germany) using 4',6-diamidino-2-phenylindole (DAPI) staining. DNA was extracted from diploid or tetraploid regenerants to test the possibility of spontaneous chromosome doubling using available SSR markers (Gong et al. 2008).

RESULTS AND DISCUSSION

Embryo formation rate after pollination with irradiated pollen

Exposure to irradiation resulted in a decrease in in vitro germination as well as lower embryo formation rate in fruits harvested 4 weeks after pollination. In contrast to reports in squash (Kurtar et al. 2002) and winter squash (Kurtar and Balkaya 2010) we observed embryo formation even at irradiation doses as high as 350 Gy, possibly due to genotype differences or lower penetration ability of soft x-rays in comparison to gamma rays which were used in the before mentioned studies. The lowest in vitro pollen germination and embryo formation rate was observed after irradiation with 300 Gy, which suggested that this might be the appropriate dose for haploid induction in different genotypes.

Efficiency of different irradiation doses for haploid induction

The frequency of haploid embryos was influenced by the doses used for anther irradiation. Our results revealed that different doses (50, 100, 200 and 300 Gy) were successful in inducing parthenogenesis, whereby 200 Gy has been most effective when using 'Gleisdorfer Ölkürbis' as female donor plants. In contrast to our results other authors (Kurtar et al. 2002; Kurtar and Balkaya 2010) reported that haploid embryos of pumpkins were obtained only from up to 100 Gy irradiation doses, most likely explained by lower penetration ability of soft x-rays which were used

for irradiation of anthers and genotype differences. In our studies no spontaneous doubled haploids were confirmed by SSR markers.

Comparison of haploid induction in different genotypes

The frequency of haploids in different genotypes used as females ranged from 0 in 'Slovenska golica' to 3,92 in PI 615102 per 100 embryos, which confirms the genotype dependence of haploid induction.

ACKNOWLEDGEMENTS

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Development of suitable sourcess of resistance to ZYMV in *Cucurbita pepo*

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Keywords: ZYMV, resistance, summer squash, *Cucurbita pepo*

Abstract

This study was carried out between 2008-2010 years, in Alata Horticultural Research Station. The hybrid summer squash variety Otto F_1 was used as donor for resistance source of ZYMV. The resistance of the variety was introduced by backcross method to our pure lines Alata6 and Alata50 to develop suitable material for the breeders. For this purpose we realized the hybridizations and at the end we obtained BC_4 populations. Before establishment of each backcross populations we realized a selection for resistance by mechanical inoculation then plants were screened according to symptom cale classes.

INTRODUCTION

Cucurbita pepo L. belongs to *Cucurbitaceae* family in which there are approximately 115 genera (Jeffrey 1980). *Citrullus*, *Cucumis*, *Cucurbita* and *Lagenaria* are four major cucurbitaceaous genera. Pumpkins and squash, *Cucurbita* species, are economically important vegetable crops grown on arable land throughout the world. China, Ukraine, Argentina and Turkey are the leader squash and pumpkin producer countries where 45% of world production is realized (Pitrat et al. 1999). In most regions of the world four major viruses affect cucurbit production: Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV), Papaya ringspot virus-W (PRSV-W) and Cucumber mosaic virus (CMV) (Zitter et al. 1996). The most serious threat to production of pumpkins and squash is ZYMV in many regions (Lisa and Lecoq 1984; Desbiez and Lecoq 1997). The virus is a highly virulent member of Potyviridae group and transmitted by aphids in non-persistent manner (Lisa et al. 1981; Desbiez and Lecoq 1997). ZYMV was firstly described on summer squash (Lisa et al. 1981; Lecoq et al. 1981). The severity of this disease led to efforts to identify sources of resistance because of cultural controls show limited effectiveness. No sources of resistance to ZYMV have been found within the *C. pepo*. A wide range of genetic resources was tested including different species of the genus. Resistance to ZYMV has been found in several accessions of two cultivated

species, *Cucurbita moschata* Duchesne, referred as "Nigerian Local", and *C. ficifolia* Bouché (Paris et al. 1988). Resistance to ZYMV in *C. moschata* is coferred by a single dominant gene, *Zym* (Munger and Provvidenti 1987; Paris et al. 1988). Resistance genes in different accessions derived from *C. moschata* were designed as *Zym-0*, *Zym-1* and *zym*^{mos} (Paris and Brown 2005). Despite ZYMV resistance is conferred by a single dominant gene, much difficulty has been encountered in transfer of resistance to *C. pepo* due to barriers to interspecific crossing (Whitaker and Davis 1962). Level of resistance can be lost during backcrossing, this being attributed to a change in the mode of inheritance through interspecific transfer. We have used a commercial *C. pepo* hybrid variety Otto F_1 to eliminate the barriers in interspecific crossing. In the study we aimed to transfer ZYMV resistance gene/s of Otto F_1 to our summer squash lines Alata6 and Alata50.

MATERIALS AND METHODS

This study was carried out between 2008 and 2010 years in Alata Horticultural Research Institute. Otto F_1 and our Alata6 and Alata50 summer squash lines were used in the experiment.

Otto F_1 has fruits that are cylindrical and light green. The variety is suitable for open field growing in main growing season and carries resistance to Watermelon Mosaic Virus (WMV) and moderately resistance to Zucchini Yellow Mosaic Virus (ZYMV). Our summer squash lines Alata6 and Alata50 are suitable for open field and plastic tunnels and greenhouses carrying light green and dark green colors of fruits respectively and recommended for early spring and spring growing seasons.

F_1, BC_1, BC_2 and BC_3 families were generated from the cross between Alata6 and Alata50 by Otto. Resistance tests were applied for every generation by mechanical inoculation using ZYMV-Ad (Gene bank Ac. N. JF 317296.1) isolate (Özer et al. 2012).

Seeds were sown to the 1 l pods including soil and sand mixture (1:1 v:v). They were kept in green house condition to germination and emergence.

Virus isolate was mechanically inoculated to the cotyledons at the first true leaf stage of the plant by rubbing Carborundum-dusted with extracts from 1 g of infected plant material prepared in 0.02 M phosphate buffer and containing 0.1% 2-mercaptoethanol (pH:7.0) and added activated carbon. After inoculation cotyledons were washed using tap water. Plants were left to grow in greenhouse conditions.

Symptom evaluation was realized by Yilmaz et al. (1994) according to the following symptom scale; 0: No symptom, 1: very weak vein clearing, 2: very weak

mosaic symptom on the leaf, 3: weak vein clearing with weak mosaic, 4: moderately mosaic and light yellowing, 5: mosaic symptoms on the leaves and shorten in plant height, 6: severe mosaic and mild deformations on the leaves with shorten in plant height, 7: severe mosaic, mottling, moderately deformation of leaves, 8: severe mosaic, filamentous appearance of leaves, moderately fruit deformations, 9: severe dwarfing of the plants, very severe mosaic and yellowing, severe fruit deformation, filamentous appearance of leaves.

After symptom evaluation the plants in 0, 1, 2 and 3 classes of symptom scale were used to generate the following generation.

RESULTS AND DISCUSSION

In the study we intended to introduce the ZYMV resistance source from Otto F_1 to our summer squash lines Alata6 and Alata50 by backcross method. For this purpose we obtained BC_1, BC_2 and BC_3 populations between 2008 and 2010 growing seasons. The overall results were summarized in Table 1, Table 2 and Fig. 1.

As we see in Table 1 the percentage of resistant plants increased in the successive backcross generations. While approximately 7.5% of the plants were found symptomless in BC_1 generation, it was 11% and 14% in BC_2 and BC_3 generations, respectively.

As shown in Table 2 symptomless plant ratio in Alata50 cross was lower than that of Alata6 cross in BC_3 generation.

The overall results showed that Otto F_1 could be used as donor for ZYMV resistance in summer squash because of hybridization drawbacks with highly ZYMV resistant wild relatives. There is not highly resistance source to ZYMV in *C. pepo* lines. Interspecific hybridization leads inconveniences because of linkage drag. It is very difficult to eliminate undesired characters coming from interspecific hybridization.

Segregation of ZYMV resistance observed at different populations of Otto F1 cross indicates up to four genes responsible for resistance. The fourth gene seems to operate late after infection and provides extended resistance and durability of plant resistance Table 3.

Zucchini yellow mosaic virus (ZYMV) is a very damaging cucurbit virus worldwide. Interspecific crosses using resistant *Cucurbita moschata* have resulted in the release of “resistant” zucchini squash (*C. pepo*) F1 hybrids (Lecoq et al. 1992). However, although the resistance is almost complete in *C. moschata*, but commercially used F1 hybrids of *C. pepo* varieties have commonly tolerance level of resistance as Otto F1.

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Table 1. Resistance tests of populations obtained from the cross Alata6 × Otto F1.

	Number of plants												
Population	0	1	2	3	4	5	6	7	8	9	Total	Resistant %	Ratio of resistance
F1	5	3	2	3	6	6	1	1	0	0	27	29,6	3/10
BC1	14	7	2	3	0	17	16	35	28	66	188	11,0	1/10
BC2	6	10	0	2	0	2	1	1	4	30	56	28,5	3/10
BC3	25	68	16	15	12	5	17	8	5	5	176	52,0	1/2

Table 2. Resistance tests of line Alata50 × Otto F_1.

Population	Number of plants												
	0	1	2	3	4	5	6	7	8	9	Total	Resistant%	Ratio of resist.
F1	1	3	1	4	15	17	14	8	1	0	64	6,0	1/16
BC1	47	17	2	4	10	121	178	168	85	161	793	8,0	1/12,4
BC2	15	43	3	8	12	23	14	13	32	103	266	21,8	1/4,6
BC3	39	134	38	36	20	25	23	14	4	8	341	51,0	1/2

Table 3. Otto segregation.

Scale	Number of plants												
	0	1	2	3	4	5	6	7	8	9	Total	Resistant%	Ratio of resistance
First observation data at May 10, 2007	5	12	9	6	2	8	-	-	-	-	42	40,4	1/2,5
Second observation data at May 15, 2007	-	2	5	6	12	10	1	2	-	-	38	5,3	1/19

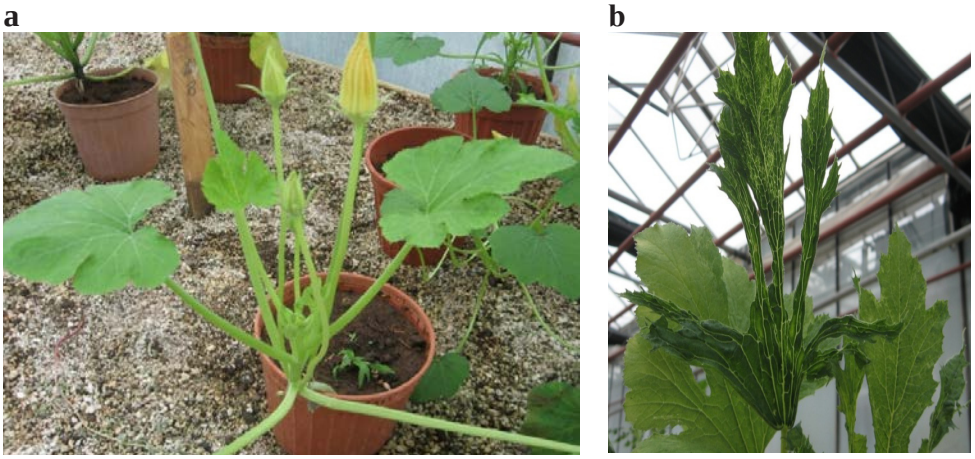

Fig. 1. Young plant of squash showing resistant (a) and sensitive (b) symptoms.

Ethylene production by fruits of zucchini cultivars differing in postharvest fruit quality and tolerance to chilling injury

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Keywords: Selection criteria, postharvest fruit quality, chilling injury, ethylene, zucchini

Abstract

Zucchini is a non-climacteric fruit that is harvested immature while reaching about 18 cm in length. We have compared the progression of certain postharvest fruit quality parameters with the production of ethylene and the expression of ethylene biosynthesis genes in the fruits of different cultivars stored at 4, 12 and 20ºC. Ethylene production remained at very low levels when fruits were stored at 12 or 20°C for a period of 14 days. However, in fruits stored a 4ºC for 7 days, and then conditioned at room temperature for a minimum of 4 hours, ethylene production was dramatically induced. This chilling induced ethylene varied among the different cultivars studied, being highest in the fruits of cultivars with lower post-harvest quality and lower tolerance to chilling injury (CI). Chilling induced ethylene does not appear to be responsible for triggering CI, since visible symptoms were evident some days before the burst of ethylene. However, our findings suggest that this chilling induced ethylene may be used as a selection criterion in screening for genotypic differences in shelf life, postharvest fruit quality and tolerance to CI. The expression profiles of *CpACS1* and *CpACO1* indicated that these genes are induced throughout the period of cold storage.

INTRODUCTION

In contrast to fruits that are physiologically mature at harvest, others as green beans, cucumbers and zucchini, are harvested and consumed physiologically immature. Regardless of their behaviour during ripening, immature fruits produce low levels of ethylene during postharvest storage and are therefore considered non-climacteric. In the immature fruit of cucumber (Wang and Adams 1982), as occurs in other non-climacteric fruit such as citrus (Zacarías et al. 2003), cold storage induces enormously the production of ethylene. This cold induced ethylene, as well as the induction of ACC content and ACS activity, does not occur during the period

of cold storage, but increases rapidly after transferring the fruit to room temperature (Wang and Adams 1982).

The zucchini fruit undergoes a series of physiological changes (loss of water, softening, and chilling injury) that devalue their market value during cold storage (Carvajal et al. 2011). Brendan-Quintana et al (2003) found that cold storage induces $C0_2$ and ethylene with a maximum at 12 d of storage. However, the involvement of ethylene in the deterioration of the fruit during cold storage is unknown. This paper compares the evolution of post-harvest fruit quality of five cultivars of zucchini squash with the evolution of ethylene production and the expression of the genes *CpACS1* and *CpACO1*.

MATERIALS AND METHODS

All fruits used in this study come from the same field trial with different commercial cultivars, which were grown in a greenhouse under standard conditions of Almería, Spain. Homogeneous fruits of 18-20 cm in length were randomly divided into different batches and then stored at 4, 12 or 20 ° C for a total of 14 days.

Twelve fruits were analysed for each cultivar, time and storage temperature, with evaluations immediately after harvesting, and at 7 and 14 days of storage. The fruit firmness was determined by a penetration test using the Stable Microsystem Txt2 equipment, with a probe of 4 mm in diameter at a speed of 1 mm/s to 10 mm in deep. To assess chilling injury (CI) on the surface of fruits (pitting), the fruit was classified according to the following scale: 0 = no damage, 1 = 1-3% damage, 2 = 3 -10% damage, 3 = 11-30% damage, 4 = 30-50% damage and 5 = more than 50% damage. Ethylene production was determined by gas chromatography on a Varian-3900 GC equipped with a flame ionization detector (FID). Gene expression was analyzed by quantitative real-time RT-PCR, following the methodology described in Manzano et al (2010).

RESULTS AND DISCUSSION

First we studied the loss of weight and firmness in 'Tosca' fruit stored for 14 days at 4, 12 and 20°C. The greatest losses of both weight and firmness occurred in the fruits stored at 20°C (data not shown). Cold stored fruits, although lost less weight and firmness, showed, however, a number of CI symptoms (pitting) that devalued fully its commercial value (Table 1). In fruits stored at 4°C, CI symptoms were apparent after 3 days, and the fruit lost its commercial value after 7 days of storage, while in fruits stored at 12 ° C, the lost of commercial value by CI occurred after 14 days of storage (Table 1).

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Ethylene production was also greatly influenced by storage temperature. Immediately after harvest, ethylene production was very low (Fig. 1). In the fruits stored at 12°C and 20°C, ethylene production hardly changed during the storage period (Fig. 1). However, in the fruit stored at 4°C ethylene production was induced up to 200 times at 7 days of storage (Fig. 1). Although previous studies have found that cold storage may induce the production of ethylene in zucchini squash (Brendan-Quintana et al. 2003), the factors that regulate this induction are unknown.

In this study we demonstrate that cold-induced ethylene is not produced during the cold storage, but after transferring the fruit to 20°C (Fig. 2), and that the production level depends on both the period length of cold storage and the time length of conditioning at room temperature. In fruits stored for 7 days at 4°C, induction of ethylene began after heating the fruit for a minimum of 4 hours, and reached the maximum production of ethylene after 10 hours of heating at 20°C (Fig. 2) Ethylene induction also increased with duration of cold storage, reaching a peak at 7 days of cold storage (Fig. 2). This cold ethylene production has also been observed in immature fruits of cucumber (Wang and Adams, 1982), although their role in the development CI is not known. The fact that zucchini cold surface damage appear in the cold chambers before reheating the fruit at room temperature indicates that the ethylene produced by cold is not necessary to induce the appearance of CI, although it could accelerate the development of CI once the fruit is transferred from the cold storage chamber to room temperature.

The analysis of the ethylene biosynthesis genes *CpACO1* and *CpACS1* by quantitative real time RT-PCR indicated that the expression of both genes is induced during the cold storage (Fig. 2). Since the level of transcripts of both genes increases even after 7 days of cold storage, when ethylene production is beginning to decrease, it is likely that either the biosynthesis or the activity of the two enzymes was irreversibly reduced by the cold damages.

After 7 days of cold storage, ethylene was induced in the fruits of all studied cultivars (Fig. 3). However, the fruits of those cultivars that were more susceptible to cold damage such as those of 'Sinatra' and 'Celeste' were those which produced more ethylene after cold storage, while the more tolerant cultivars to CI, such as 'Nature' and 'Blas', produced less ethylene after cold storage. These data indicate a correlation between the cold-induced ethylene production and the sensitivity of the fruit to CI. These differences may be used as a selection criterion for cold tolerance in zucchini squash.

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Table 1. Chilling injury Index (CI) in'Tosca' fruits stored at 4ºC y 12 ºC for 14 days.

Time	CI Index (average ± ds)	
	4ºC	12ºC
3 days	2,54±0,58 b	--
7 days	4,00±0,82 c	1,65±0,73 a
10 days	4,54±0,33 d	--
14 days	5,00±0,00 *	4,04±0,69 c

Average and standard deviation of 4 replicates with 5 fruits each. They were compared with a t-test.

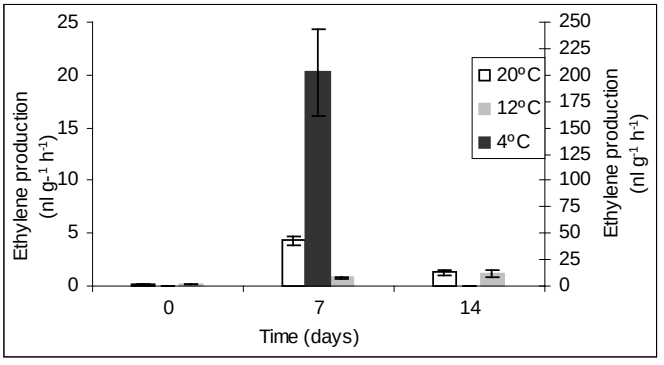

Fig. 1. Evolution of ethylene production in 'Tosca' fruit stored at different temperatures for a total of 14 days. 20ºC and 12ºC, left scale, 4°C, right scale.

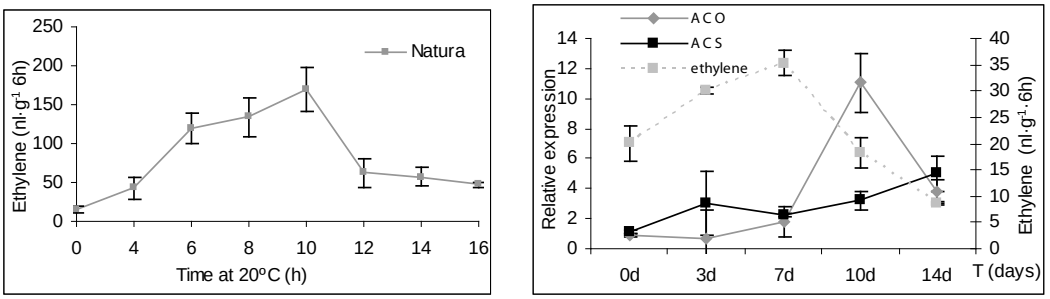

Fig. 2. Ethylene production in 'Natura' fruits stored for 7 days at 4°C and then transferred to 20°C for 0 to 16 h (left). Ethylene production and expression of *CpACO1* and *CpACS1* genes in 'Natura' fruits stored at 4°C for different periods of time and then transferred to 20ºC for 6 h (right).

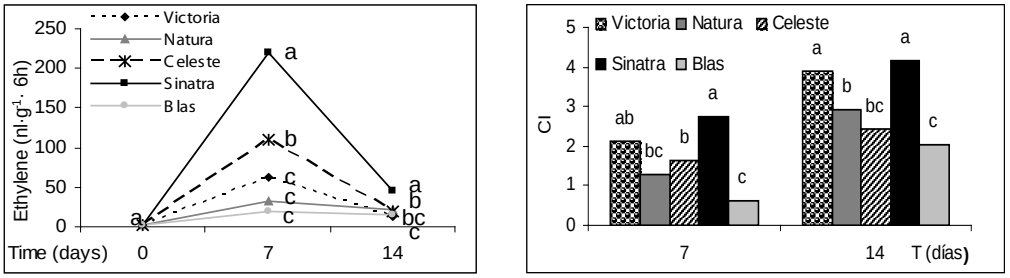

Fig. 3. Ethylene production (left) and chilling injury (right) during postharvest storage at 4 ° C in fruits of five varieties of zucchini.

Bottle gourd germplasm collection in Turkey

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Keywords: *Lagenaria siceraia*, germplasm, collection, core collection

Abstract

Bottle gourd [*Lagenaria siceraria* (Mol.) Stanley] is one of the first plant species domesticated for human use as food, medicine and various utensils and instruments made from the large hard-shelled fruit. The five wild species of bottle gourd are native to the northern half of Africa, *L. siceraria* is the only cultivated species of the 6 *Lagenaria* species and belongs to the family Cucurbitaceae. The cultivated species originated in Africa has been spread to all over the world and grown in Turkey for centuries with various purposes such as cooked vegetable, household containers, musical instruments, and decorations. The preservation of bottle gourd germplasm is important to maintain genetic diversity in Turkey where rapid and progressive genetic erosion occurs. A primary germplasm collection was initiated in 2003-2004 by collection of 182 accessions in southern part of the country. Tremendous morphological variations for fruit size and shape among preserved accessions were recorded. A new project proposal to collect bottle gourds germplasm for the rest of the country and to investigate genetic diversity in germplasm was supported by TUBİTAK in 2011. Since then seeds of 180 new accessions displaying unique morphological characteristics for fruits were gathered from all over the country other than Mediterranean region. As expected the fruits were found to show bottle, club-shaped or globular with vast variation. Size of fruits varied from less than 50 grams to 15 kg. Seeds of old and new collections of Turkish bottle gourds and 38 accessions obtained from gene banks of USA, Russia and India have been planted in Mersin in the spring of 2012 for detailed morphological characterization and more seed production for preservation. This collection representing genetic diversity of bottle gourds of Turkey will be a good core collection for preservation and moreover it will be good source to breeding and physiological researches of bottle gourd.

INTRODUCTION

Cultivated bottle gourd [*Legenaria siceraria* (Mol.) Standley] was one of the

first domesticated plant species for human utilization and it is commonly known as the white-flowered gourd (Fig. 1). It is an annual monoecious, vigorous climber (Fig. 2) species and five wild perennial diocious species, *L. brevifilora* (Benth) Roberty, *L. abyssinica* (Hook F.) Jeffrey, *L. rufa* (Gilg) Jeffrey, *L. spherica* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey (Motimoto et al. 2005). *L. siceraria* is diploid with 2n = 22 chromosomes (Beevy and Kuriachan 1996). All six species are present in Africa, where a center of genetic diversity for *L. siceraria* is believed to exist, although its wild progenitors have not yet been reported (Whitaker 1971). The *L. siceraria* is believed to be among the earliest domesticated plant species (Cutler and Whitaker 1967). The name *Lagenaria* is originated from '*lagena*', name of Florence flask in the Latin, associating to the form of the *L. siceraria* fruit. The species name '*siceraria'* refers to its dry (*siccus*) mature fruit used by people throughout the world as containers, bowl, music instrument, decorative purposes or in some cases, fishing floats. It is commonly known as the 'bottle gourd' due to its bottle like shape (Decker-Walters et al. 2001; 2004; Erickson et al. 2005). Bottle gourd has a wide distribution in both hemispheres. Remains of *L. siceraria* were found in Egyptian tombs dated about 3,000-3,500 B.C. Similarly bottle gourd remains were found in Spirit caves of Thailand (10,000-6,000 B.C.), in Mexico (7,000-5,000), in Peru (4,000-3,000) and in China (500 A.D.). Archaeological finding predicts a time depth of at least 12,000 years for *Lagenaria* remains, both in new and old worlds. A theory of trans-oceanic drift of bottle gourd has been suggested to explain pre-Colombian distribution in tropical America. It was suggest that *Lagenaria* had a pan-tropic distribution and was independently domesticated in both old and new worlds (Bose and Som 1986), while Whitaker (1971) reverified his hypothesis that bottle gourd was indigenous to tropical Africa (south of Equator) and has been distributed by trans-Oceanic drift or human transport to other parts of the world. *L. siceraria* mature dry fruits can float on seas for long time without losing seed viability (Decker-Walters et al. 2004).

The fruits are generally eaten as a vegetable in Africa and Asia. Immature fruits are eaten by boiling, frying or stuffing like fruit of *Cucurbita pepo*. Shoots, tendrils and leaves are also cooked and the seeds are removed for oil extraction or for use in cooking. Seeds, tendril and young leaves are also used for some medical purposes (Herklots 1972; Moerman 1998; Manandhar 2002). *L. siceraria* fruit has been traditionally used for its cardioprotective, cardiotonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion string. It cures pain, ulcers, fever and it was used for pectoral cough, asthma and other bronchial disorders-especially syrup prepared from the tender fruits (Nadkami 1992; Duke 1985).

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Modern pharmacological investigation showed that bottle gourd fruits extracts has various beneficial effects on lowering cholesterol, triglyceride and low density lipoproteins in rats (Ghule at al. 2006a), pain and inflammation in mice (Ghule at al. 2006b), free radicals and oxidation (antioxidative effect) (Deshpane et al. 2007) and liver damage during chemotherapy (Fard et al. 2008). Furthermore, *L. sciceraria* is used as rootstocks for watermelon against various biotic and abiotic stress factors. Grafting of watermelon onto bottle gourd was first performed in Korea and Japan in the late 1920s (Ashita 1927) and has been practiced in Eastern Asia for many years, and in recent years there has been an increased interest in Europe and in the USA in adopting this practice as one of the alternatives for methyl bromide fumigation for the control of Fusarium wilt (Miguel et al. 2004; Cohen et al. 2007). *L. siceraria* shows high compatibility rate with watermelon as rootstock (Lee 1994; Oda 1995; Yetisir and Sari 2003).

Bottle gourd can grow in a wide range of soil types, including alluvial sandy soils along river banks, red silt, clay loam soils and rocky soils. It is more tolerant to high water table (Yetisir et al. 2006) and to salt than watermelon (Colla et al. 2005, Yetisir and Uygur 2010) and Fusarium wilt (Yetisir et al. 2003). The ability of *Lagenaria* to survive in different soils types and its resistance to soil-borne diseases makes it desirable rootstocks for different cucurbit crops susceptible to soil pathogens. In a recent study (Ling and Levi 2007), a number of plant introductions (PIs) of *L. siceraria* were resistant to zucchini yellow mosaic virus (ZYMV), and were moderately resistant to powdery mildew (Kousik et al. 2008). Edelstein et al (2000) reported that *Lagenaria* rootstocks may confer resistance to the carmine spider mite (*Tetranychus cinnabarinus*) in the grafted scion of *Cucmis melo* cv. Brava. In addition, *L. siceraria* rootstocks appeared to enhance lycopene content in watermelon fruits of grafted vines (Perkins-Veazie et al. 2007). For these reasons, there is an increased interest by researchers and plant breeders in exploring the utility of *L. siceraria* germplasm for improving watermelon yield and quality. Recently, several studies on collection and evaluation of *L. siceraria* germplasm have been conducted in United State of America (Decker-Walters et al. 2001; Decker-Walters et al. 2004; Levi et al. 2009), Kenya (Morimoto et al. 2005; Morimoto et al. 2006), Serbia (Mladenovic et al. 2012) and Turkey (Yetisir et al. 2007; Yetisir et al. 2008; Özarslandan et al. 2011; Karaca et al. 2012).

STUDIES ON *Legenaria siceraria* IN TURKEY

Turkey is not center of genetic diversity for *L. siceraria* but the landraces of *L. siceraria* show great diversity, particularly in fruit size and shape (Fig. 3) due to suitable soil and climatic conditions of Turkey for bottle gourd. In most of small cities and villages of Turkey, a number of different landraces had been commonly grown as vegetable, music instrument, decorative and containers, corresponding to

characteristic of the fruit, before equipment and tools made from plastic were used intensively by people. *L. siceraria* is not a commercial grown species in Turkey. It was possible to see bottle gourd genotypes in many part of Turkey about 25 years ago but genotypes have gradually disappeared. It is difficult to acquire genetic diversity presented 25 years ago in Anatolia.

Studies on *L. siceraria* in Turkey were started about 15 years ago with a grafting experiment. In the experiment, one Turkish bottle gourd landrace was used as rootstocks for watermelon to compare with commercial rootstocks for Fusarium wilt resistance, plant growth, fruit yield and quality. This landrace showed promising results for investigated parameters (Yetisir and Sari 2003; Yetisir et al. 2003; Yetisir and Sari 2010). This promising study leaded to other studies. Production of haploid bottle gourds via irradiated pollen techniques was investigated. Four landraces and four different irradiation doses were used to induce haploid embryo. According to preliminary results, induction of haploid embryo by pollination with irradiated pollen is possible in *L. siceraria*. It was found that a suitable irradiation dose in bottle gourd was 100 Gy (Unpublished data).

Researchers were encouraged by studies mentioned above and a study to collect bottle gourd genotypes in Mediterranean region of Turkey was carried out from 2003-2007. In this project, 182 bottle gourd genotypes were collected from southern part of Turkey and their potential watermelon rootstock in terms of Fusarium wilt resistance, graft compatibility and plant growth was investigated (72 genotypes). The highest graft compatibility was 99% while the lowest graft compatibility was 70%. All the grafted plants showed better performance than control plants for plant growth. All bottle gourd genotypes were tested against known 3 races of FON and all of them showed resistance (Yetisir et al. 2007).

Turkish *L. siceraria* genotypes collected by Yetisir et al. (2007) were morphologically characterized following the international standards for crop descriptors set by Biodiversity International. The descriptive statistics revealed that the whole collection exhibits a great deal of morphological diversity and a subset core collection represents most of the variability. Among the studied accessions, no apparently distinct patterns such as geographical origin were detected. This may suggest that the accessions have been introduced to Turkey from multiple locations and/or their diversity had been distributed almost evenly across the Mediterranean region of this country. Based on our results from the morphological characterization, 30 genotypes were selected to develop a subgroup (core) collection in order to represent most of the genetic diversity of all accessions collected from southern part of the Turkey (Yetisir et al. 2008).

Yetisir and Uygur (2009 and 2010) studied rootstocks potential of different gourd for watermelon under salinity stress. They used genotypes belong to *Cucurbita*, *Lagenaria*, *Benincasa* and *Luffa* genera and genotypes belong to *Cucurbita* and

Lagenaria genera showed better performances than ungrafted watermelon for plant growth and ion regulation under salt stress. Two Turkish bottle gourd landraces used presented similar plant growth and ion regulation performances of commercial rootstocks.

Özarslandan et al (2011) studied resistance of 57 bottle gourd landraces collected from Mediterranean region of Turkey against *Meloidogyne incognita* (Kofoid&White) Chitwood and *Meloidogyne javanica* (Treub) Chitwood. It was found that all bottle gourds genotypes were susceptible to root-knot nematodes. Watermelon plants grafted onto bottle gourd rootstocks showed better plant growth and produced higher yield than ungrafted watermelon plants in field contaminated with root-knot nematodes. It was concluded that bottle gourds rootstocks were not directly resistant to nematodes but they can tolerate nematodes with their rapid root growing ability at low soil temperature when nematodes are problem in the soil.

Previous studies on rootstocks of the Turkish bottle gourds produced limited information about early developing period of the grafted watermelon. Therefore, Karaca et al (2012) studied rootstock potential of subset of the Turkish bottle gourd germplasm (21 genotypes selected based on results of Yetisir et al. 2008) as regarded to plant growth, fruit yield and quality in 2008-2009. The Crimson Tide watermelon cultivar was used as a scion and 2 commercial rootstocks (*L. siceraria*) were also used for comparison. The survival rates of the grafted plants varied from 83% to 100%. Survival rates of those with commercial rootstocks and local rootstocks were similar. Plants grafted onto 20-02, 31-09, 31-43, 35-01, and 46-03 were more vigorous than ungrafted control plants. Compared to the control plants, grafted plants had 37% to 80% higher plant dry weight. All of the grafted plants except CT/Macis produced a higher yield than the control plants. The plants grafted onto 6 local rootstocks had significantly greater total yields than those grafted onto the commercial rootstocks. Among the local bottle gourd landraces tested, 01-16, 07-45, 20-06, 31-09, 31-15, and 46-03 were found to be promising genotypes for total yield. Early yield was not significantly affected by rootstock. The quality parameters of the fruits harvested from the grafted and control plants were similar except for a limited number of graft combinations. It was concluded that germplasm from Turkish bottle gourds has a high rootstock potential for watermelon with regard to the investigated parameters.

Currently, a project started in 2011 for collection of all Turkish bottle gourd germplasm is being conducted by Yetisir and his co-workers. In this project, 200 new genotypes, in addition to previously collected 182 bottle gourd accessions, were collected from different part of the country other than Mediterranean region. Thirty eight bottle gourd accessions from USA (24), India (8) and Russia (6) were also included to the collection for genetically and morphologically comparison. All accessions were planted for morphological characterization and total DNA

was extracted from all genotypes for genetic characterization by DNA markers. When the project is completed at the end of the 2013, core collection representing Turkish bottle gourd germplasm will be established. Recently some studies of *L. siceraria* as cucumber rootstocks have been conducted and promising results were reported. More research on rootstock potential of Turkish bottle gourd germplasm under different biotic and abiotic stress conditions should be conducted. Breeding programs for rootstock and bottle gourd varieties as vegetables should be carried out.

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Fig. 1. Flowers of bottle gourd (*L. siceraria* (Mol) standley).

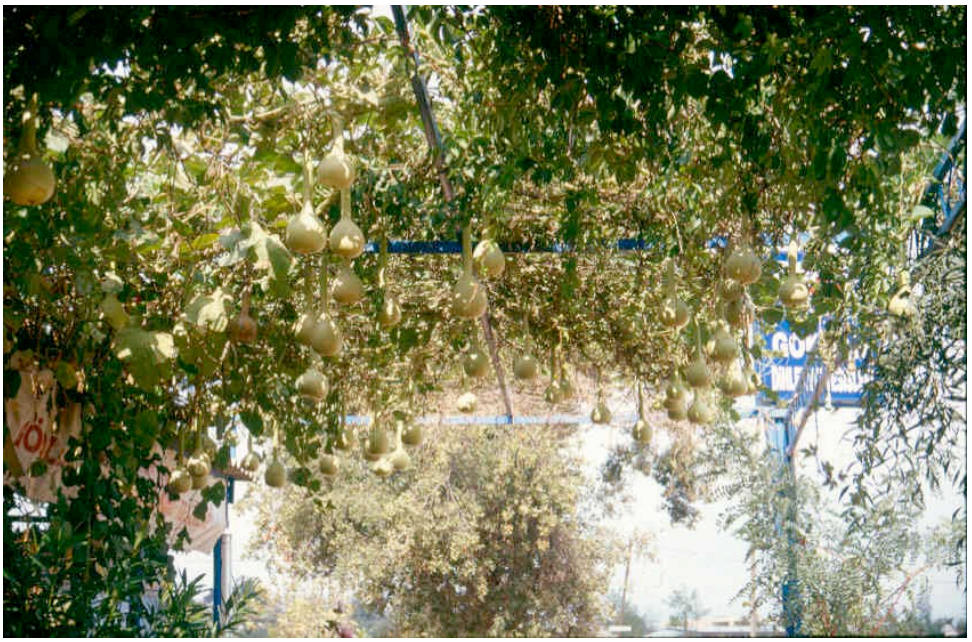

Fig. 2. Bottle gourd plant with fruits on trellis.

Fig. 3. A set of Turkish bottle gourds.

Marker aided detection of seedling stage purity in bottle gourd hybrids

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Keywords: *Lagenaria siceraria*, pedate-leaf, narrow-petals, DUS-test

Abstract

Bottle gourd (*Lagenaria siceraria*) is a highly amenable crop for heterosis breeding. Marker aided detection of seedling stage purity in its F_1 plants strengthens the quality of the hybrids. Such a distinct 'pedate leaf shape' monogenic marker with a proposed designated gene *'P'* having incomplete dominance over 'normal leaf shape' has been detected in an indigenous land race (now a cultivar) 'Narendra Shishir'. The F_1 seedlings between pedate leaf shape and normal leaf shape parents can easily be distinguished from either of the parents at the third to fourth true leaf stage. Consequently, the undesirable plants appearing due to chance selfing of the parents or otherwise can be rogued out from the production plots of the F_1 hybrid. The beauty of this genetic marker is that either of the pedate leaf or normal leaf parents can be chosen as the male or female parent, since F_1 plants are distinctly intermediate to both types of parents. The 'pedate leaf shape' gene *'P'* is tightly linked with another monogenic trait, 'small narrow petals' with a proposed designated gene, *'S^P'*. The 'small narrow petal' trait has shown near incomplete dominance over the large broad petal of normal leaf bottle gourd genotypes. With a little work experience,- with F_1 petal size, the left over undesirable plants can also be rogued out at the flowering stage. Both 'pedate leaf shape' and 'small narrow petal' traits were found to be inherited together, independently from fruit shape characters; therefore, they can be transferred to various fruit shape backgrounds as distinctive feature for the DUS test.

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] is a widely grown crop of tropical, sub-tropical and frost- free temperate regions over the world. With an exceptional andromonoecious genotype 'Andromon-6' (Singh *et al*. 1996),

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bottle gourd is monoecious. The crop is highly amenable for heterosis breeding. Marker aided detection of seedling stage purity in F_1 plants improves the quality of the hybrids. Early stage useful leaf markers are reported in watermelon (Mohr 1953), muskmelon (Djutin 1967) and other cucurbits. Leaf colour markers are also reported in winter squash (Scott and Riner 1946), summer squash (Scarchuk 1954) and pumpkin (Coyne, 1970). No such distinct seedling stage stable genetic markers are reported in bottle gourd. Ram *et al*. (1997), in a cross combination of PBOG-54 - a segmented leaf genotype of bottle gourd with a relatively large corolla and PBOG-22- a normal leaf shape genotype, recorded dominance of normal leaf shape over segmented leaf shape.

In the year 1995, the first author of the article collected an indigenous round fruited bottle gourd genotype endowed with peculiar 'pedate -leaf shape'(Fig. 1b) and distinct 'small narrow -petals' in its flowers (Fig. 1c). The genotype was named NDBG-202 and kept under critical observations. After purification and evaluation it was released as the winter type cultivar 'Narendra Shishir'. The leaf shape and petal size of common bottle gourd genotypes are here referred to as 'normal leaf shape' (Fig. 2b) and 'large broad -petals' (Fig. 2c), respectively. The objectives of the present study was to work out the genetics of (i) 'pedate- leaf shape' vs 'normal leaf shape' and (ii) 'small narrow –petals' vs 'large broad –petals', and the utility of these genetic markers in purity detection of F_1 plants and also as stable distinctive traits for the DUS test.

MATERIALS AND METHODS

The experiments were conducted at the Narendra Deva Univ. of Agri. & Techn., Kumarganj, Faizabad (India), which has a humid sub-tropical climate. The parent 'Narendra Shishir' was homozygous for 'pedate leaf shape' (Fig. 1b), 'small narrow- petals'(Tab. 1 & Fig. 1c) and round fruit shape. The other parent, 'Narendra Rashmi' was homozygous for 'normal leaf shape' (with shallow lobes) (Fig. 2b), 'large broad -petals' (Tab. 1 & Fig. 2c), and long fruit shape. However, the segregation data for fruit shape have not been considered in the present study. The two parents were crossed in spring 2005 to produce F_1 seeds. A few F_1 plants along with parental lines were raised in spring 2006. The F_1 plants produced 'intermediate pedate leaves' and near 'small narrow- petals' highly inclined towards Narendra Shishir. Four F_1 plants were selfed and another four were backcrossed by Narendra Rashmi to obtain F_2 and BC_1 seeds, respectively.

In spring 2007, 400 F_2 seeds from one fruit, 400 BC_1 seeds also from one fruit, and 25 seeds each of the parents and F_1 were separately sown in 3 m long rows at spacing of 3 m x 50 cm. Care was taken to grow a maximum number of healthy plants. A total of 370 F_2 plants and 382 BC_1 plants and 16 each of the parents and F_1 plants were retained till flowering and later stages. Observations of leaf

shape were recorded from the seedling emergence stage. Petal size was observed at the flowering stage both in staminate and pistillate flowers. The chi-square test was used to test the goodness of fit of F_2 and BC_1 segregation data. The relevant background results recorded over the years have also been utilized to make the results more fruitful.

RESULTS AND DISCUSSION

In bottle gourd genotypes like Narendra Rashmi, having normal leaves, the first few leaves (generally 20 -30) are non-lobed (Fig 2a). Lobing begins only in later stages of plant growth (Fig. 2b). In Narendra Shishir- the genotype with pedate leaves, the deep lobing may begin either in the first (Fig. 1a) or in the second true leaf stage (Fig. 4), which is dependent on the temperature prevailing at the time of planting. When sown at above 25 ^{O}C average day-night temperatures, the first true leaf is acutely lobed (Fig. 1a) and when sowing is done at a temperature of below 25 ^{O}C on the average, the first true leaf had no lobes and the second true leaf was acutely lobed (Fig. 4). However, the second true leaf is inevitably deeply lobed (Fig. 1a) in all seasons of plantings. The leaves from the third to the fourth true leaf and later stages acquire sharpened true pedate structure (Fig. 1a & b) in all temperature conditions of plant growth. The F_1 plants between pedate leaf shape and normal leaf shape may produce mildly lobed second true leaves (Fig. 3a), but the third, fourth and later true leaves are 'intermediate pedate' (Fig. 3a & b), and are clearly distinct from the pedate leaf parent (Fig. 1a & b) and the normal leaf parent (Fig. 2a & b). A gene *'P'* is being proposed to designate 'pedate leaf shape' and a counterpart *'p'* for normal leaf shape in bottle gourd. The F_2 plants segregated in close agreement with 1 Pedate (*PP*) : 2 Intermediate pedate (*Pp*) : 1 Normal (*pp*) leaf shape, whereas BC_1 plants segregated into the expected ratio of 1 Intermediate pedate (*Pp*) : 1 Normal (*pp*) leaf shape (Tab. 2), which clearly indicated monogenic incomplete dominance of pedate leaf shape over normal leaf shape.

The 'small narrow petals' of both staminate and pistillate flowers (Fig. 1c) are being reported for the first time in bottle gourd. Segmented leaf genotype PBOG-54 by Ram et al. (1997) had a relatively larger corolla. It is remarkable to note that in pedate leaf genotypes the narrow petals of staminate flowers were smaller in size as compared to pistillate flower petals (Tab. 1and Fig. 1c). This is in clear contrast with the normal genotypes, where staminate flower petals are always larger than pistillate flower petals (Tab. 1& Fig. 2c). The comparison of the parents' petal size (Fig. 1c & 2c) with that of the F_1 plants (Fig. 3c) revealed that 'small narrow petals' also had incomplete dominance over 'large broad petals', and were more inclined towards small narrow-petals. However, it was important to note that staminate flower petals in F_1 plants were larger in size as compared to pistillate flower petals, as in normal genotypes. The gene 'S^P' is being proposed to designate 'small narrow petals' and the

counterpart 's^p' - 'large broad petals'. The F_2 population segregated in accordance with the ratio of 1 Small narrow petals (S^PS^P) : 2 Intermediate narrow petals(S^P-) : 1 Large broad petals (s^ps^p) and the BC_1 generation segregated in agreement with 1 Intermediate narrow petals (S^P-) : 1 Large broad petals (s^ps^p) (Tab. 2). Thus F_2 and BC_1 data indicated monogenic incomplete dominance of small narrow petals over large broad petals. Here it may clearly be stated that although intermediate narrow petals of F_1 plants were more inclined towards small narrow petals of Narendra Shishir, they were distinct in shape and size (Fig. 3c) from both the parents (Fig. 1 c & 2 c). With a little experience field workers can easily distinguish petal shape and size in F_1 plants from that of the narrow petal and broad petal parents and can rogue out the undesirable plants from the field. It may be noted that 'pedate leaf shape' was tightly linked with 'small narrow –petals' and 'normal leaf shape' was tightly linked with 'large broad petals'. It was recorded that the 'pedate leaf shape' and 'small narrow -petal' traits were found to be inherited together, independently from fruit shape character, the data of which not presented here due to lack of space. Hence, both of these traits, i.e., pedate leaf shape and small narrow petals can be transferred together to various fruit shape backgrounds, as distinctive features for the DUS test.

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Table 1. Leaf shape and dimension as well as petal size and dimension of the parents.

Parents	Leaf shape	Leaf dimension		Petal size	Flower petal dimension			
					Staminate		Pistillate	
		L	B		L	B	L	B
Narendra Shishir	Pedate	29	32	Narrow	4.3	1.4	5.0	1.7
Narendra Rashmi	Normal	28	31	Broad	4.9	3.7	4.6	2 .8

L stands for Length and B stands for Breadth

Table 2. Segregation patterns of leaf shape and petal size in F_2 and BC_1 generations.

Specific observations and ratios recorded in F_2 and BC_1 generations	F_2	BC_1
Total number of plants maintained	370	382
Plants with pedate -leaf and small narrow-petals (a)	101	-
Plants with intermediate pedate -leaf and intermediate narrow-petals	172	198
Plants with normal leaf and large broad-petals	97	184
Observed ratio in F_2 = 1.04 (a) : 1.77 (b) : 1.00 (c)	-	-
Observed ratio in BC_1= 1.08 (b) : 1.00 (c)	-	-
Chi-square value	1.91	0.52
Probability	0.3-0.5	0.3-0.5

Fig.1. Pedate leaf (a, b) and small narrow petals (c) in Narendra Shishir (P1)

Fig. 2. Normal leaf (a, b) and large broad petals (c) in Narendra Rashmi (P2)

Fig.3. Intermediate pedate leaf (a, b) and narrow petals (c) in F1 (P1 x P2)

Fig.4. Narendra Shishir sown in low temperature conditions showing normal non lobed first true leaf

Morphological characterization of *Cucurbita pepo* L. germplasm collection

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Keywords: *Cucurbita pepo* L., seed coat anatomy, scanning electron microscopy, morphological characterization, germplasm collection

Abstract

Cucurbita pepo L. is the economically most important cultivated species of the genus *Cucurbita*. It is grown worldwide and therefore characterized by high polymorphism of plant and fruit characteristics. Fruits are used as vegetables for human consumption or as livestock fodder whiles the seeds are processed to extract oil. Production of pumpkin seed oil was broaden and intensified by the discovery of a mutation causing development of seeds without a seed coat. The mutant phenotype segregated from normal field pumpkin in the end of the 19th century and was later bred to produce numerous cultivars of oil pumpkin known today. The aim of our work was to collect available seeds with mutated seed coats in order to analyse the anatomy of different seed coats and to describe plant, fruit and seed characteristics. Seeds of 65 accessions (cultivars or landraces) were collected and their seeds were categorized in groups based on the visual appearance of their seed coat. The anatomy of different seed coats was further analysed using scanning electron microscopy of dried ripe seeds. Our results confirmed features regarding *C. pepo* seed coat anatomy already published and additionally supplemented them with 3D images of the seed coat and its layers. Furthermore, a new phenotype of white seeds without any seed coat tissue layers was discovered and described. Seeds of some accessions were planted in the field and their morphological traits were characterized using the ECPGR descriptors for *Cucurbita* spp. (2008). The accessions differed considerably in fruit and seed characteristics while almost all showed a prostrate plant growth habit.

INTRODUCTION

The mutated seed coat phenotype has been the topic of numerous studies due to its high value for pumpkin seed oil production (Singh and Dathan 1972; Lott

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1973; Stuart and Loy 1983; Teppner 2000, 2004; Bezold et al. 2003; Zraidi et al. 2003). The seed coat anatomy was studied by light and fluorescent microscopy while the seed coat traits have also been mapped on a *C. pepo* gene map (Gong et al. 2008). The mature seed coat of wild type seeds is differentiated into five clearly distinguishable zones (Singh and Dathan, 1972): seed epidermis, hypodermis, sclerenchyma, aerenchyma, chlorenchyma and (on some seeds) the remnants of placental epidermis can also be present.

The aim of our work was to collect cultivars and landraces with mutated seed coats in order to describe plant, fruit and seed characteristics and to study the structure of different seed coat types with scanning electron microscopy.

MATERIALS AND METHODS

A total of 65 cultivars and landraces were collected from the seed companies Semenarna Ljubljana (Slovenia) and Saatzucht Gleisdorf (Austria); from the Institute of Special Crops, Agricultural Research Center Styria, Austria; from Arche Noah Association, Austria; from Crop Research Institute, Czech Republic; from Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Italy; from USDA genebank, USA and from the breeder Anton Ivančič (Slovenia). Seeds were visually examined and classified in categories based on external seed coat characteristics. Seeds representing a range of seed coat types were selected, transversely sectioned in the middle of the seed with razor blades and mounted to specimen stubs with Dotite silver colloid paste. Air dried seed specimens were sputter coated with platinum using a Bal-Tec (Leica) SCD 050 sputter coater and examined using a FE scanning electron microscope JEOL JSM 7500F operating at 3.0 kV. Images were analyzed using LUCIA Cytogenetics 2 (Laboratory Imaging, s.r.o., Prague, Czech Republic) software.

Seeds of some cultivars or landraces were planted in the field and plant, fruit and seed characteristics were evaluated using the ECPGR descriptors for *Cucurbita* spp. (2008): plant growth habit, peduncle transectional shape, fruit shape, fruit ribs, predominant and secondary fruit skin colour at maturity, secondary fruit skin colour pattern, fruit skin texture, fruit weight, flesh thickness, flesh colour and flesh texture.

RESULTS AND DISCUSSION

The examined seeds were characterized in 12 different classes based on the visual appearance of their seed coats as described in Murovec et al. (2012). The external morphology of the seeds ranged from thick white coloured ‘Wild type’ seeds through several levels of testa thickening to ‘White hull-less type’ of seeds without any testa layer. The seed coats varied among accessions and variability was also noted within some accessions. The results of the analysis of seed coat structures with scanning electron microscopy were mostly in accordance with

previous reports on *C. pepo* seed coat anatomy and complement these results with a three dimensional view of the seed coat and its layers, as shown in Fig. 1.

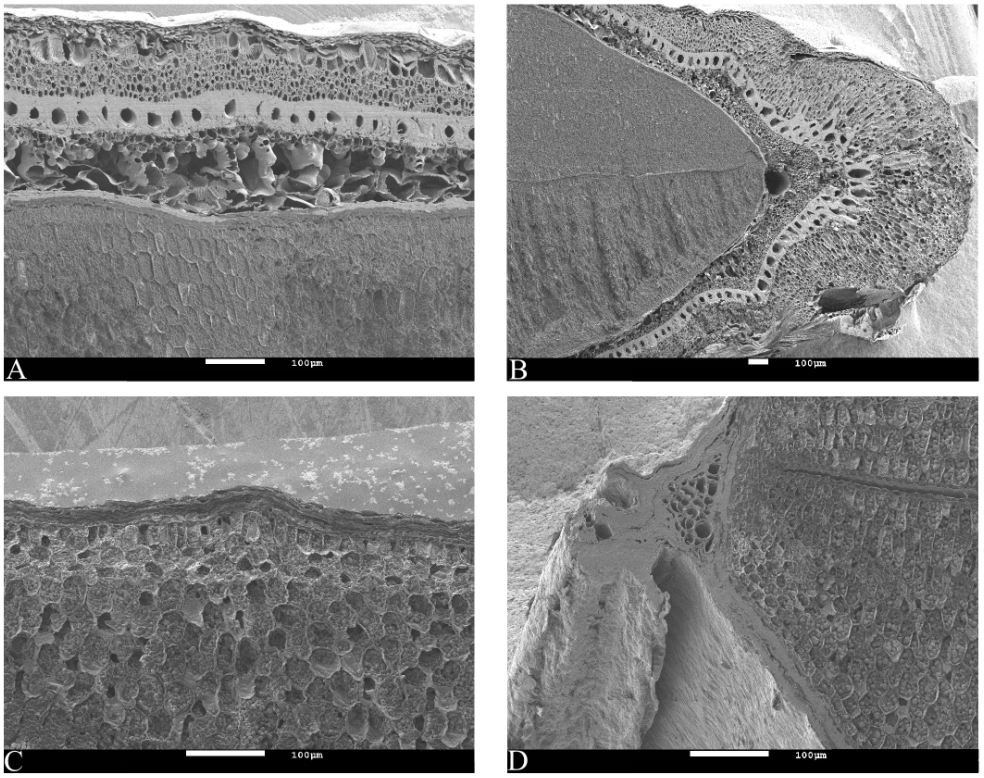

Fig. 1. Scanning electron micrographs of *Cucurbita pepo* 'Wild type'(A, B) and 'Thin-coated' seeds (C,D): at the main seed surface ('Wild type' in A, 'Thin-coated' in C) and on seed margins ('Wild type' in B, 'Thin-coated' in D).

The accessions differentiated considerably in fruit and seed characteristics while almost all showed a prostrate plant growth habit.

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Genetic variation of the leaf laminae of *Cucurbita pepo*

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Keywords: cultivar-groups, gourd, pumpkin, squash, morphotypes, leaf morphology

Abstract

A set of 49 accessions of *Cucurbita pepo*, which included representatives of the eight edible-fruited cultivar-groups (fruit-shape morphotypes) and ornamental and wild gourds, was observed in the field at Olomouc, Czech Republic. The goal was to characterize and compare the leaf laminae among accessions. Most accessions of *C. pepo* subsp. *pepo* but none of *C. pepo* subsp. *texana* and *C. pepo* subsp. *fraterna* had silver mottling. Almost all accessions had deep, narrow basal sinuses, but the basal sinuses of advanced cultivars of the Zucchini and Cocozelle Groups differed in shape from the others. Acute shape of the lamina apex was frequent in subsp. *texana*, but obtuse or rounded was prevailing in subsp. *pepo*. Leaves were less spiculately harsh in the zucchini and acorn accessions and also in an accession of wild subsp. *texana* and subsp. *fraterna*. Overall, subsp. *texana* was more homogeneous for leaf lamina traits than subsp. *pepo*.

INTRODUCTION

Cucurbita pepo L. is a highly polymorphic cucurbit native to North America that was domesticated independently at least twice (Nee 1990). One domesticated lineage, *C. pepo* subsp. *texana* (Scheele) Filov, derives from the United States. Another, *C. pepo* subsp. *pepo*, derives from Mexico. Wild plants of a third lineage without any known cultigens, *C. pepo* subsp. *fraterna*, occur in northeastern Mexico. The fruits of wild *C. pepo* are small gourds, several cm in diameter, spherical, oblate, ovate, or pyriform, usually green-striped, with a lignified rind and thin, fibrous, light-colored flesh enclosing as many as several hundred, flat oval seeds.

Edible-fruited domesticates, the squash and pumpkins, have fruits that are larger than those of their wild counterparts, with a wide variety of shapes and colors (Paris et al. 2012). On the basis of the fruit shape, the edible-fruited cultigens are

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classified into eight cultivar-groups or morphotypes (Paris 1986). Analyses of DNA sequence polymorphisms have indicated that the Cocozelle, Pumpkin, Vegetable Marrow and Zucchini Groups belong within *C. pepo* subsp. *pepo* while the Acorn, Crookneck, Scallop and Straightneck Groups belong within *C. pepo* subsp. *texana* (Paris et al. 2003). Of the cultivated ornamental "fancy" gourds, some belong within the former and others within the latter subspecies.

The polymorphism of *Cucurbita pepo* observed today is the result of thousands years of domestication and cultivation in different regions and from intensive breeding. There is considerable comparative evidence for the parallel, independent evolution under domestication of the two cultivated subspecies of *C. pepo*. The common interest in the selection and breeding has been the improvement of fruit traits. However, there are some vegetative traits that differentiate between the two subspecies (Paris et al. 2012).

The leaves of *Cucurbita* are simple but can be strongly lobed, the prominence of the lobing varying among species and cultivars (Whitaker and Davis 1962). They also show developmental changes, for example, although the first few leaves of *C. pepo* tend to be three-lobed, leaves of mature plants are often 5-lobed. Several genes affecting leaf shape, harshness, and color have been identified (Paris and Brown 2005).

From a large collection of *Cucurbita pepo*, 49 accessions were selected for study of phenotypic variation. These accessions include representatives of the edible-fruited cultivar-groups as well as some gourds. Results pertaining to some vegetative and reproductive traits have already been described (Lebeda et al. 2010; Paris et al. 2012). Presently, we will focus on variation of the leaf laminae exhibited by these accessions.

MATERIALS AND METHODS

The 49 accessions of *Cucurbita pepo* (Table 1) are derived from the germplasm collection of the Agricultural Research Organization, Newe Ya'ar Research Center, Ramat Yishay, Israel (Paris, 2001). Seeds of these accessions were sown in the field at the campus of Palacký University, Olomouc – Holice, Czech Republic, in early May of 2006, 2007, and 2008. The seeds were sown in "hills" (groups) 0.5 m apart in rows 6 m apart, but between two neighbouring accessions within-row spacing was 1 m. The resulting seedlings were thinned to two per hill. Each accession was represented by ten plants (5 hills) in a single block; the blocks were not replicated. The plants were grown using standard cultivation practices except that no chemical protection was used.

At time of fruit set, five fully developed leaves per accession were taken from the middle part of the main stems and assessed for 10 morphological traits of the leaf laminae: colour, glossiness, density and colour of mottling, quality of

trichomes, shape of the apex of terminal lobe, presence of awn on the apex, shape of lamina base, number of leaf lobes and depth of incisions between the terminal and neighbouring lateral lobes. Phenotypic expression of traits was expressed by numbers. Herbarized leaves are kept by the Department of Botany of Palacký University in Olomouc, Czech Republic.

RESULTS AND DISCUSSION

The leaf laminae showed marked variation in color, glossiness, harshness, and shape among accessions (Table 2). Some of the differences observed among the accessions are depicted in Fig. 1- 4.

Silver mottling of the leaf laminae was observed on most cultivars of *C. pepo* subsp. *pepo*, including representatives of all of its morphotypes, but on none of the accessions of *C. pepo* subsp. *texana* and *C. pepo* subsp. *fraterna* (Table 2). The mottling occurred over covered much of the leaf surface in the accessions of the Zucchini Group. In some accessions, the appearance of the mottling was silvery but in others it was light-green.

Only one accession, U-GU-MNB, had light green leaf laminae (Table 2). Two accessions of *C. pepo* subsp. *texana* and seven of *C. pepo* subsp. *pepo* had medium green leaf laminae. All accessions of the Cocozelle Group and the Zucchini Group (both subsp. *pepo*), and the Straightneck Group (subsp. *texana*), and wild *C. pepo* subsp. *texana* and *C. pepo* subsp. *fraterna* had dark green leaf laminae.

The light green leaf laminae of U-GU-MNB had a matte surface, as did the dark green laminae of P-GP-FLA, T-SC-WBS, and the wild accessions of *C. pepo* subsp. *texana* and *C. pepo* subsp. *fraterna* (Table 2). The laminae of all accessions of the Cocozelle, Zucchini, and Straightneck Groups, with one exception, were glossy.

The 49 accessions exhibited marked variation in the spiculate harshness of the leaves (Table 2). This variation was readily palpable in both subspecies. Relatively harsh leaves prevailed in the Cocozelle Group (*C. pepo* subsp. *pepo*) and the Straightneck Group (*C. pepo* subsp. *texana*), even though these cultivar-groups are considered to be relatively advanced (Paris 2000). Accessions of the Zucchini Group possessed relatively non-harsh, smooth leaves. Accessions of the Acorn Group had less harsh foliage than others of subsp. *texana*. A wild accession of *C. pepo* subsp. *texana*, T-GT-ARK, and the wild subsp. *fraterna* accession, F-GF-WM2, also had less harsh leaves.

The shape of the leaf base and of the basal sinuses was circular or deep and narrow in most accessions (Fig. 1; Table 2). The leaf base was “V” shaped or shallow and open in the Zucchini and most Cocozelle accessions. The Zucchini accessions were also uniform for rounded shape of the apex of the terminal lobe (Fig. 2) and by the absence or reduction of an “awn” (Fig. 3). Rounded apices occurred also within

the other groups of subsp. *pepo* and one of the wild accessions of *C. pepo* subsp. *texana*. The majority of accessions from both *C. pepo* subspecies had intermediate depth of incisions between the terminal and adjacent lateral lobes. The Zucchini accessions and two Cocozelle accessions had very deep incisions and the highest number of leaf lobes (Fig. 4).

Among the 49 *C. pepo* accessions, no two accessions had identical leaf phenotypes. Absence of silver mottling, deep and narrow basal sinuses, acute apex of the terminal lobe, and five lobes are typical traits of the leaf laminae *C. pepo* subsp. *texana. C. pepo* subsp. *pepo* is more heterogeneous for these traits. (Table 2).

Overall, *C. pepo* subsp. *texana* was fairly homogeneous for leaf lamina traits, as was the *C. pepo* subsp. *pepo* Zucchini Group. The other groups and gourds of subsp. *pepo* were more heterogeneous. The accession Cocozelle VNI distinctly differed from all others in shape of the leaf laminae (Table 2). Some differences in the leaf laminae between two wild accessions of the *C. pepo* subsp. *texana* were apparent.

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Table 1. Set of *Cucurbita pepo* accessions used for this study.

Accession designation	Abbreviation[1]	Origin
Black Zucchini	P-ZU-BZU	U.S.A. (Field)
Blanche non-coureuse	P-VM-BNC	France (Gondian)
Citrouille de Touraine	P-PU-CIT	France (Ducrettet)
Cocozelle Tripolis	P-CO-CTR	Germany (Samenzüchter)
Connecticut Field	P-PU-CTF	U.S.A. (Ledden)
Creamy[2]	T-SN-CRE	U.S.A. (Park)
Dagestan	P-PU-DAG	Dagestan (National Institute of Plant Breeding, Russia)
Early Crookneck	T-CN-ECN	U.S.A. (Field)
Early Prolific Straightneck	T-SN-EPS	U.S.A. (Ledden)
Early Summer Crookneck	T-CN-ESC	U.S.A. (Ledden)
Flat[3]	U-GU-FLA	Canada (Stokes)
Fordhook Zucchini	P-ZU-FZU	U.S.A. (Burpee)
Gleisdorfer Ölkürbis	P-PU-GLE	Austria (Saatzücht Gleisdorf)
Golden Bush Scallop	T-SC-GBS	U.S.A. (Dessert)
Little Gem	P-GP-LGM	South Africa (Mayford)
Long Cocozelle	P-CO-LCO	U.S.A. (Ledden)
Lungo Bianco di Sicilia	P-CO-LBS	Italy (S.A.I.S.)
M2546	P-VM-546	Israel (Agric. Res. Org., Plant Intro. Serv., Israel)
Miniature Ball	U-GU-MNB	Canada (Stokes)
Nero di Milano	P-ZU-NER	Italy (C.C.S.A.)
Nonkadi	P-PU-NOK	Uzbekistan (Inst. Veg. Seed Quality, Uzbekistan)
Orange Ball	P-GP-ORB	Canada (Stokes)
Orange Warted	P-GP-OWA	Canada (Stokes)
PI 442309	P-GP-309	Mexico (North Central Plant Intro. Sta., U.S.A.)
PI 442313	P-GP-313	Mexico (North Central Plant Intro. Sta., U.S.A.)
Rolet	P-GP-ROL	South Africa (unknown)
Romanesco	P-CO-ROM	Italy (Semitalia)
Royal Acorn	T-AC-RAC	U.S.A. (Twilley)
Seneca Butterbar[2]	T-SN-SBU	U.S.A. (Gurney)
Shenot´s Crown of Thorns	T-GT-SCT	Canada (Stokes)
Small Sugar	P-PU-SSU	U.S.A. (Ledden)
Striato d´Italia	P-CO-STI	Italy (S.A.I.S.)
Striped Pear	T-GT-SPR	Canada (Stokes)
Sweet Dumpling	T-AC-SWD	U.K. (Thompson & Morgan)
Table Dainty	P-VM-TDA	U.K. (Sutton's)
Table Queen	T-AC-TQE	U.S.A. (Northrup King)
Tender and True	P-PU-TAT	U.K. (Sutton's)
Tondo di Nizza	P-PU-TON	Italy (Ingegnoli)
Tondo Verde Scuro di Piacenza	P-PU-TOS	Italy (C.C.S.A.)
True French	P-ZU-TRF	U.K. (Thompson & Morgan)
Vegetable Spaghetti	P-VM-VSP	Japan (Sakata)
Verte non-coureuse d´Italie	P-CO-VNI	France (Abondance)
Verte Petite d'Alger	P-VM-VPA	France (Abondance)
White Bush Scallop	T-SC-WBS	U.S.A. (Field)
Wild Arkansas	T-GT-ARK	U.S.A. (Univ. Arkansas)
Wild Mexico 2	F-GF-WM2	Mexico (Texas A&M Univ.)
Wild Texas	T-GT-WTX	U.S.A. (Texas A&M Univ.)
Yellow Bush Scallop	T-SC-YBS	U.S.A. (Gurney)
Yellow Summer Crookneck	T-CN-YSC	U.S.A. (Ferry Morse)

[1]Tripartite designation is for subspecies, group, and abbreviated accession name, respectively. For subspecies: F = *fraterna*, P = *pepo*, T = *texana*, U =Uncertain. For Group: AC = Acorn, CN = Crookneck, CO = Cocozelle, GF = *fraterna* Gourd, GO = Ovifera Gourd (cultivated subsp. *texana* gourd), GP = *pepo* Gourd, GT = *texana* Gourd, GU = Uncertain Gourd, PU = Pumpkin, SC = Scallop, SN = Straightneck, VM = Vegetable Marrow, ZU = Zucchini.

[2]Hybrid cultivar; [3]Derived by self-pollinating a single plant from the mixture 'Small Warted Blend'

Table 2. Phenotypes of leaves of 49 *Cucurbita pepo* accessions.

Accession abbreviation	Lamina colour and surface					Lamina shape				
	Spots[1]	Spot colour[2]	Colour[3]	Gloss[4]	Harsh-ness[5]	Base[6]	Awn[7]	Apex[8]	Inci-sions[9]	Lobes[10]
U-GU-MNB	1	2	1	0	2	4	0	1	3	5
U-GU-FLA	1	1	3	0	0	3	1	1,2	3,5	5
P-GP-ORB	0	0	3	1	2	3	1	1,2	7	5
P-GP-LGM	1	1	2	3	3	3	0	2,3	5	5
P-GP-OWA	1	1	2	2	3	4	0	3	3	5
P-GP-ROL	1	2	3	2	3	3	1	1	5	5
P-PU-309	1	1	3	1	3	3	0	3	7	5
P-PU-313	0	0	3	1	3	3,4	0,1	2,3	3,5	5
P-PU-CIT	1	2	2	2	3	3	1	2-3	7	5
P-PU-NOK	0	0	2	1	2	4	1	2	3,5	5
P-PU-DAG	1	1,2	2	2	2	4,5	1*	2	5	5
P-PU-GLE	0	0	3	1	3	2,3	0	2,3	7,9	5,3
P-PU-TAT	1	1	3	1	2	3	0	2	3	7*
P-PU-TOS	0	0	3	1	1	4	1*	2	5	7*
P-PU-TON	3	1	3	1	2	4	nd	3	5	5
P-PU-CTF	1	1	2	1	3	3	1*	2	7,9	5,7
P-PU-SSU	0	0	3	1	1	4	nd	2	< 3	5*
P-VM-546	0	0	3	2	2	2,3,4	0	1,2	5,7	5
P-VM-VSP	0	0	3	1	1	4	1*	3	5	3
P-VM-TDA	1	2	3	1	3	4	0	3	< 3	5*
P-VM-BNC	1	1	2	2	3	3	1*	3	3,5	5
P-VM-VPA	2	2	3	1	2	4	0	3	3	5*
P-CO-ROM	0	0	3	2	3	2	0	2,3	5-7	5
P-CO-LBS	0	0	3	2	3	1,4	0	2,3	5,7	5
P-CO-CTR	2	1	3	2	2	3	1*	2	9	7
P-CO-LCO	1	1	3	3	3	1,3	0	2,3	9	7
P-CO-VNI	0	0	3	1	1	4	0	3	< 3	0
P-CO-STI	1	1	3	2	2	2	0,1	2,3	9	7
P-ZU-BZU	3	1	3	3	1	1	1*	3	9	7
P-ZU-FZU	3	1	3	2	0	1,3	0	3	9	7
P-ZU-NER	3	1	3	3	1	2	0	3	7	7
P-ZU-TRF	2	1	3	2	1	2	1*	3	9	7
F-GF-WM2	0	0	3	0	0	4	0	1	9	7
T-GT-WTX	0	0	3	0	2	4	0	1	7	5
T-GT-ARK	0	0	3	0	0	4	0	3	3	5
T-GO-SPR	0	0	2	1	1	4	0	1	< 3	5*
T-GO-SCT	0	0	3	1	2	4	1*	1	7	5
T-SC-GBS	0	0	nd	nd	1	4	0	1	5	5
T-SC-WBS	0	0	3	0	1	4	0	1,2	5	5
T-SC-YBS	0	0	3	2	3	3-4	0	1	7	5
T-CN-ECN	0	0	3	3	0	4	1*	1	5	5
T-CN-ESC	0	0	2	3	2	4	1*	1-2	5	5
T-CN-YSC	0	0	3	1	2	4	0	1	3	5
T-AC-RAC	0	0	3	2	1	4	0	2	3	5
T-AC-SWD	0	0	nd	nd	0	4	0	1	5	7
T-AC-TQE	0	0	3	1	0	4	0	1-2	5	5
T-SN-CRE	0	0	3	2	3	2,3	1	1	5	5
T-SN-EPS	0	0	3	3	2	4	1*	1	7	7
T-SN-SBU	0	0	3	2	3	4	1,0	1	5	5

Two or more numbers in one box, separated by comma, indicate different phenotypical expressions of the trait (heterogeneity).

Two numbers separated by - in one box indicate intermediate phenotypic expression of the trait.

nd = trait not determined

[1]Mottled area of leaf lamina: 0 = without spots, 1 = covered on ≈ 1/3 of surface by spots, 2 = covered on ≈ 2/3 of surface by spots, 3 = covered on > 2/3 of surface by spots;
[2]Colour of mottling: 1 = silver, 2 = light-green;
[3]Colour of the upper side of leaf lamina: 1 = light green, 2 = medium green, 3 = dark green;
[4]Glossiness of the upper side of leaf lamina: 0 = matt, 1 = low level of glossiness, 2 = medium glossiness, 3 = high glossiness;
[5]Harshness/quality of trichomes (assessed by palpation and by touching the leaves and petioles by hand and arm): 0 = smooth, 1 = low level of harshness, 2 = medium harshness, 3 = very sharp (traces of trichomes persist on the skin);
[6]Shape of base and basal sinus of the leaf lamina: 1 = „V“ shaped, 2 = shallow and open, 3 = circular, 4 = deep, narrow;
[7]Awn on the apex of terminal lobe: 0 = absent, 1 = present, 1* = reduced length;
[8]Shape of apex of terminal lobe: 1 = subacute, 2 = obtuse, 3 = rounded;
[9]Depth of incisions between terminal lobe and neighbouring lobes: < 3 = almost no incisions, 3 = palmatelylobed (less than 1/3 from leaf lamina margin to the centre of the lamina, 5 = palmatelyfid (to 1/2 from leaf lamina margin to the centre of the lamina), 7 = palmatelypart (to 2/3 from leaf lamina margin to the centre of the lamina), 9 = palmatelysect (more than 2/3 from leaf lamina margin to the centre of the lamina);
[10]Number of leaf lobes: 0 = almost no lobes, 3,5,7,9 - number of lobes, * limited length of lobes.

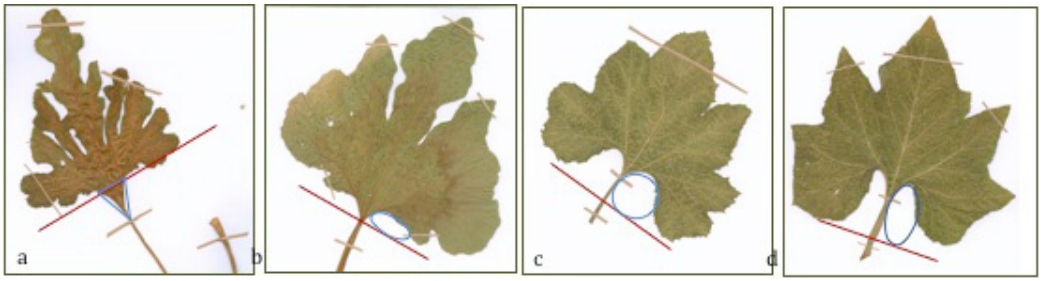

Fig. 1. Shape of base and basal sinus of the leaf lamina: a = „V“ shaped (1) (P-ZU-BZU), b = shallow and open (2) (P-CO-LBS), c = circular (3) P-GP-LGM), d = deep, narrow 4) (T-SC-GBS).

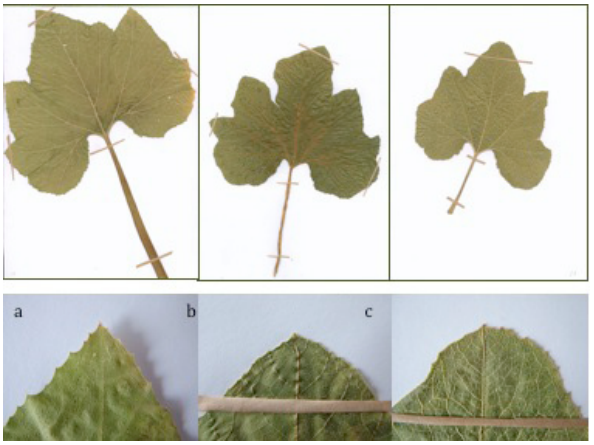

Fig. 2. Shape of apex of terminal lobe: a = subacute (1) (T-SC-YBS), b = obtuse (2) (P-PU-DAG), c = rounded (3) (P-GP-OWA).

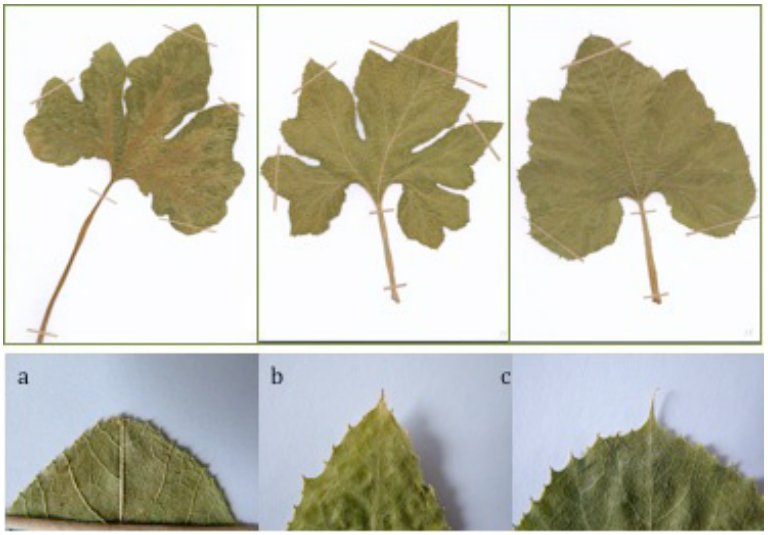

Fig. 3. Awn on the apex of terminal lobe: a = absent (0) (P-PU-309), b = reduced length (1*) (T-GO-SCT), c = present (1) (P-PU-NOK).

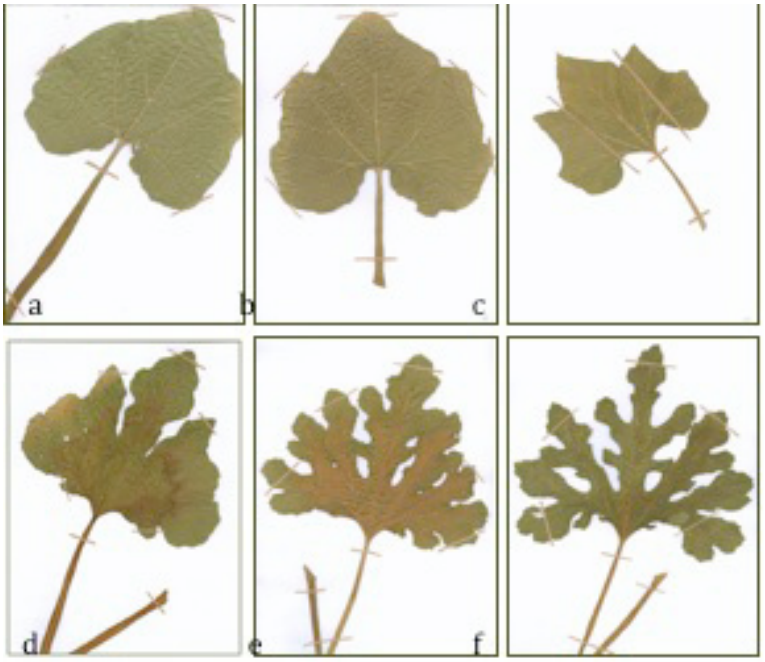

Fig. 4. Depth of incisions between terminal lobe and neighbouring lobes: a,b = almost no incisions (< 3) (P-CO-VNI, P-PU-SSU), c = shallowly incised/palmatelylobed (3) (U-GU-MNB), d = deeply incised/palmatelyfid (5) (P-CO-LBS), e = very deeply incised/palmatelypart (7) (P-ZU-NER), f = very deeply incised/palmatelysect (9) (P-CO-LCO).

Generation and evaluation of a preliminary EMS mutant library in zucchini squash

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Keywords: EMS collections, TILLING, zucchini, M_1 fertility

Abstract

The ultimate objective of this investigation is the construction and analysis of an EMS-mutant zucchini-squash collection. In order to develop a protocol for the treatment of zucchini seeds with the mutagenic chemical ethyl methanesulphonate (EMS), seeds were treated with different concentrations of EMS ranging from 0.3% to 2.0%. The highest concentrations, 1.5% and 2.0%, reduced seed germination and plant viability by 30 to 50%, and affected quite negatively the fertility of the M_1 plants. Pollination stimulated normal fruit growth, but most of the fruits had no seeds. In a second experiment, seeds were treated with 0.3% to 1.0% EMS. The results indicated that germination percentage was not the best indicator for establishing the most appropriate dosage of EMS but rather the fertility of the M_1 plants. We have now established the most appropriate dosage of EMS treatment for generating the greatest number of mutations while reducing fertility as little as possible in the M_1 plants. The resulting library is expected to be useful not only for establishing a TILLING platform for reverse genetic analysis in this important vegetable crop, but also for the identification of new mutant phenotypes that could be of interest in modern breeding programs.

INTRODUCTION

Forward screening of chemically induced mutants has been traditionally used to identify the genes involved in a specific biochemical or developmental process of interest. More recently, chemically induced mutants have become a major resource for reverse genetics studies thanks to the development of TILLING (Targeting Induced Local Lesions IN Genomes) (Colbert et al. 2001), a methodology that enables the identification and selection of single point mutants for a specific gene. This strategy was first applied to an *Arabidopsis* mutant collection induced with ethyl methanesulphonate (EMS) (Till et al. 2003; Greene et al. 2003), but

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afterwards EMS collections for TILLING were developed in a large number of plant species including rice, maize, barley, sorghum, wheat, *Brassica napus*, *B. oleracea*, *Medicago truncatula* and tomato (Menda et al. 2004; Watanabe et al. 2007). EMS mutant populations of melon have been developed in Israel, Spain and France, and the establishment of efficient TILLING resources for reverse genetic studies is in progress (García Mas 2008; Dahmani-Mardas et al. 2010; González et al. 2011). Nevertheless, to our knowledge, there has been no initiative to generate an EMS mutant library in zucchini squash until now, despite the importance of this vegetable crop worldwide. The purpose of the present work was to determine the optimal dosage of EMS for mutagenesis in zucchini squash.

MATERIALS AND METHODS

The experiments were initially carried out with the commercial zucchini hybrid 'Tosca', but later extended to an inbred line designated MUC16, kindly provided by the Seed Bank from the Polytechnic University of Valencia (COMAV). Lots of 200 mature seeds were immersed in bottles containing 200 ml EMS diluted to particular concentrations with deionized water, and placed on a shaker overnight for 12 h at 22ºC in the dark. After the EMS treatment, the seeds were washed twice in 200 ml of 3% sodium thiosulfate buffer for 30 min at room temperature, with gentle shaking, followed by three washes in 200 ml distilled water as above. Control seeds were treated with distilled water in the same manner. Treated seeds were sown in the nursery according to standard zucchini horticultural practice. For each EMS treatment, the lethal dose (LD) was determined by calculating the survival rate of the plants: the number of M_1 seedlings in each EMS treatment was divided by the number of control seedlings. After evaluation of germination rate and LD, the seedlings were transplanted to a greenhouse and grown under standard conditions in Almeria, Spain. Plants were self-pollinated, and at the end of the fruit ripening phase, M_2 seeds were collected from individual M_1 plants and stored. The frequency of fertile M_1 plants was quantified as the percentage of plants producing more than 20 viable seeds per fruit.

RESULTS AND DISCUSSION

The effects of EMS and the efficiency of the mutagenesis treatment were estimated by quantifying three parameters on M1 plants: seed germination, frequency of albino chimeras, and fertility. No significant reduction in the germination rate was observed in any of the applied concentrations of EMS from 0.3% to 1.0% (Table 1; Fig. 1). Most M_1 treated plants exhibited growth retardation as seedlings, but all of them recovered, except for the 1.5% and 2.0 % EMS fraction where the LDs in each EMS treatment were approximately 30 and 50 respectively. The frequency of M_1 albino chimeras increased with concentration (data not shown).

There was an inverse correlation between M_1 plant fertility and mutagen dosage. The number of fruits without viable seeds increased linearly with EMS concentration, whereas the frequency of fertile fruits rapidly decreased (Table 1). In addition, low seed production was observed at 2% EMS, with 80% of the plants producing less than 20 seeds per fruit. At 1.5% EMS, 70% of the plants yielded less than 20 seeds per fruit.

The results indicate that the most appropriate dosage of EMS for the construction of a mutant population in zucchini squash is 0.3% EMS. This dosage did not affect the germination rate of the M_1 plants. Although it reduced fertility of 'Tosca' to 69%, most of the fruits contained more than 100 seeds, thus not compromising by much the production of the M_2 generation. The 0.3% concentration was also the most suitable for EMS mutagenesis in the inbred line MUC16 (Fig. 2). In 0.3% treated seeds of MUC16, no significant reduction in the germination rate was observed, and the fertility was reduced to about 50%.

The EMS treatments in zucchini cause a higher level of seed lethality than in melon. EMS dosages of 1%, 1.5% and 2% were used to prepare mutagenized populations in melon. These dosages resulted in an acceptable level of M_1 seed survival and fertility in M_1 plants, thereby allowing production of large populations of viable M_2 seeds (Dahmani-Mardas et al. 2010; González et al. 2011).

Overall, our results indicate that the percentage of seed germination is not a completely reliable indicator of the optimal EMS dosage. Evaluation of the fertility of the M_1 plants is a necessity. There was an association between EMS dosage and plant fertility, 0.3% being the most favorable for obtaining mutant populations of zucchini squash. By using this concentration, we are now obtaining about 5000 M_2 EMS families for reverse-genetic studies and plant breeding in *C. pepo*. At the moment, we have more than 2500 available families for mutation screening.

ACKNOWLEDGEMENTS

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Table 1. Effects of EMS treatments on germination and fertility of M1 zucchini plants 'Tosca'.

EMS dose	Number of M1 seeds treated	M1 seedlings (%)	LD	Fertile M1 plants (%)
Distilled water	200	100	Control	95
0.3%	200	100	0	69
0.5%	200	100	0	36
0.7%	200	98	2	46
1.0%	200	94	6	33
1.5%	200	77	23	30
2.0%	200	56	44	15

The lethal dose (LD) for each EMS concentration was determined by calculating the survival rate of 200 plants. The frequency of fertile M1 plants among all of the surviving plants was assessed by the percentage of plants producing fruits with more than 20 viable seeds.

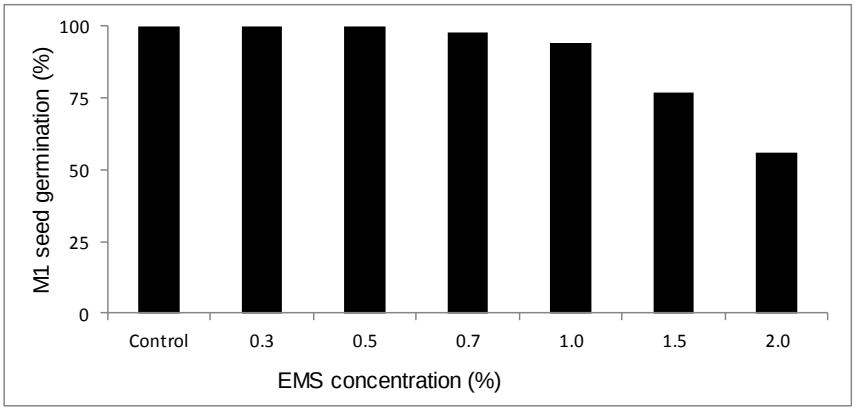

Fig. 1. Dosage effects of EMS mutagenesis on seed germination of zucchini ‘Tosca’.

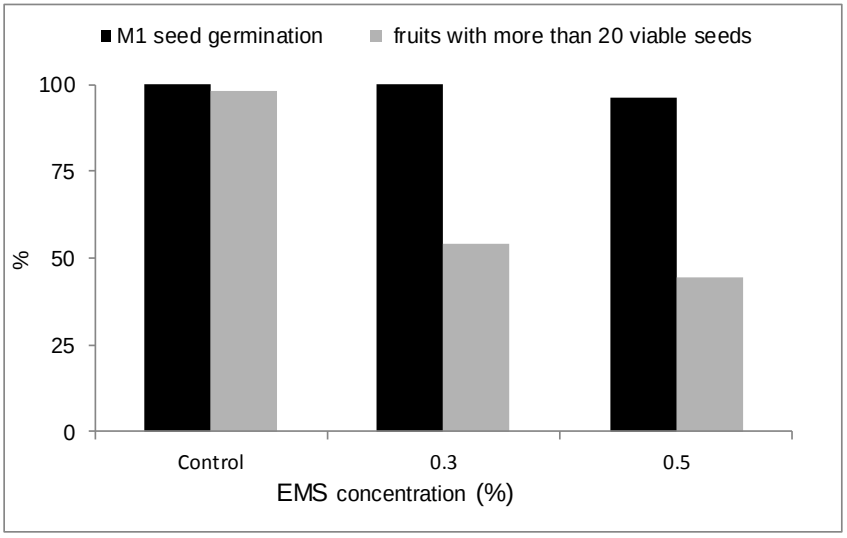

Fig. 2. Dosage effects of EMS mutagenesis on seed germination and fertility of M1 plants of the *C. pepo* inbred line MUC16.

Involvement of *CpACS2/7* gene in monoecy instability of zucchini squash

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Keywords: sex expression, monoecy instability, ethylene, *ACS* gene, zucchini

Abstract

Although no hermaphrodite or andromonoecious genotypes have been described in *Cucurbita pepo*, we have identified a number of genotypes in which female flowers are partially or completely converted into bisexual flowers when grown under high temperatures. This unstable monoecy is associated with a reduction in ethylene production in young floral buds. In fact, anti-ethylene treatments of the apical meristems with AVG and STS were able to induce the conversion of female into bisexual flowers in the absence of inductive conditions in all the tested genotypes, while ethylene treatments reduced the conversion of female into bisexual flowers under inductive conditions. The inbred lines *Bolognese* (*Bog*) and *Vegetable Spaghetti* (*Veg*), contrasting for both sex expression and ethylene production and sensitivity, are both stable for monoecy. However, the F2 populations from the cross *Bog* × *Veg* segregated in a 15:1 ratio of stable/unstable monoecy under inductive conditions, indicating that monoecy instability derived from this cross is conferred by two independent recessive genes. The conversion of female into bisexual flowers in andromonoecious and hermaphroditic genotypes of melon (*Cucumis melo*) and cucumber (*Cucumis sativus*) is the result of a mutation in the *CmACS7* or *CsACS2* genes, two orthologs encoding an ACC synthase (ACS) that arrest stamen development by regulating the production of ethylene in young female floral buds. An *ACS* gene, having the highest similarity with *CmACS7* or *CsACS2*, has been cloned and characterised in *Cucurbita pepo*. As occurs in the other two cucurbit species, the gene, named *CpACS2/7*, is only expressed in female but not in male floral buds. The possible function of this gene in monoecy instability was studied by comparing its expression in stable and unstable genotypes of segregating population of *Bog* × *Veg*, as well as in other unstable genotypes of *C. pepo*.

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INTRODUCTION

Sex determination in monoecious species of the *Cucurbitaceae* is known to be controlled by genetic, environmental, and hormonal factors. In fact, ethylene and low temperature conditions induce femaleness, while anti-ethylene treatments, gibberellins, and high temperature induce maleness in *Cucumis sativus*, *Cucumis melo* and *Cucurbita pepo*. It has been reported that *CmACS7* in *C. melo* and its ortholog in *C. sativus* (*CsACS2*) are responsible for stamen arrest, and their loss-of-function mutations promote the conversion of female into bisexual flowers, and therefore a change of monoecious into andromonoecious plants (Boualem et al. 2008; 2009). Although cultivars of *C. pepo* are all monoecious, under extreme high temperatures some cultivars behave as partially andromonoecious. We have cloned an *ACS* gene from *C. pepo* (*CpACS2/7*) which shows the highest homology with both *CsACS2* and *CmACS7*. The goal of this work is to analyse the role of ethylene and *CpACS2/7* in the instability of monoecy in *C. pepo*.

MATERIALS AND METHODS

The effects of ethylene on instability of monoecy in *Cucurbita pepo* was assessed by treating the plant apices with ethephon as well as with the ethylene inhibitors aminoethoxyvinylglycine (AVG) and silver thiosulphate (STS). Plants from two cultivars that show different monoecy stability, 'Cavili' and 'Cora', were treated every three days with 200 ppm ethephon, 1mM AVG, or 0.25M STS for three weeks. A randomized block design was used with three blocks and 4 repetitions per block. The number of bisexual flowers was recorded for each control and treated plant. Simple and factorial analyses of variance (ANOVA) at $P<0.05$ were performed by the Statgraphics Plus v 5.1 software, and every mean pair was compared with the method of Fisher's least significant difference (LSD). The percentage of bisexual flowers was also evaluated under inductive conditions in the F2 populations of the cross 'Bolognese' (Bog) × 'Vegetable Spaghetti' (Veg).

An *ACS* gene from *C. pepo* (*CpACS2/7*), having the highest similarity with *CmACS7* or *CsACS2*, has been cloned by using degenerated primers derived from these two genes. The homology with other *ACS* genes was analyzed by using Basic Alignment Search Tool (Altschul et al. 1990) available at NCBI. Gene expression analysis was performed in flowers buds of different size by real time RT-PCR as described in Manzano et al. (2010b).

RESULTS AND DISCUSSION

Monoecy instability in *Cucurbita pepo* is dependent on environmental, hormonal and genetic factors

The 'Cora' and 'Cavili' cultivars differed in monoecy instability. When cultured under fall-winter conditions, both cultivars behave very stable (data not

shown). Nevertheless, when cultured under greenhouse spring-summer conditions, 'Cora' plants were very stable and produced no bisexual flowers, but 'Cavili' plants produced about 60% of bisexual flowers (Fig. 1). Ethephon treatments reduced the percentage of bisexual flowers of 'Cavili', and AVG increased significantly the percentage of bisexual flowers in the two cultivars (Fig. 1), indicating that a reduction in ethylene production makes the monoecy more unstable under high temperature conditions. The higher monoecy stability in 'Cora' could therefore be caused by a higher production of ethylene in the apical shoots, which also could make this cultivar more sensitive to AVG and STS treatments, as we have observed. Therefore, the stability of monoecy in *C. pepo* appears to depend on the ethylene production in each genotype.

As 'Bolognese' and 'Vegetable Spaghetti' contrast greatly for ethylene production and sensitivity (Manzano et al. 2010a), we have analysed monoecy stability in these two lines. These two cultivars as well as their F1 showed a very stable monoecy when cultured under inductive conditions. Nevertheless, the F2 populations segregated for monoecy stability. A total of 170 F2 plants were evaluated for the presence of stamens in the female flowers. Considering unstable plants as those which were scored >2 on a scale of 0–3 for stamen development in female flowers, the F2 generation segregated in a proportion of approximately 1:15 for unstable:stable plants. This segregation, therefore, fits with a model in which the monoecy instability in this population is controlled by two recessive genes (χ^2=0.712, *P*=0.39). Therefore, although monoecy instability appears to be induced under high temperatures, this instability only affects specific genotypes.

Cloning and characterization of *CpACS2/7*

Given that the genes *CmACS7* of melon and *CsACS2* of cucumber are responsible for stamen arrest in female flowers (Boualem et al. 2008; 2009), we have cloned and characterised a gene from *Cucurbita pepo*, *CpACS2/7*, which showed the highest sequence identity with *CmACS7* and *CsACS2*. The gene in melon and cucumber, *CpACS2/7*, contains 3 exons and 2 introns, encoding for a protein of 445 aa which shares 89% identity with *CmACS7* and *CsACS2*. Other homologous ACS proteins in *Cucurbita* and *Cucumis* were aligned and used to perform neighbor-joining analyses. The result showed that *CpACS2/7* clusters together with *CsACS2* and *CmACS7*, but is separated from other *ACS* genes in the *Cucurbitaceae*, and thus these three genes could be orthologs.

Gene expression pattern of *CpACS2/7* is also similar to those of *CsACS2* and *CmACS7*. The gene is specifically expressed in the carpels of female flowers, but not in male flowers or in vegetative organs (Fig. 2). Throughout the development of the female flowers, the gene is mainly expressed in floral buds of less than 2 mm in length (stage S0), decreasing its expression throughout the development of the

female flower (Fig. 2). At stage S5 (buds of 22-27 mm in length) and at anthesis, the transcripts of *CpACS2/7* were undetectable (Fig. 2). Within the female flower bud, the gene was mainly expressed in the ovary, although expression was also detected in the style and the stigma of the flower. No expression was observed in petals or nectaries (Fig. 2).

The function of *CpACS2/7* in stamen arrest and therefore in monoecy stability was studied by comparing its expression in female and bisexual flowers derived, respectively, from stable or instable plants for monoecy in the F2 population of 'Bog' × 'Veg'. Flowers at stage S2 and S3 were used for this comparative analysis, since at earlier stages it was not possible to differentiate between the two types of flowers. In both S2 and S3 stages of development, the transcript was nearly undetectable in the bisexual flowers, but was expressed more than 50 times higher in the female flowers. These data indicate that monoecy instability and the development of bisexual flowers in *C. pepo* is associated with a drastic reduction in *CpACS2/7* expression. Therefore, this gene seems to have the same function as *CmACS7* and *CsACS2* (Boualem et al. 2008; 2009), arresting stamen development during female flower development, and therefore controlling monoecy in this species.

ACKNOWLEDGEMENTS

This work was supported by projects AGL2008-05619-C02-02/ALI and AGL2011-30568-C02-02/ALI. C.M. and Z.M. acknowledge FPU program scholarships from MEC, Spain. S.M. is funded by grant PTA2011-479-I from the Spanish Ministry of Science and Innovation.

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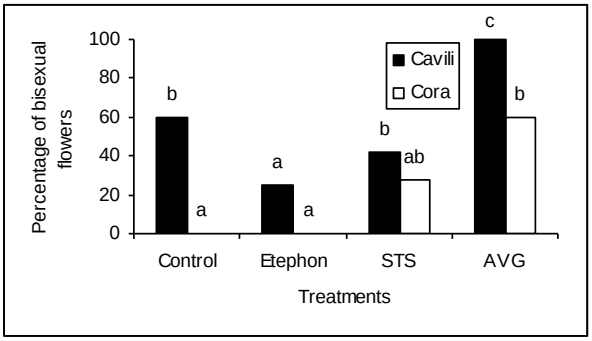
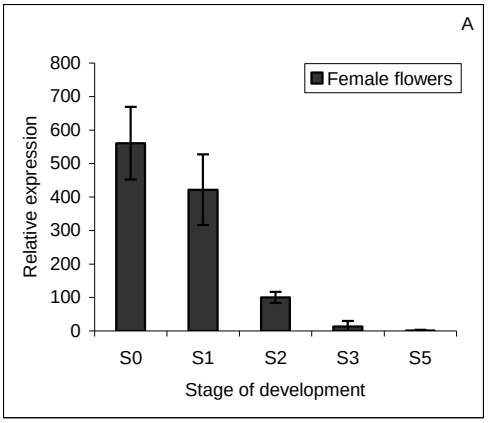
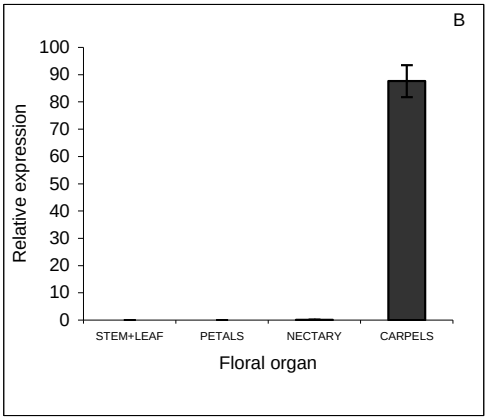


Fig. 1. Effects of ethephon, AVG and STS treatments on the percentage of bisexual flowers in 'Cora' and 'Cavili' grown under inductive spring-summers conditions in greenhouses. Different letters indicate significant differences among treatments of the same cultivar.





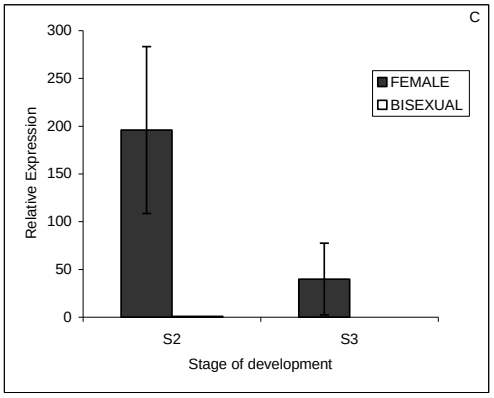


Fig. 2. Expression pattern of *CpACS2/7* different stages of female flower development (A), separated on the base of petals length (S0, <2mm; S1, 2-4 mm, S2: 4-8 mm, S3: 8-12 mm, S5: 22-27 mm), as well as in different organs (B). In (C) it is compared the expression of *CpACS2/7* between carpels of female and bisexual flowers at stages S2 and S3 of development.

Accumulation and distribution of iron, zinc and manganese ions in pumpkin (*Cucurbita* spp.) and gourd (*Lagenaria siceraria*) accessions subjected to drought stress

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Keywords: Drought stress, gourd, pumpkin, ion distribution, plant organs

Abstract

The aim of this study was to observe the distribution of ions in different organs of pumpkin and gourd accessions commonly grown in Turkey, in order to better assess drought stress-tolerance mechanisms. Seedlings of 38 accessions of pumpkin (*Cucurbita* spp.) and gourd **(***Lagenaria siceraria***)** were cultured in cups containing Hoagland's nutrient solution in a controlled climate chamber. To simulate drought stress, 15% polyethylene glycol (PEG6000), equivalent to -0.60 Mpa of osmotic potential, was added to the nutrient solution. Following its application, the accumulation and distribution of iron (Fe), zinc (Zn), and manganese (Mn) ions in the roots, shoots, and leaves of the seedlings were observed using atomic absorption spectrometry (AAS) flame photometry. Ion accumulation increased in plants subjected to drought stress in comparison to the control plants. The greatest increase occurred in the leaves.

INTRODUCTION

Drought stress adversely affects plant growth and productivity by inducing competition for water between vegetative and generative organs of the plant and disrupting intracellular structures, photosynthesis, and nitrogen metabolism (Kocaçalışkan 2003). Due to the functional characteristics of water, such as constituting a large portion of cell content, acting as a transporter, and fulfilling the role of solvent for cellular reactions and functions, normal cellular regulation cannot continue in case of water loss from the cells, causing cellular metabolism to become disrupted. The accumulation of ions that takes place in parallel to the loss of water can damage the cell by disrupting the integrity of the cellular membrane and the structure of proteins (Kalefetoğlu and Ekmekçi 2005). Just as it leads to a reduction in the overall intake of nutrient elements, drought also decreases the transportation of nutrients from roots to shoots. This is due to the decrease in transpiration and the disruption of active transport and membrane permeability (Viets 1972; Alam 1999).

Drought conditions can prevent the intake of ions of micronutrient elements such as Mn, Fe, and Zn, leading plants to display symptoms associated with ion

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deficiency (Havlin et al. 1999). The accumulation of micronutrient elements under conditions of drought or salt stress varies among plant taxa. As a response to drought or salt stress, ion accumulation has been observed to decrease in some plants and increase in others (Hasson et al. 1970). The objective of the present work was to observe the response of locally grown pumpkin and gourd accessions to drought stress.

MATERIALS AND METHODS

Thirty-eight cucurbit accessions grown in various parts of Turkey were included in this work (Table 1). These included 35 pumpkins of *Cucurbita pepo* **L.,** *C. moschata* Duchesne, and *C. maxima* Duchesne, and three gourds of *Lagenaria siceraria* (Mol.) Standl.

Seeds were planted in foam pots filled with pumice and in a control-climate chamber having a photoperiod of 16/8-hour light/dark. When the seedlings developed two true leaves in pumice, they were transplanted to Hoagland water culture (Hoagland and Arnon 1938). The seedlings were grown in the water medium for a week and then, when they had 3 to 4 true leaves, simulated drought conditions were begun by adding 15% polyethylene glycol (PEG 6000) to the Hoagland nutrient solution (Turkan et al. 2005). Fifteen plants from each accession were sampled, in three replicates.

For ion content determination, fresh samples of roots, stems, and leaves were extracted in 0.1N nitric acid. Fe, Zn and Mn contents were measured by atomic absorption spectrometry (AAS) flame photometry (Taleisnik and Grunberg 1994). The data were subjected to analysis of variance using the SAS statistical program (SAS Institute 1985). Means were subjected to Duncan's multiple range test.

RESULTS

Accumulation of iron (Fe) ions

Drought-stressed plants exhibited a general decrease of Fe ions in the roots, little or no change in the stems, and an increase in the leaves (Table 2). However, there were some marked differences among accessions. One pumpkin accession had a slightly increased Fe concentration in the roots. Some accessions showed marked increases in Fe accumulation in the stems or leaves or both, yet others showed marked decreases. The reason for the increase of Fe ions in the leaves could be leaf shrinkage due to the drought conditions. This causes the area of the leaves to decrease, which may have been perceived as an increase in the ion concentration. However, the widely differing results among accessions suggests that other factors also influenced ion concentration.

Accumulation of zinc (Zn) ions

Drought-stressed plants generally exhibited a decrease of Zn ions in the roots, an increase in the leaves, with a mixed reaction in the stems (Table 3). The highest accumulation occurred in the leaves, while the lowest occurred in the stems. Accessions differed in zinc accumulation under drought stress, with Zn accumulation decreasing in the organs of certain accessions and increasing in others.

Accumulation of manganese (Mn) ions

Drought-stressed plants exhibited a decrease of Mn ions in the roots and an increase in the stems and leaves (Table 4). However, there were some marked differences among accessions. In some, Mn accumulation decreased in the roots, stems, and leaves while in others Mn accumulation increased.

DISCUSSION

Drought stress in plants affects the intake of mineral elements, the growth of roots, the flow of nutrients from the soil and the effects of nutrient intake (Fageria et al. 2002; Samarah et al. 2004). The manner of nutrient intake under stress plays an important role in the drought tolerance of these plants (Samarah et al. 2004). Accessions that are developed by selection and capable of adapting to drought conditions are superior with regards to productivity (Baligar et al. 2001).

Chickpea accessions subjected to drought stress differed in their intake of Fe and Zn (Ali et al. 2002). Zn intake of the plants under drought stress showed significant differences between accessions. Except for the Uzunlu-99 accession, Zn intake decreased significantly during the 14 day drought stress. However, in the same study, the application of short period droughts of seven days was noted to have no effect on the intake of nutrient elements. Similarly, the Menemen variety grown under the application of a 7-day drought was observed as having a significant increase in the intake of Zn. This variety was hence identified as being drought tolerant. The results obtained in our study for the application of long term stress were in parallel with the above-mentioned results for Zn.

Accessions that are more drought tolerant have a higher accumulation of nutrient elements. The studies of Ali et al. (2002) on the chickpea plant and of Özpay (2008) on the bean plant, have displayed significant differences with regards to the intake of Mn. It was observed that Mn intake decreases significantly in plants subjected to a long period of drought stress.

For plants subjected to drought stress for a short period of time, decrease in the intake of Mn was very low. When analysing plants for the relation between the intake of Mn and plant development, it was determined that the relation between the two is negative when long-term drought stress is applied, while the relation is correlative when short-term drought stress is applied. In drought resistant varieties, mineral nutrient element intake during both short-term and long-term drought conditions tended to be higher. At the same time, in both stress period conditions, the negative influence between plant development and nutrient elements was found to be less in comparison to the other, more sensitive accessions. Similarly, in the study conducted by Ali et al. (2002) on chickpeas, plants that were more tolerant to drought conditions had milder decreases in nutrient elements. With a decrease in the amount of water under conditions of drought, a significant decrease was observed in both the total quantity and the concentration of mineral elements in plants (Marschner 1995; Baligar et al. 2001).

In the study conducted by Özpay (2008), mineral element accumulation in drought tolerant bean accessions was observed to be generally higher than the accumulation in drought-sensitive varieties. Samarah et al.'s (2004) study,

a significant interrelation was identified between the degree of mineral element intake and drought-tolerance for soybeans grown under drought stress conditions. In conclusion, the drought-tolerant pumpkin and gourd varieties have a high level of nutrient element intake and tend to direct the nutrient elements obtained from their roots to the upper sections of the plant. As such, tolerant accessions have displayed a better performance with regards to plant growth and nutrient element intake and distribution.

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Table 1. Assigned code, taxon and location of collection of the 38 pumpkin and gourd accessions used in this study.

Code	Taxon	Location
A1	*Cucurbita maxima*	Kundur/Adana
A3	*Cucurbita pepo*	Rize
A8	*Cucurbita maxima*	Keskin-Kırıkkale
A9	*Cucurbita pepo*	Elazığ
A10	*Cucurbita maxima*	Kovanlık-Antalya
A12	*Cucurbita pepo*	Hakkari
A13	*Cucurbita pepo*	Nevşehir
A18	*Cucurbita pepo*	Adana
A19	*Cucurbita moschata*	Bingöl
A20	*Cucurbita maxima*	Rize
A24	*Cucurbita moschata*	Rize
A26	*Cucurbita pepo*	Kadirli-Osmaniye
A31	*Cucurbita maxima*	Mersin
A32	*Cucurbita moschata*	Van
AB2	*Cucurbita pepo*	Van
AB5	*Cucurbita maxima*	Elazığ
AB6	*Cucurbita maxima*	Adana
AB18	*Lagenaria siceraria*	Türkoğlu-Kahramanmaraş
AB20	*Cucurbita pepo*	Bursa
AB32	*Cucurbita pepo*	Bingöl
AB44	*Cucurbita moschata*	Tokat
AB49	*Cucurbita pepo*	Bursa
AB51	*Cucurbita maxima*	Kadirli-Osmaniye
AB54	*Cucurbita maxima*	Yenice-Adana
AB57	*Cucurbita maxima*	Bingöl
AB58	*Cucurbita pepo*	Mersin
AB63	*Cucurbita maxima*	Rize
AB68	*Lagenaria siceraria*	Mersin
AB69	*Lagenaria siceraria*	Adana
C3	*Cucurbita maxima*	Çukurca-Hakkari 3
C5	*Cucurbita moschata*	Çukurca-Hakkari 5
C6	*Cucurbita moschata*	Çukurca-Hakkari 6
C8	*Cucurbita maxima*	Çukurca-Hakkari 8
C10	*Cucurbita moschata*	Çukurca-Hakkari 10
C11	*Cucurbita maxima*	Çukurca-Hakkari 11
C13	*Cucurbita moschata*	Çukurca-Hakkari 13
C18	*Cucurbita maxima*	Çukurca-Hakkari 18
C20	*Cucurbita moschata*	Çukurca-Hakkari 20

Table 2. Effect of drought stress on iron ion accumulation (µg/mg wet weight) in pumpkin and gourd accessions.

Code	Roots			Stems			Leaves		
	Control	Applied	Difference	Control	Applied	Difference	Control	Applied	Difference
A1	411.60 h-n	384.26 bc	-27.34	6.470 c-h	7.142 f-j	0.672	15.884 c-f	14.693 ı-n	-1.191
A3	400.32 ı-n	221.39 f-j	-178.93	5.097 f-k	8.836 de	3.739	15.095 d-h	16.674 h-k	1.579
A8	664.72 ab	128.00 mn	-536.72	11.888 ab	2.561 r	-9.327	54.386 a	11.521 l-p	-42.865
A9	487.86 d-l	150.62 k-n	-337.24	6.570 c-h	4.304 n-q	-2.266	16.569 c-e	11.576 l-p	-4.993
A10	527.98 c-ı	360.01 cd	-167.97	10.816 b	10.339 bc	-0.477	14.256 e-j	13.420 j-o	-0.836
A12	592.79 b-d	237.88 f-ı	-354.91	7.291 c-f	5.842 j-o	-1.449	8.639 m-o	21.898 e-f	13.259
A13	512.90 c-k	139.93 l-n	-372.97	8.233 c	4.670 m-q	-3.563	17.017 c-e	43.493 a	26.476
A18	674.261 a	421.62 ab	-320.99	12.937 a	10.490 a-c	-2.447	17.802 cd	22.677 e	4.875
A19	593.58 b-d	206.19 g-k	-387.39	5.672 d-j	6.127 ı-m	0.454	16.910 c-e	45.936 a	29.026
A20	535.27 c-h	136.71 l-n	-398.56	4.544 h-k	8.266 d-g	3.721	12.133 g-m	34.487 b	22.354
A24	554.49 b-f	150.97 k-n	-403.52	7.507 c-e	8.327 d-g	0.820	23.604 b	25.171 c-e	1.567
A26	229.57 pq	110.46 n	-119.11	3.735 ı-k	4.975 m-p	1.239	14.573 d-ı	28.061 c	13.488
A31	638.56 a-c	143.62 k-n	-494.94	7.852 cd	5.554 j-p	-2.298	11.653 h-m	23.124 e	11.471
A32	551.91 b-f	329.27 cd	-222.64	7.090 c-g	8.705 e-f	1.614	18.829 c	25.178 c-e	6.349
AB2	574.98 b-e	474.04 a	-100.94	7.190 c-f	6.915 g-k	-0.274	12.322 g-l	17.044 h-j	4.722
AB5	333.11 n-p	266.35 e-g	-66.76	3.463 jk	5.391 k-p	1.928	7.896 o	10.722 n-p	2.826
AB6	218.59 pq	168.51 j-n	-50.08	3.866 ı-k	4.660 m-q	0.793	15.865 c-f	12.789 k-o	-3.076
AB18	459.52 e-m	220.11 f-j	-239.41	4.926 g-k	4.891 m-p	-0.349	10.784 j-o	15.695 h-k	4.911
AB20	418.35 g-n	256.43 f-h	-161.92	5.750 d-j	6.822 g-l	1.072	9.903 l-o	11.189 m-p	1.286
AB32	546.45 b-g	248.12 f-h	-298.33	6.413 c-h	11.123 ab	4.710	15.561 c-g	15.474 h-l	-0.087
AB44	413.88 h-n	195.15 h-l	-218.73	5.696 d-j	7.855 e-h	2.159	8.732 m-o	27.104 cd	18.372
AB49	359.73 l-o	222.38 f-j	-137.35	5.939 d-ı	6.661 h-l	0.722	11.757 h-m	27.773 c	16.016
AB51	384.07 k-n	149.53 k-n	-234.54	3.335 k	5.235 l-p	1.899	10.125 k-o	14.794 ı-n	4.669
AB54	517.44 c-j	137.65 l-n	-379.79	7.665 c-e	3.211 q-r	-4.454	7.642 o	14.983 h-m	7.341
AB57	625.24 bc	181.05 ı-m	-444.19	7.480 c-e	3.587 ef	-3.892	10.406 k-o	10.000 op	-0.406
AB58	375.53 l-n	247.24 f-h	-128.29	7.543 c-e	3.969 p-r	-3.573	13.908 e-j	23.564 de	9.656
AB63	442.58 f-n	274.47 ef	-168.11	8.293 c	9.486 cd	1.193	14.903 d-ı	13.810 j-o	-1.093
AB68	434.57 f-n	131.88 l-n	-302.69	10.283 b	7.169 f-j	-3.113	14.114 e-j	23.446 de	9.332
AB69	256.97 o-q	167.12 j-n	-89.85	5.478 e-k	11.752 ab	6.273	14.133 e-j	16.252 h-k	2.119
C3	315.29 n-p	435.90 ab	120.61	3.941 ı-k	11.896 a	7.954	8.075 no	14.712 ı-n	6.637
C5	546.95 b-g	141.35 l-n	-405.60	8.260 c	4.215 o-q	-4.044	11.412 ı-n	21.294 e-g	9.882
C6	392.11 j-n	395.34 bc	3.23	5.608 d-j	4.821 m-p	-0.786	9.978 l-o	18.876 f-h	8.898
C8	244.91 o-q	136.03 l-n	-108.88	5.423 e-k	10.564 a-c	5.141	10.828 j-o	18.061 g-ı	7.233
C10	446.79 f-m	315.31 de	-131.48	11.359 ab	7.464 e-ı	-0.389	16.718 c-e	23.026 e	6.308
C11	443.53 f-n	173.41 j-n	-270.12	4.015 ı-k	6.739 g-l	2.723	14.122 e-j	15.375 h-l	1.253
C13	162.83 q	129.75 mn	-33.08	4.762 h-k	5.910 ı-n	1.148	13.610 e-k	14.135 ı-n	0.525
C18	427.52 f-n	159.57 j-n	-267.95	4.901 g-k	4.991 m-p	0.899	12.621 f-l	8.047 p	-4.574
C20	622.43 bc	164.54 j-n	-457.89	4.655 h-k	5.565 j-p	0.909	7.343 o	11.092 m-p	3.749
T.Av.	**458.092 A**	**224.004 B**	**-234.088**	**6.630 A**	**6.764 A**	**0.134**	**14.213 B**	**19.399 A**	**5.186**

Lower-case letters indicate mean separations in columns by Duncan's multiple range test, 5% level and upper-case letters indicate mean separations between applications, 5% level

Table 3. Effect of drought stress on zinc ion accumulation (µg/mg wet weight) in pumpkin and gourd accessions.

Code	Roots			Stems			Leaves		
	Control	Applied	Difference	Control	Applied	Difference.	Control.	Applied	Difference.
A1	24.456 k-m	26.816 b-e	2.36	12.695 h-j	11.823 ı-j	-0.872	24.106 h-ı	64.513 b	40.407
A3	42.276 cd	30.300 b-d	-11.976	25.669 b	7.269 k-m	18.4	39.514 ab	35.575 g-ı	-3.939
A8	26.053 j-l	18.682 ı-o	-7.371	20.322 c-f	14.452 e-h	-5.87	27.555 f-g	29.298 j-l	1.743
A9	14.082 qr	23.169 e-k	9.087	2.872 n-p	16.455 d-f	13.583	8.290 r	43.211 de	34.921
A10	12.713 r	26.644 b-e	13.931	6.254 l-o	31.473 a	25.219	10.209 qr	20.742 mn	10.533
A12	16.848 n-r	30.504 bc	13.656	5.703 l-p	16.413 d-f	10.71	19.591 j-l	65.788 b	46.197
A13	32.048 gh	24.272 e-ı	-7.776	11.500 h-k	15.354 d-g	3.854	20.096 j-k	32.924 ı-k	12.828
A18	63.848 a	24.704 e-h	-39.144	33.442 a	6.920 k-m	-26.522	37.521 b	40.100 e-h	2.579
A19	21.550 l-n	16.843 l-o	-4.707	1.889 o-p	8.066 kl	6.177	14.033 n-p	24.640 lm	10.607
A20	32.793 gh	15.101 m-o	-17.692	7.968 j-n	13.874 f-ı	5.906	39.330 ab	57.103 c	17.773
A24	32.713 gh	14.903 n-p	-17.81	9.360 ı-m	15.269 d-g	5.909	28.740 e-f	24.923 lm	-3.817
A26	20.668 m-p	21.366 e-l	0.698	10.719 h-l	8.315 k	-2.404	29.310 e-f	42.268 d-f	12.958
A31	39.797 de	25.327 c-g	-14.47	19.748 c-f	1.612 pq	-18.136	30.118 e-f	26.934 kl	-3.184
A32	46.219 c	22.509 e-k	-23.71	20.857 b-e	16.670 c-e	-4.187	31.188 de	42.619 de	11.431
AB2	43.564 cd	40.563 a	-3.001	19.426 d-f	8.708 k	-10.718	21.310 ı-j	26.821 kl	5.511
AB5	51.317 b	22.694 e-k	-28.623	24.439 b-d	8.873 k	-15.566	36.809 bc	40.607 e-g	3.798
AB6	19.323 n-p	20.325 g-n	1.002	4.723 m-p	9.009 k	4.286	13.998 n-p	36.114 g-ı	22.116
AB18	15.694 p-r	19.166 h-n	3.472	2.731 n-p	19.038 c	16.307	15.138 m-o	19.088 n	3.95
AB20	44.693 c	31.395 b	-13.298	24.363 b-d	25.091 b	0.728	33.664 cd	56.351 c	22.687
AB32	33.479 f-h	18.464 j-o	-15.015	14.384 g-ı	12.625 hı	-1.759	41.883 a	34.438 h-j	-7.445
AB44	19.387 n-p	14.734 n-p	-4.653	6.665 k-o	5.732 l-n	-0.933	24.983 gh	36.681 f-ı	11.698
AB49	17.419 n-r	17.725 k-o	0.306	6.118 l-o	0.844 q	-5.274	16.385 l-n	36.207 g-ı	19.822
AB51	17.851 n-q	20.603 f-m	2.752	1.325 o-p	13.466 g-ı	12.141	11.577 pq	53.449 c	41.872
AB54	21.680 l-n	20.179 g-n	-1.501	0.648 p	4.778 m-o	4.13	16.244 l-n	29.747 j-l	13.503
AB57	46.104 c	23.894 e-j	-22.21	5.672 l-p	8.906 k	3.234	20.372 jk	16.977 no	-3.395
AB58	18.924 n-q	26.217 b-f	7.293	6.665 k-o	0.982 q	-5.683	12.235 o-q	28.841 j-l	16.606
AB63	37.701 e-f	24.997 d-g	-12.704	24.897 bc	3.328 n-q	-21.569	19.294 j-l	32.113 ı-k	12.819
AB68	15.758 p-r	20.693 f-m	4.935	1.838 o-p	15.676 d-g	13.838	15.405 m-o	32.660 ı-k	17.255
AB69	29.050 h-j	13.233 op	-15.817	14.417 g-ı	2.781 o-q	-11.636	20.657 jk	17.297 no	-3.36
C3	31.202 g-ı	22.084 e-k	-9.118	20.604 b-e	17.656 cd	-2.948	36.830 bc	26.959 kl	-9.871
C5	45.294 c	9.836 p	-35.458	18.017 e-g	5.068 m-o	-12.949	36.591 bc	54.136 c	17.545
C6	21.445 l-o	23.452 e-k	2.007	2.417 o-p	24.989 b	22.572	15.338 m-o	46.312 d	30.974
C8	16.084 p-r	21.813 e-l	5.729	10.469 h-l	9.562 j-k	-0.907	17.915 j-m	29.333 j-l	11.418
C10	26.958 ı-k	21.123 e-l	-5.835	24.253 b-d	4.065 n-p	-20.188	31.044 de	74.538 a	43.494
C11	17.663 n-q	23.344 e-k	5.681	10.413 h-l	13.604 g-ı	3.191	17.168 k-n	38.940 e-h	21.772
C13	16.547 o-r	20.562 f-m	4.015	2.057 o-p	15.103 d-h	13.046	18.514 j-m	24.919 lm	6.405
C18	34.140 f-g	19.955 g-n	-14.185	15.472 f-h	17.696 cd	2.224	14.305 n-p	12.198 o	-2.107
C20	30.228 g-j	18.384 j-o	-11.844	2.299 op	14.388 e-ı	12.089	17.306 k-n	17.477 no	0.171
T.Av.	**28.883 A**	**22.015 B**	**- 6.868**	**11.929 A**	**11.735 A**	**-0.194**	**23.278 B**	**36.127 A**	**12.849**

Lower-case letters indicate mean separations in columns by Duncan's multiple range test, 5% level and upper-case letters indicate mean separations between applications, 5% level

Table 4. Effect of drought stress on manganese ion accumulation (µg/mg wet weight) in pumpkin and gourd accessions.

Code	Roots			Stems			Leaves		
	Control	Applied	Difference	Control	Applied	Difference	Control	Applied	Difference
A1	142.73 n-p	172.47 h-m	29.74	27.141 j-o	48.126 g-k	20.985	129.86 h-k	173.81 f-h	43.95
A3	280.11 e-d	304.50 bc	24.39	64.723 b	161.201 a	96.478	384.23 a	284.37 a	-99.86
A8	277.49 e-d	122.35 m-o	-155.14	142.242 a	32.870 m-o	-109.372	220.60 b	166.66 f-ı	-53.94
A9	251.84 e-f	185.05 f-j	-66.79	26.476 j-p	55.668 f-h	29.192	82.44 mn	166.12 f-ı	83.68
A10	318.56 cd	202.57 e-h	-115.99	34.695 h-l	57.251 e-g	22.556	89.59 l-n	187.73 d-g	98.14
A12	204.48 g-m	131.15 k-o	-73.33	37.584 g-j	131.632 b	94.048	80.23 n	130.69 ı-o	50.46
A13	344.37 c	147.68 ı-o	-196.69	35.192 h-l	43.826 ı-l	8.634	100.19 k-n	152.66 g-l	52.47
A18	575.25 a	325.72 b	-249.53	132.810 a	106.555 c	-26.255	186.46 cd	182.77 d-g	-3.69
A19	184.41 h-o	162.26 h-n	-22.15	40.931 e-h	96.487 d	55.556	180.39 c-e	212.76 cd	32.37
A20	240.81 e-h	140.58 j-o	-100.23	13.941 r-t	26.247 o-s	12.306	165.77 d-g	175.94 e-h	10.17
A24	250.23 e-f	117.66 no	-132.57	50.698 c-f	44.594 ı-l	-6.104	170.51 d-f	119.76 l-o	-50.75
A26	256.99 e-g	223.65 d-g	-33.34	51.639 c-e	88.933 d	37.294	219.80 b	165.15 f-j	-54.65
A31	237.05 e-ı	129.66 l-o	-107.39	28.998 ı-m	26.770 r-t	-2.228	210.08 bc	126.08 k-o	-84.0
A32	242.99 e-h	164.22 h-n	-78.77	43.712 e-h	66.887 e	23.175	135.45 g-j	107.18 no	-28.27
AB2	363.10 c	451.24 a	88.14	58.279 bc	37.253 k-n	-21.026	155.11 d-h	210.83 c-e	55.72
AB5	165.26 l-p	131.61 k-o	-33.65	37.380 g-j	49.464 f-j	12.084	78.21 n	156.32 g-k	78.11
AB6	143.33 n-p	147.31 ı-o	3.98	10.733 s-t	26.737 n-s	16.004	160.44 d-h	157.96 g-k	-2.48
AB18	132.95 n-p	172.34 h-m	39.39	29.031 ı-m	43.997 ı-l	14.966	167.65 d-g	163.98 f-j	-3.67
AB20	226.61 e-j	190.86 g-k	-35.75	41.614 e-h	53.606 f-ı	11.992	151.33 e-h	156.85 g-k	5.52
AB32	464.70 b	228.79 d-f	-235.91	54.963 b-d	43.056 ı-m	-11.907	113.26 ı-m	180.19 d-h	66.93
AB44	214.04 f-l	228.81 d-f	14.77	20.594 m-s	46.207 h-l	25.613	142.51 f-ı	174.07 f-h	31.56
AB49	172.83 j-p	146.50 ı-o	-26.33	32.609 h-l	27.383 n-r	-5.226	91.38 l-n	143.75 h-m	52.37
AB51	124.27 op	109.67 o	-14.6	17.631 n-t	18.633 q-t	1.002	184.97 cd	128.31 j-o	-56.66
AB54	453.54 b	227.67 d-f	-225.87	14.722 q-t	36.272 l-o	21.55	45.72 o	143.60 h-m	97.88
AB57	430.27 b	243.22 de	-187.05	47.333 d-g	12.346 t	-34.987	143.91 f-ı	117.92 l-o	-25.99
AB58	115.11 p	173.16 h-l	58.05	12.299 s-t	15.831 s-t	3.532	95.86 l-n	98.66 o	2.8
AB63	256.47 ef	146.79 ı-o	-109.68	40.722 e-h	19.957 q-t	-20.765	157.36 d-h	113.71 m-o	-43.65
AB68	166.10 k-p	136.64 j-o	-29.46	15.369 p-t	21.459 p-t	6.09	105.71 j-n	186.50 d-g	80.79
AB69	236.96 e-ı	168.73 h-m	-68.23	25.311 k-q	20.228 q-t	-5.083	138.71 f-ı	144.22 h-m	5.51
C3	136.81 n-p	265.54 cd	128.73	39.949 f-ı	43.141 ı-m	3.192	137.27 g-j	226.90 bc	89.63
C5	225.74 e-k	150.89 ı-o	-74.85	16.519 o-t	26.515 r-t	9.996	132.12 h-j	257.11 ab	124.99
C6	141.87 n-p	269.14 cd	127.27	13.839 r-t	17.296 q-t	3.457	116.55 ı-l	196.20 c-f	79.65
C8	176.25 j-p	252.20 d	75.95	24.294 l-r	28.012 n-q	3.718	130.63 h-k	135.53 ı-n	4.9
C10	266.11 ef	244.18 de	-21.93	28.030 j-n	59.946 ef	31.916	151.12 e-h	152.87 g-l	1.75
C11	180.50 ı-o	134.43 k-o	-46.07	16.588 o-t	31.481 n-p	14.893	134.53 g-j	212.43 cd	77.9
C13	146.71 m-p	160.46 h-n	13.75	9.006 t	50.726 f-j	41.72	28.21 o	135.61 ı-n	107.4
C18	188.49 h-n	194.85 f-ı	6.36	14.932 q-t	42.131 j-m	27.199	166.45 d-g	176.25 e-h	9.8
C20	203.61 g-m	152.82 ı-o	-50.79	35.725 h-k	43.730 ı-l	8.005	138.44 f-ı	178.18 d-h	39.74
T.Av.	**240.49 A**	**190.98 B**	**-49.51**	**36.533 B**	**47.433 A**	**10.900**	**142.711 B**	**165.779 A**	**23.068**

Lower-case letters indicate mean separations in columns by Duncan's multiple range test, 5% level and upper-case letters indicate mean separations between applications, 5% level

Confectionary pumpkin breeding studies in Turkey

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Keywords: Confectionery pumpkin, *Cucurbita pepo*, selection, morphological traits, seed yield

Abstract

Confectionery pumpkin (*Cucurbita pepo* L.) seeds are one of the important snacks preferred by consumers in Turkey due to higher nutrient contents. Production areas are mostly located Middle Anatolia and the Trakya region. Although pumpkin seeds are produced and consumed widely, there are no registered cultivars and certified seed production in Turkey. To develop new cultivars of confectionery pumpkin, breeding efforts were initiated at the Trakya Agricultural Research Institute (TARI) in Edirne in 2007. Germplasm was collected from various parts of Turkey and selection was applied using the single seed descent method. Plants were selfed in each generation and selected on the basis of morphological, seed quality, and yield traits in 2008, 2009 and 2010. They were classified in three main groups, large-seeded like 'Urgup Sivrisi', small-seeded like 'Edirne', and long-seeded like 'Hanim Tırnagi'. Hull-less seeded pumpkins were also included in the breeding nursery, but they are mostly for oil extraction. From each group, lines with increased homogeneity were selected and crossed with one another in 2010 to produce hybrids for evaluation. Sixteen hybrids and three standard cultivars were entered into trials in 2011, and evaluated for morphological, seed quality and yield traits. Experiments were conducted with irrigation in the 2011 growing season in the field at Edirne. The seed yields of hybrids ranged from 600-1020 kg ha^{-1}. Three of the hybrids exhibited higher yield performance than the standards and the parent lines of these hybrids were selected. Also, more crosses were made in 2011, and the resulting hybrids will be tested in 2012 too. The most promising lines were selfed through the F_6 generation in 2012. After final evaluation of homogeneity, the best ones will be sent for registration.

INTRODUCTION

Pumpkin, sunflower, peanut, hazelnut, pistachio, and chickpea are among the

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most consumed crops as confectionery food in Turkey. While chickpea, confectionery sunflower and pumpkin seeds are mostly produced in the Middle Anatolia Region, hazelnut is produced around the Black Sea, pistachio in Southeastern Anatolia, and peanut in Mediterranean areas.

Confectionery pumpkin seeds, both hulled and hull-less, are consumed in Turkey. However, while hull-less ones are used mostly for oil extraction and sprinkled on cakes and breads, the hulled seeds are consumed mostly as a confection. Both of them are produced in Turkey but the production and consumption of hulled-seed pumpkins is more widespread (Abak et al. 1994, 1997).

Village populations of pumpkins are used generally in confectionery pumpkin production because no improved cultivar with certified seed production has been developed in Turkey (Yegul et al. 2012). These populations with hulled seeds consist of three main groups, large-seeded like 'Urgup Sivrisi', small-seeded 'Edirne' type, and long-seeded like 'Hanim Tırnagi'. Seeds for planting are produced in non-isolated areas so there is much heterogeneity in the pumpkin populations. This situation results in economic losses, both in production and also in the confectionery industry (Düzeltir and Yanmaz 2004).

Although confectionary pumpkins have been widely grown in Turkey for many years, little work has been performed both on breeding (Abak et al. 1994; Düzeltir and Yanmaz 2004; Seymen et al. 2011) and on production techniques (Abak et al. 1997). Confectionery pumpkins are grown both under irrigated and dryland conditions in Turkey. Therefore, there is much variation in seed yield and seed quality (Yegul et al. 2012).

The lack of certified seed and high yielding varieties are the most important problems in confectionery pumpkin production in Turkey. Therefore, our goal at TARI in Edirne, Turkey was to develop improved confectionery pumpkin germplasm and hybrids.

MATERIALS AND METHODS

Breeding for improved confectionery pumpkin, using single-seed descent, was initiated in the field at TARI in 2007 with germplasm collected from various parts of Turkey. Seeds were planted in 2007 and plants were selfed in each generation and selected for morphological traits, seed quality, and yield. The process was repeated in 2008, 2009 and 2011.

Seeds of the various pumpkin accessions were sown, 3-4 seeds per hole, at the beginning of April. Subsequently, they were thinned to one plant per hole, resulting in a 1 × 1 m plant spacing. They were irrigated before and after flowering. The selected plants were harvested in September, and then their seeds were threshed, dried and cleaned. After all these processes, seeds of all breeding materials were evaluated based on desired seed characteristics together with earliness, desirable

fruit and vine type, good fruit set, uniformity, and high yield, and the best ones selected for planting the following year.

These breeding materials with hulled seeds were classified into three main groups as described above. Additionally, the hull-less material were also grown in the breeding nursery for possible use in oil production and also for snacks, cakes, and breads.

From each group with hulled seeds, lines having increased homogeneity were selected in the F_4 generation and crossed to produce hybrids for evaluation in the 2010 summer season. Sixteen hybrids were prepared and placed in yield trials with 3 standards in the field at Edirne in 2011. The plots were irrigated before and after flowering. Composite fertilizer (20-20-0) at 250 kg ha^{-1} was applied before planting, and ammonium nitrate 33% at 150 kg ha^{-1} was applied when plants were 25 cm long. The pumpkin hybrids were tested in a randomized complete block design with 3 replications. The plants were planted in four rows at 1 × 1 m plant density and plot size was 5 × 4 m. Seeding was in April and harvesting was in September. The trials were sown and harvested by hand. The pumpkins were evaluated for morphological homogeneity, seed quality and yield traits. Seed yield (kg ha^{-1}), 1000 seed weight (g), seed length (mm) and seed width (mm) were observed and recorded.

RESULTS

Variation in the confectionery pumpkin breeding pool, as evaluated at TARI, and seed characteristics of breeding lines are presented in Table 1. From these breeding materials, the best plants having desired traits were selected in each year.

The first hybrids were produced in 2010 and they were trialled in 2011 under irrigated conditions. The seed yields of the hybrids ranged from 600-1020 kg ha^{-1} while the average yield of controls was 926 kg ha^{-1} (Table 2). Although these hybrids were formed in the first year and in earlier generations, improved yield was obtained in some and higher homogeneity was observed. Especially in seed characteristics, overall better results than controls were obtained in many hybrids. The average yield of controls was 185.1 g for 1000 seed weight, 19.9 mm seed length and 9.4 mm seed width.

After evaluation of seed characteristics and other yield traits of the breeding material in 2011, nine populations were selected for F_1 generation, 21 for F_3, 25 for F_4, 11 for F_5 and 16 populations were selected for F_6 generation for the 2012 growing season.

DISCUSSION

The homogeneity of the germplasm was increased year by year, gradually achieving the development of improved pumpkin varieties. The higher yielding and seed-quality hybrids indicate that the breeding program at TARI has been successful. The new homogeneous lines were also crossed with others to develop new hybrid combinations quite recently. After evaluation, the best hybrids and parental lines will be sent for registration and commercialized.

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Table 1. Seed traits of confectionery pumpkins in all generations in 2011.

	Yield Traits	#	Min	Max	Mean	Std Dev.	Median	25% Quartile	75% Quartile
General	Seed Length (mm)	104	14.5	24.7	19.3	2.13	19.4	17.8	21.0
	Seed Width (mm)	104	6.6	14.4	9.4	1.38	9.2	8.5	10.3
	1000 Seed Weight (g)	104	100.2	308.0	197.8	46.5	193.8	164.7	229.7
With Hull	Seed Length (mm)	74	16.6	24.7	20.2	1.73	20.0	19.1	21.4
	Seed Width (mm)	74	7.8	14.4	10.0	1.18	9.8	9.1	10.8
	1000 Seed Weight (g)	74	203.5	308.0	213.3	42.4	208.5	183.4	240.4
Husllless	Seed Length (mm)	30	14.5	20.2	17.1	1.32	16.8	16.5	17.9
	Seed Width (mm)	30	6.6	9.2	8.0	0.74	8.2	7.4	8.6
	1000 Seed Weight (g)	30	100.2	233.0	159.7	32.5	152.6	136.7	182.2
F_2	Seed Length (mm)	37	15.1	24.7	19.2	2.11	19.1	17.8	20.9
	Seed Width (mm)	37	6.6	14.4	9.4	1.37	9.2	8.5	10.1
	1000 Seed Weight (g)	37	100.2	302.2	197.9	48.0	185.9	165.8	233.3
F_3	Seed Length (mm)	33	15.6	24.6	19.6	2.21	19.6	17.5	21.2
	Seed Width (mm)	33	7.0	11.6	9.3	1.35	9.1	8.3	10.5
	1000 Seed Weight (g)	33	112.7	308.0	198.7	50.2	197.3	156.3	228.1
F_4	Seed Length (mm)	11	17.4	22.4	19.6	1.50	19.5	18.3	20.3
	Seed Width (mm)	11	7.8	10.4	9.2	0.73	9.2	8.6	9.8
	1000 Seed Weight (g)	11	128.0	265.0	202.9	35.8	205.4	179.2	228.9
F_5	Seed Length (mm)	23	14.5	24.0	18.9	2.37	18.7	17.0	20.9
	Seed Width (mm)	23	6.6	12.3	9.7	1.68	10.0	8.2	11.1
	1000 Seed Weight (g)	23	114.2	278.6	194.1	45.6	195.7	156.2	233.9

Table 2. Seed yields of confectionery pumpkin hybrids in 2011.

	Name of Hybrids	Seed Yield (kg ha^{-1})	Seed Length (mm)	Seed Width (mm)	1000 Seed Weight (g)
1	Village Population (C)	950	19.6	9.5	209.0
2	Village Population (C)	1000	20.2	8.8	136.6
3	Urgup Sivrisi (C)	830	19.8	9.9	209.8
4	F_4 SN:19 x F_4 SN:9 B_3	880	21.8	12.5	224.8
5	F_4 SN: 5 x F_4 SN:1 B_1	980	18.5	11.0	226.2
6	F_4 SN: 1 x F_4 SN:4 B_2	850	19.0	10.5	210.9
7	F_4 SN: 5 x F_4 SN:19 B_3	760	19.5	10.7	248.6
8	F_4 SN: 5 x F_4 SN:9 B_2	920	17.5	10.7	240.6
9	F_4 SN: 8 x F^4 SN:15 B_1	740	21.8	11.8	180.7
10	F_4 SN:14 x F_4 SN:19 B_1	860	19.6	10.0	216.8
11	F_4 SN:19 x F_4SN:15 B_3	770	20.2	11.5	270.1
12	F_4 SN: 9 x F_4 SN:1 B_5	600	17.5	9.8	166.6
13	F_4 SN:19 x F_4 SN:8 B_2	880	19.0	10.5	245.4
14	F_4 SN: 4 x F_4SN:19 B_2	760	16.0	9.0	160.1
15	F_4 SN:18 x F_4 SN:14 B_2	680	18.6	10.0	240.1
16	F_4 SN: 4 x F_4 SN:9 B_1	850	20.2	11.2	280.5
17	F_4 SN:14 x F_4 SN:15 B_1	1020	21.8	11.5	224.2
18	F_4 SN:19 x F_4 SN:14 B_1	710	22.8	11.6	266.1
19	F_4 SN: 9 x F_4 SN:14 B_2	940	23.0	12.0	304.2
Mean		841	19.8	10.7	224.3

The identification of SSR markers in RIL mapping population of winter squash (*Cucurbita maxima* Duch.)

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Abstract

The aim of work was the identification of SSR molecular markers in the recombinant inbred line F6 mapping population of winter squash (*Cucurbita maxima* Duch.). The population consisting of 102 RILs was developed from the cross between two inbred lines F8 *Cucurbita maxima* (Duch.) – 800 and 801, representing different genetical pools, originated from the germplasm collection of Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences. The parental lines were differentiated in respect to dry mass and carotenoid content in the fruit flesh and some morphological characters. 120 SSR primer pairs, which sequences originated from *C.pepo* and *C.moschata* genomes, previously used for the construction of genetic map of *C. pepo* (Gong et al. 2008), were applied in the research. PCR amplification, the SSR products size-separation on 6% polyacrylamide denaturating gels and silver staining were conducted as described by Pillen et al. (2000).

Out of 120 SSR primer pairs screened on parental lines, for 117 the amplicons were obtained, and 31 were found to be polymorphic between the two parents. Two types of markers: codominant and dominant, were observed. Next, out of the selected 39 primer pairs tested on 8 random RILs from mapping population, for 14 the segregating amplicons were identified. For all mapping population (102 RILs), in the result of amplification with the use of the selected 14 primers pairs, 10 SSR markers - products of primer pairs: CmTp17, CMTp98, CMTp187, CMTp201, CMTp256, CMTm7, CMTm11, CMTm19, CMTm52, CMTm54, CMTm111, segregating in 1:1 ratio, were obtained. The identificated SSR markers are potentially useful for the construction of molecular map of *Cucurbita maxima*.

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Inheritance of fruit skin colour in summer squash (*Cucurbita pepo* L.)

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Keywords: *Cucurbita pepo* L, inbreding, backcross, dark green fruit, light green fruit

Abstract

In this study, 10 inbreeding squash lines (*C. pepo* L.) which have light and dark fruit skin colored were used in order to clarify the inheritance of fruit skin colour. Summer squash lines (I6 level) were classified from light green to dark green for the criteria of fruit skin colour at mature stage of development. Inbred lines with light green fruits were crossed reciprocally with the dark green fruited lines in order to determine whether fruit skin colour inheritance had parental effect. In each cross combination, the F_1 plants were selfed to obtain F_2 and also backcrossed to each parent to produce BC_1. All F_2 and BC_1 generations were grown in the field and subjected to open pollination. Fruits at mature stage were harvested and their colours were scored in each individual plant. The Chi-square test was used to analyze the data. In this research, it was possible to say that genotype of the line 27 was l-1/l-1 l-2/l-2, line 39 was L-1/L-1 L-2/L-2.

INTRODUCTION

Fruit skin colour of *Cucurbita pepo* L. vary from dark green to light green with various kinds of striping, mottling and bicolour patterns. Fruit skin colour changes during the development (Paris et al. 1985). Paris (2000) reported that developmental fruit coloration in *C. pepo* was affected by thirteen *loci*. Some of them are D (Dark), l-1 (light coloration-1) and l-2 (light coloration-2). According to Paris and Nerson (1986) these three genes are inherited independently. Paris (1992) reported that Fordhook Zucchini possesses genotype D/D L-1/L-1 L-1/L-2 (Dark colored fruit) Beirut d/d l-1/l-1 l-2/l-2 (Light colored fruit), and Vegetable Spaghetti d/d l-1/l-1 l-2/l-2.

The aim of the present work was to clarify the inheritance of fruit skin colour in summer squash.

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MATERIALS AND METHODS

Experiments were conducted at the Agricultural Faculty of Akdeniz University. Plants were grown in both spring and fall seasoned, so that two breeding generations could be done in a year. Seeds of 70 inbred lines which were at I6 inbreeding level, were sown (20 plants perline). 10 lines were selected according to the criteria of very dark green and light green fruit color. These 10 lines were subjected to an additional inbreeding generation. These 10 lines were crossed reciprocally according to the fruit skin colour, ie dark green with light green, light green with dark green, in order to determine whether fruit skin colour inheritance had parental effect. In each cross combination, the F_1 plants were selfed to obtain F_2 and also backcrossed with the each parent to produce BC_1. All F_2 and BC_1 generations were grown in the field and subjected to open pollination. Fruits at mature stage were harvested and their colours were scored in each individual plant.

Pistillate and staminate flowers in the appropiate statge of development were tagged in the afternoon prior to anthesis to be used in pollination the following morning. They were prevented from opening by using pens at the tips of the corolla tube. The following day early in the morning, soon after dehiscence the polen sacs, transfer of the polen from anther to stigma were conducted. After selfing, the pistillate flowers were labelled and tied to prevent uncontrolled polination. In this research, the first pistillate flowers of the plants were used for pollination since the fruit set was usually better. If open pollinated fruits were already formed, they were removed to improve the fruit set of the controlled pollinations.

The results obtained from segregating progenies were subjected to chi-square analysis.

RESULTS AND DISCUSSION

In this study, dark green fruited lines were D16, D39, D49 and D57, while light green fruited lines were L25, L27, L28, L47, L52 and 65 (Fig.1). The results of crossings light and dark green fruited lines and backcrosses with parents are presented in Tables 1 and 2. In the reciprocal crosses of the light and dark green fruited lines, only dark green fruits were observed in the progeny regardless of the direction of the cross (Table 1). When the light fruited line was crossed with another light fruited line, F_1 plants were light fruited.

Table 1. The results for segregations in crosses of dark and light green fruited lines.

Crossings	Light green	Dark green
D 16 x L 25	0	10
L 25 x D 16	0	10
D 39 x L 27	0	10
L 27 x D 39	0	10
L 65 x D 39	0	10
D 39 x L 65	0	10
D 57 x L 25	0	10
L 52 x D 57	0	10
L 47 x D 49	0	10
L 27 x L 28	10	0

When dark fruited line 39 was backcrossed with (27x39), all resulting plants had dark fruited phenotype. When the line 39 was backcrossed with (65x39), 15 dark and 36 light fruited phenotypes was observed. This result is not in accordance with the 3:1 one gene ratio (x^2=0.529). When the line 39 was backcrossed with (39x65), dark and light fruit phenotypes segregated according to a 1:1 ratio (x^2=2.143).

When the light fruited line 25 was backcrossed with (25x16), (16x25) and (57x25) only dark fruited phenotypes was observed. The F_2 also segregated in accordance with a 1:0 ratio.

In this research, it was possible to say that genotype of the line 27 was l-1/l-1 l-2/l-2, line 39 was L-1/L-1 L-2/L-2 and there was a possitive correlation (78%) between the stem colour and the fruit skin colour. However, the genotypes of the other lines remain unknown and more studies must be conducted to fully understand the genetics of fruits color in summer squash.

Table 2. The results for segregations in backcrosses with parents.

Line Description	Number of Dark fruited	Plants Light Fruited	Expected ratio	Observed ratio	X^2
(27x39) x 39	84	0	1:0	1:0	-
(65x39) x 39	15	36	1:0	1:3	0.529
(39x65) x 39	45	60	1:0	1:1	2.143
(39x27) x 27	3	15	1:3	1:3	0.667
(25x16) x 25	60	0	1:3	1:0	-
(16x25) x 25	51	0	1:3	1:0	-
(57x25) x 25	30	0	1:3	1:0	-
(39x27)	15	0	7:9	1:0	-
(25x16)	0	102	7:9	1:0	-

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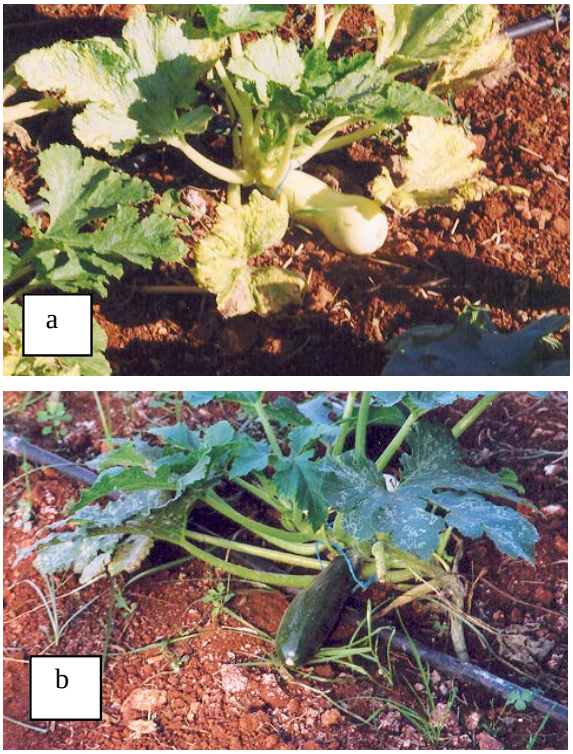

Fig. 1. Light fruited line 27 (a) and dark fruited line 39 (b).

Inheritance of resistance to *Papaya ringspot virus* in tropical pumpkin is controlled by at least two genes

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Keywords: *Cucurbita moschata,* squash, plant breeding, genetics, disease, potyvirus

Abstract

Tropical pumpkin (*Cucurbita moschata*) in Puerto Rico is often infected with viruses of the *Potyviridae* family. One of the most common is *Papaya ringspot virus* (PRSV). A previous study concluded that the African landrace 'Nigerian Local' (NL) carries a single recessive gene for resistance to PRSV. The study used the susceptible cultivar 'Waltham', and classified the F_2 into two phenotypic classes. When using NL to introgress PRSV resistance into cultivars of tropical pumpkin, we observed more than two classes of disease severity. Thus, the purpose of this study was to re-investigate the inheritance of PRSV resistance from NL. 'Waltham' and three susceptible tropical pumpkin cultivars were crossed with NL. Plants in the parental, F_1 and F_2 populations were classified for PRSV symptom severity on a 0 (no symptoms) to 3 (severe symptoms) scale. The four phenotypic classes (0, 1, 2 and 3) were grouped in several ways, and 1-gene and 2-gene models were tested. As in the previous study, a single recessive gene model (3:1 susceptible:resistant) fit the data from 'Waltham' x NL when the resistant class consisted of plants with a severity of 0 or 1 and the susceptible class combined plants with a severity rating of 2 or 3. But no single method of class grouping resulted in good fits of this 1-gene model to the tropical pumpkin F_2 populations. A 13:3 (susceptible:resistant) model (dominant suppression epistasis) provided the best overall fit of the data for both 'Waltham' and the tropical pumpkin populations. Plants with severity = 0 were classified as resistant, and plants with severity = 1, 2 or 3 were classified as susceptible. These results suggest that PRSV resistance in both 'Waltham' and tropical pumpkin cultivars is controlled by at least two genes: a dominant gene for resistance together with an epistatic dominant suppressor gene.

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INTRODUCTION

Resistance to *Papaya ringspot virus-watermelon strain* (PRSV-W=*Watermelon mosaic virus*-1=WMV-1) has been identified in *Cucurbita moschata* (Duchesne) (Costa 1974; Brown 2003), *C. maxima* (Maluf et al. 1984, 1985, 1986, 1997), *C. ecuadorensis* (Provvidenti et al. 1978) and *C. foetidissima* (Provvidenti et al. 1978; Maluf et al. 1986). Studies with *C. maxima* and *C. ecuadorensis* conclude that the inheritance of resistance within these sources is polygenic (Herrington et al. 1989; Maluf et al. 1985, 1997). Oliveira et al. (2003) also reported quantitative variation in a F_2 population when attempting to introgress genes from resistant *C. moschata* 'Duma' (a Brazilian cultivar) into *C. pepo*. In contrast to these previously mentioned studies, Brown et al. (2003) concluded that resistance from *C. moschata* 'Nigerian Local' (an African landrace) is controlled by a single recessive gene. Their study used *C. moschata* 'Waltham' (a butternut squash adapted to temperate areas) as the susceptible parent, and F_2 progeny were classified into only two phenotypes: resistant and susceptible. They suggested *prv* as the symbol for this source of resistance.

While attempting to introgress PRSV resistance from 'Nigerian Local' into tropical pumpkin cultivars, we observed more than just two phenotypic classes in F_2 populations, and deviations from the 3:1 (susceptible:resistant) segregation expected of *prv* as proposed by Brown et al. (2003). This led us to the objective of this research which was to re-investigate the inheritance of PRSV resistance from 'Nigerian Local' using both 'Waltham' and several tropical cultivars as susceptible parents.

MATERIALS AND METHODS

F_2 populations were developed from crossing PRSV-resistant 'Nigerian Local' with the susceptible parent 'Waltham' (as previously tested in Brown et al. (2003)) and three susceptible tropical pumpkin cultivars from the University of Puerto Rico tropical pumpkin breeding program: 'Soler', 'Taína Dorada' and 'Verde Luz'. 'Nigerian Local' was derived from seed originally provided by R. Provvidenti (formally of the New York Agricultural Experiment Station, Geneva).

The cotyledons of 7 day old seedlings were dusted with carborundum and rubbed with a mixture of PRSV infected tissue and buffer (1 g fresh wt. of inoculum per 10 ml of phosphate buffer). The PRSV isolate was collected in Puerto Rico and maintained on seedlings of 'Waltham'. Plants were kept in a greenhouse in Mayaguez, Puerto Rico with no artificial lighting. The experiment took place in late December through January when days are generally sunny. Daytime greenhouse temperatures were from 32 to 35 ˚C. The symptoms on each leaf were noted beginning on the 3rd true leaf at 7 days post-inoculation and continuing about every 3 days until 28 days post-inoculation (6th or 7th true leaf). Symptom severities

reported are from 28 days post-inoculation and were classified on a 0 to 3 scale (no symptoms to severe symptoms) as described in the footnote in Table 1. At day 28, each plant was tested with commercial ELISA (enzyme-linked immunosorbent assay) kits from Agdia (Elkhart, Indiana, USA) to confirm that plants were infected with PRSV and not with *Zucchini yellow mosaic virus* (ZYMV), the other common potyvirus in Puerto Rico and a potential contaminant.

Data from the four phenotypic classes of severity (0, 1, 2, 3) were grouped in various ways (for example, class 0 vs. classes 1+2+3) to test gene models with 2 or 3 phenotypic classes. A total of 11 models were tested using chi-square (α=0.05): two 1-gene models (3:1, 1:2:1) and nine 2-gene models (9:3:3:1, 9:4:3, 9:6:1, 7:6:3, 12:3:1, 15:1, 13:3, 11:5, 9:7).

RESULTS AND DISCUSSION

Symptoms began to appear on the 3rd true leaf about 7 days post-inoculation. Severity ratings in Table 1 are from 28 days post-inoculation. ELISA tests detected the presence of PRSV in most plants and did not detect contamination by ZYMV (data not shown). All plants of 'Nigerian Local' remained free of symptoms throughout the study and their ELISA tests were negative. However, some F_2 plants had positive ELISA readings despite severity ratings of 0. Plants of the susceptible cultivars had ratings greater than 0, and most plants had ratings from 2 to 3. Plants in the F_1 populations were susceptible (ratings > 0), with the exception of 'Taína Dorada' x 'Nigerian Local'. In the F_2, a 3:1 (susceptible:resistant) model fit 'Waltham' x 'Nigerian Local' (p=0.078) when plants were grouped into severity class = 0 *vs.* severity class = 1+2+3 and had an even better fit (p=0.609) when grouped into severity class = 0+1 *vs.* severity class = 2+3. However, these groupings did not always result in a good fit for the other populations. Of the eleven models tested, the best overall model was 13:3 (susceptible:resistant) (p=0.495, 0.415, 0.095, and 0.227 for the 'Waltham', 'Soler', 'Taína Dorada' and 'Verde Luz' F_2 populations, respectively). In this case, plants were grouped into severity=0 as the resistant class and severity=1+2+3 as the susceptible class. The results in the F_1 populations, with the exception of 'Taína Dorada' x 'Nigerian Local', also fit the 13:3 model.

The 13:3 model is known as "dominant suppression epistasis." A possible explanation for this 2-gene model is a dominant gene for resistance working together with a dominant suppressor gene negating the dominant resistance. Resistance would only be expressed when at least one dominant gene for resistance is present together with the homozygous recessive state at the suppressor locus.

In practical terms, selection for PRSV resistance has proven successful in our breeding program at the Agricultural Experiment Station at the University of Puerto Rico, Mayaguez. We have been able to develop PRSV-resistant F_4 families with good horticultural attributes by crossing our best tropical pumpkin cultivars with 'Nigerian Local' followed by several generations of backcrossing and selfing.

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Table 1. Number of plants in each severity class in parental, F_1 and F_2 populations of *Cucurbita moschata* inoculated with *Papaya ringspot virus*.

Population	No. of plants	Severity[z] 0	1	2	3
Parents:					
Nigerian Local	16	16	0	0	0
Waltham	18	0	0	0	18
Soler	20	0	2	11	7
Taína Dorada	19	0	0	2	17
Verde Luz	19	0	0	1	18
F_1:					
Waltham x Nigerian Local	6	0	0	0	6
Soler x Nigerian Local	20	0	0	12	8
Taína Dorada x Nigerian Local	14	3	6	3	2
Verde Luz x Nigerian Local	16	0	7	7	2
F_2:					
Waltham x Nigerian Local	53	8	4	9	32
Soler x Nigerian Local	130	28	21	37	44
Taína Dorada x Nigerian Local	124	16	14	25	69
Verde Luz x Nigerian Local	124	18	15	31	60

[z] The degree of symptom severity was rated 28 days post-inoculation on the 3rd to the 7th true leaf of seedlings using the following 0 to 3 scale: 0 = absence of virus symptoms on all leaves; 1 = small chlorotic flecks or spots on <10% of the leaf area with symptoms diminishing on newer leaves; 2 = large chlorotic flecks extending to complete vein clearing and/or coalescing chlorotic spots (mottling) on 10 to 25% of leaf surface, symptoms similar in each new leaf; 3 = mosaic, vein clearing, blistering and/or leaf distortion on more than 25% of the leaf area, new leaves with similar or more severe symptoms.

Effects of NaCl application on physiological, morphological and pomological features of Sanliurfa summer squash genotypes

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Keywords: Genetic resources, abiotic stress, *Cucurbita pepo*, salinity

Abstract

This research was conducted at GAP Agricultural Research Center of Turkish Ministry of Agriculture and Rural Affairs in Şanlıurfa-Turkey between 2011 and 2012 in pots under non heated greenhouse condition to determine effects of salinity on eight *Cucurbita pepo* genotypes collected from different locations of Sanliurfa-Turkey. At the 4-5 true leaves, salt treatments were applied. Non salt plots continued from sowing to harvest as a control plot. Both quality and physiologic/ vegetative characteristics were measured such as leaf temperature, leaf area, yield, TSSC, mean fruit weight, mean fruit height, fruit diameter and dry root weight/ fresh root weight were tested at two years. Also final drought resistance tests were examined. The research had four salinity categories in order to determine salt effect on genotypes (50 mmol, 100 mmol, 150 mmol and the control at 0 mmol) were applied. Class A pan used for determining amount of water for irrigation. At the end of the experiment all genotypes died at 100 mmol and 150 mmolsalt. Applying 50 mmol salt injured most of genotypes except number 8 (Sanlıurfa Mrkz.).

INTRODUCTION

The world squash production is about 22 million t from 1.7 million ha. Table 1 shows *Cucurbita pepo* production of different countries in the world (FAO 2010). In spite of improvement in new cultivars, it is necessary to study resistance to diseases and insects (Levi et al. 2001). So much irrigation, high ground water and poor quality irrigation water cause salinity. Southeastern Anatolia Region has severe climatic conditions with high temperatures (maximum temperature was observed in August as 46.8 ^{o}C) and very little rain (the lowest relative humidity was 33% in July) and still it has very little irrigated areas. With the beginning of irrigation in Sanliurfa-Harran, salin areas have been increasing. Total *Cucurbita pepo* production as a main crop in Sanliurfa is about 210 t and has been increasing (TUIK 2009).

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MATERIALS AND METHODS

This research was conducted at Koruklu research station of GAP Agricultural Research Centre (GAPTAEM) of Turkish Ministry of Food, Agriculture and Livestocks in Sanliurfa-Turkey. This experiment was carried out during the 2011 and 2012 production seasons. The altitude, latitude and longitude of the experimental site were 410 m, 36°42'N and 38° 58'E, respectively. Average field capacity, permanent wilting point, dry bulk density and pH of the site for 90 cm soil depth were 32.21%, 21.61%, 1.41 g/cm^3 and 7.55, respectively. Water quality at the site was good enough for irrigation with EC = 0.52 dS/m and pH = 7.26. Weather conditions at the site was hot and dry from May to September with air temperatures up to 46 °C and relative humidity averaging about 34%. Annual rainfall is about 380 mm.

Eighty summer squash genotypes were used in this study. *Cucurbita pepo* genotypes were collected from farmer's fields for this study from different regions of Sanliurfa-Turkey. The lists of genotypes are shown in Table 2. These genotypes are open pollinated.

In total, 480 pots were used for salinity tolerance test 30, 60 and 90 mmol NaCl was used as salinity applications. All pots were irrigated with non salt water until NaCI was applyed (4-5 true leaves). After formation of 4-5 true leaves, salinity treatments were applied to all pots except controls (S_0) designated to receive non salt water during vegetation. All pots were filled with white peat bedding substrate (for salt sensitive plants). Irrigation treatments were based on water content of each pot. Soil moisture content of pots was measured by WET Sensors. Irrigation water was applied by a measuring cup for each pot at every irrigation. Five plants per replication were planted and the middle three plants were used for harvesting. The additional plant/pot up and down the rows was considered buffers. Evapotranspiration was determined by Class A pan. The formula, improved by Karipçin et al. (2008), was used to evaluate data as an indicator of salinity tolerance. It can be formulated as salinity tolerance (ST %) = (S_1/S_0)*100. According to S_0 treatment the changing ratio between S_1 treatments for each genotype was obtained. If any genotype's changing rate (S_1/S_0) was near 1 that genotype was more tolerant to salinity than the others. The fruit diameter and height were scaled between 0.1 and 1. 1 point (score) was given for '0.1-0.2 (score)', 2 point for '0.3-0.4', 3 point for '0.5-0.6', 4 point for '0.7-0.8', 5 point for '0.9-1'. A total of 10 points were used for these features. Weight of fruit and TSSC were evaluated using a 0-10 reference point (scale). 1 point was given for '0.1', 2 point to '0.2', 3 point to '0.3', 4 point to '0.4', 5 point to '0.5', 6 point to '0.6', 7 point to '0.7', 8 point to '0.8', 9 point to '0.9' and 10 point to '1'. TTSSC was measured by refractometer. A total of 35 points was given for leaf temperature and the last resistance test data. '0.1-0.5 and 1' reference points were used; 1 point for 0.1, 5 point to 0.5 and 20 point to 1 was given for leaf

temperature. '0.1-0.3-0.5-0.8 and 1' reference point were used for the last test to classify the genotypes as tolerant or intolerant. 1 point was given for 0.1, 2 points to 0.3, 3 points to 0.5, 4 points to 0.8 and 5 points to 1 for the last resistance test. Genotypes which were given high point were determined tolerant. Point (scale) and reference points are showed in Table 3. According to the result of total score which were given genotypes for each evaluation all genotypes pointed from 0 to 100 and selected as tolerant genotypes which have high points. For each plot, 3 fruits were measured for fruit diameters by using a manual caliper to calculate the average of a genotype's fruit diameter. A digital balance was used to weight the fruits and calculate yield. Total leaf area of each genotype was measured by using a portable leaf area meter. Length of main stem was measured on 3 plants using a measuring tape. Leaf temperature was measured with an infrared thermometer once per week between 11:00 and 16:00 hours and the mean of temperature were taken for determining the difference between the genotypes. After all observations and measurements were taken resistance data was taken until all plants lost at least 75% of all leaves according to Proebsting and Middleton (1980).

RESULTS AND DISCUSSION

One genotype was selected and classified as saline tolerant (Table 4). The genotype called "Sanlıurfa Mrkz" had the highest resistance score at 78. The other genotypes such as Halfeti-2 and Bozova Mrkz rated 61 and 60 respectively and were considered middly tolerated genotypes. Five genotypes were marked as intolerant. The most susceptible (Tutluca-1 and Yaslıca) had a score of 25 and 26 respectively. Genotypes Konak, Halfeti-1 and Tutluca-2 were determined as intermediate tolerant. The genotypes with a point of 59 or higher were classified as tolerant. The lines below 50 were classified as intolerant genotypes (Table 4). The decreases in shoot dry matter production as a consequence of the NaCl treatment were higher than the decreases in root growth. The results of this experiment showed a similarity with research and result of Mutlu and Bozcuk (2000). At the end of the experiment, it was clear that increasing amount of salinity negatively affected dry weight. This result was similar to the results of Erdal et al. (2000). Growth was inhibited by salinity as reported by Turkmen et al. (2000).

CONCLUSION

Taking into account all 9 measured features, Sanliurfa, native *Cucurbita pepo* genotypes, was determined to have potential salinity tolerance in high temperature greenhouse conditions. As a conclusion, genotypes were classified as tolerant and intolerant the genotype 'Sanlıurfa Mrkz' was found more tolerant than others. The genotypes 'Halfeti-2' and 'Bozova Mrkz' were classified as middle tolerant and the genotypes 'Konak', 'Halfeti-1', 'Tutluca-2', 'Yaslıca' and 'Tutluca-1' were selected as intolerant.

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Table 1. Pumpkins and squash production data of different countries in the World.

Countries	Amount of Production (X1000 Ton)	%
China	6150	27.4
India	4424	19.7
Russian Fed.	988	4.4
USA	779	3.5
Turkey	430	1.9
Others	9642	43.1
Total	22413	100.0

Table 2. The summer squash genotypes used in this study and their origins

Acces.No	Name	Origin
1	Tutluca 1	Tutluca-Bozova
2	Tutluca 2	Tutluca-Bozova
3	Halfeti 1	Halfeti
4	Halfeti 2	Halfeti
5	Bozova Mrkz.	Bozova
6	Yaslıca	Yaslıca-Bozova
7	Konak	Konak-Mrkz.
8	Sanlıurfa Mrkz.	Sanliurfa

Table 3. Pointing (score) and reference points at evaluation and calculation

Leaf Temp.		Fruit Weight		Leaf Area		TSSC (measured on Fruit)		Dry Root Weight / Fresh Root Weight	
Ref-Po.	Po.	Ref-Po.	Po.	Ref-Po.	Po.	Ref-Po.	Po.	Ref-Po.	Po.
0.1	1	0.1	1	0.1	2	0.1	1	0.1	2
0.5	5	0.2	2	0.2	4	0.2	2	0.2	3
1	20	0.3	3	0.3	6	0.3	3	0.3	5
Fruit Diam.		0.4	4	0.4	8	0.4	4	0.4	7
Ref-Po.	Po.	0.5	5	0.5	10	0.5	5	0.5	9
0.1-0.2	1	0.6	6	0.6	8	0.6	6	0.6	10
0.3-0.4	2	0.7	7	0.7	14	0.7	7	0.7	12
0.5-0.6	3	0.8	8	0.8	16	0.8	8	0.8	13
0.7-0.8	4	0.9	9	0.9	18	0.9	9	0.9	14
0.9-1	5	1	10	1	20	1	10	1	15

Table 4. The salt tolerance of Sanlıurfa native *Cucurbita pepo* genotypes (point-score from 99 to 70 are selected tolerant, points from 50 to 25 are selected as intolerant)

Acces. No	Point	Acces. No	Point
8	78	3	42
4	61	2	33
5	60	6	26
7	49	1	25

Effects of roasting on nutritional composition of seven lines of pumpkin (*Cucurbita pepo* L.) seeds

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Keywords: Pumpkin seed, *Cucurbita pepo* L., oil content, fatty acid, protein, vitamin E, nutrition, roasting

Abstract

In this study, changes between chemical composition of raw and roasted pumpkin seeds (*Cucurbita pepo* L.) was investigated among 7 pumpkin lines (2 naked). Studies were carried out between 2007 and 2010 in Ankara.

In this research, the following compositions were evaluated as chemical characteristics: humidity, total oil ratio and composition of oil acids, protein, vitamin E and mineral substance content (Fe, Mn, Mg, K, P).

It was concluded for the pumpkin seeds that total oil ratio was high (35-48%), oleic acid (40-58%) and linoleic acid (30-40%) were dominant oil acids, the composition of seeds were 35-40 % protein and they were rich in magnesium, potassium and phosphorus, compared to other elements. It is observed that the roasting process affected the composition of components in the lines which were sensitive to the temperature; however, through roasting, the membrane layer surrounding the seed was removed, then it facilitated cracking and brought some characteristics such as colour, aroma and texture unique to the pumpkin seed, raw taste disappeared and it became more resistant to the proper storage conditions through reduction of humidity.

INTRODUCTION

Pumpkin seeds are used for human consumption as a snack food in many countries, but are especially popular in Middle Eastern and Arabian countries, after salting and roasting (Al Khalifa 1996). It is also consumed as a snack in Turkey and it is widely grown. According to the Turkish statistical data, total pumpkin and squash production for Turkey is 430.402 tones and the pumpkin seed has a share of 26.694 tones for snack in production (Anonymous 2010 and 2011).

Pumpkin seeds are excellent sources of both oil (37.8-45.4%) and protein (25.2-37.0%) (Lazos 1986). Seed oil is composed mainly of unsaturated fatty acids

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and palmitic acid ($C_{16:0}$, 9-5-14.5%), stearic acid ($C_{18:0}$, 3.1-7.4%), oleic acid ($C_{18:1}$, 21.0-46.9%) and linoleic acid ($C_{18:2}$, 35.6-60.8%) are the dominant fatty acids. These four fatty acids make up 98% of the total acid (Murkovic et al. 1999). Pumpkin seed oil also contains high amounts of tocopherols.

Pumpkin seed oil provides many health benefits. One of the most notworth attributed reported for pumpkin seed oil is preventing the growth and reducing the size of prostates (Younis et al. 2000). Pumpkin seed has been used in traditional medicine in North America and Mexico as an antihelmintic agent and for supportive treatment in functional disorders of the bladder. Also anti-inflammatory and cardio protective activity of pumpkin seeds are discussed (Winkler et al. 2005). The content of vitamin E in pumpkin seeds is very high (Murkovic et al. 1999) and is reported to have capacity to quench free radical damage, and play a putative role in prevention of alzheimer's disease and cancer. Therefore a pumpkin variety with high Vitamin E content is desirable that can be used as a nutraceutical (Murkovic et al. 1999).

Pumpkin seed oil has been produced in Austria, Slovenia and Hungary (Murkovic et al. 1999). Although none of these oils are utilized on an industrial scale, many are used as cooking oil in some countries in Africa and the Middle East (Alfawaz 2004) and they are also used as salad oil in the South of Austria and Slovenia and Hungary (Sabudak 2007).

Very limited research has been done on pumpkin in Turkey because of its low acreage and small economic importance. Turkey has favorable climatic and agricultural conditions for the cultivation of a wide variety of pumpkins. Pumpkin is a snack that has been grown for the last 25 years in Nevsehir. Recently, it is grown in Kayseri, Aksaray, Konya, Sakarya and Edirne besides Nevsehir and is utilized across the country.

Various chemical reactions are induced by roasting. Yoshida et al. (2002) reported that polyunsaturated fatty acids in vegetable oil are higher, and the rate of quality deterioration of the oils after roasting was greater. Besides, tocopherols are particularly sensitive to heating at high temperatures (Barrera-Arellano et al. 2002). As a result, most tocopherols are lost or destroyed during roasting (François et al. 2006). Some reports suggest that nutrient retention is improved during roasting (Gould and Golledge 1989), however other studies indicated that nutrient retention is not much greater than that of unroasted seeds (Thompson 1982).

The purpose of the present study was to examine the influence of roasting on the composition of fatty acids, vitamin E, protein, mineral components and humidity of the seven different local varieties of pumpkin seeds. This study will help compare the aforementioned chemical characteristics among roasted and unroasted pumpkin seeds.

MATERIALS AND METHODS

Samples

In this study, 5 coated (3, 5, 7, 10, 14) and 2 naked (3K, 5K) seed pumpkin (*C. pepo* L.) lines which obtained from the pumpkin seed selection studies made between 1993-2006 in Ankara University, Faculty of Agriculture, Department of Horticulture were used for plant material.

Pumpkins were grown in Ankara (latitude 39º57′46″ N, longitude 32º51′56″ between 2007 and 2008. The ripe pumpkins were cut and then the seeds were separated in each of lines. The separated seeds were washed and air-dried at room temperature. The dried seeds were kept at +4ºC and used as the stock seed samples for further analyses. Laboratory analysis was conducted at Palancı Food Technology Research and Development Trade Inc.

Seeds were roasted in Palancı Food Technology Development and Trade Inc. For each experiment 500 g of seeds were roasted at 120ºC for five minutes, 24 hours before the extraction of oils. To protect tocopherols, the roasted seeds were hermetically sealed in plastic packaging.

Chemical Analysis

The moisture content was determined by the high- temperature (130ºC, 1h) oven method (Anonymous 1996).

Crude oil was extracted from dried powdered seeds. 20 g of the seeds were extracted in a Soxhlet aparatus using n-hexane as a solvent. The solvent was removed at 60 ºC and 120 rpm by a rotary evaporator (Heidolph Laborota 4000). The fatty acid composition was studied by gas chromatography.

The fatty acids were analyzed in an Agilent 7890A gas chromotography (split mode 1/80) equipped with a flame ionization detector (FID). The components were separated in an HP-88 capillary column (60m with internal diameter of 250 mm, film thickness of 0.25). The injector temperature was 250 ºC and the temperature of the detector was 230ºC. The determination involved temperature programming; 120ºC for 1 min; temperature was increased 10ºC per min up to 175ºC; 5ºC per min up to 210ºC and 210ºC for 5 min. Carrier gas Helium was used at a constant pressure of 1 bar.

The Kjeldahl method was used for protein content analysis. The protein compositions were obtained multiplying these values by the coefficient 6.25 (Anonymous, 2007).

The Vitamin E content was determined by direct injection of the oil samples into an HPLC. In brief, oil samples weighing 1 g were dissolved in a 9 ml acetone and vortex mixed for 30 second. 1.5 µl of this solution were injected onto the column. The used HPLC equipment was an Agilent Eclipase 1200 liquid chromatograph with a fluorescence detector (Agilent Technologies), equipped with an Agilent Eclipase

XDB-C18 column (5 µl, 150x4.6mm). The column was eluted with a mobile phase of 480 ml HPLC Methanol+480 ml HPLC Acetonitrile+40 ml HPLC water+ 0.2 ml phosphoric acid. The flow rate was 1.5 ml/min and the effluent was monitored at an excitation wavelength 250 nm and an emission wavelength of 410 nm for the determination of tocopherol. The relative amounts of tocopherols were calculated using an external standard method using reference samples of tocopherol and was used to calculate the peak areas (Anonoymous 1988).

Mineral elements were estimated using the AOAC (2005) method. The minerals iron, potassium, zinc and magnesium were determined by Atomic absorption spectrophotometry (Varian 240 Australia). Phosphorus was measured by converting phosphates into phosphorus molybdate blue pigment and assayed 700 nm.

Duncan multiple range test was performed using CoStat as a randomized complete block design ($p \leq 0.05$) in order to compare lines.

RESULTS AND DISCUSSION

Analysis of variance showed significant differences ($p \leq 0.05$) among the species for all chemical analysis. In the experiment, the moisture content of the seeds were similar in both the years.The water activity of raw seeds was 5.38-7.24% decreased considerably during roasting only 1.72-3.01% for two years. Thus, in both cases the seeds were stable with respect to potential growth of micro-organisms (Guiraud 1998).

The compositions of protein and vitamin E concentrates from pumpkin seeds are presented in Table 1. The protein content of raw pumpkin seeds studied ranged from 33.73-36.60% and 31.02-36.48 % in 2007 and 2008 years, respectively. The pumpkin seeds contained a high percentage of crude protein. Similar values for protein contents of pumpkin seeds were reported by Lazos (1986), Younis (2000) and Alfawaz (2004).

Generally, the concentrations of proteins depended on the line, roasting increased the crude protein content of pumpkin seeds. The level of protein content of roasted samples was found to be 32.12-39.99% in both years. The increment of protein content of samples may be due to the heat treatment.

In both years, HPLC analysis showed vitamin E content between 2.85-4.47 mg/100g. The vitamin E content in line 3K (naked line) contained signifacantly more tocopherol than the other lines. Genetic factors most explain these differences. In fact, Nakic et al. (2006) for pumpkin seeds showed that tocal tocopherol contents in vegetable oils depend on different factors (cultivar, climate, extraction conditions, method of determination).

Vitamin E has been the subject of many studies describing their content in plant materials and some papers described the effect of physical treatments like

frying (Barrera-Arellano et al. 2002), boiling (Fagbemi 2007) and subjecting them to microwave heating (Yoshida et al. 2002, 2005). Our results showed that 120ºC for 5 minutes affected the content of vitamin E and did not remain constant. The vitamin E contents by preferentially increasing for roasted seeds. Yoshida et al. (2002) and François et al. (2006) reported that vitamin E showed highest rate of loss during microwave heating. However, Lee *et al.* (2004) reported that the contents of tocopherol in safflower oil gradulally increased as roasting temperature increased up to 160ºC but then decreased up to 260 ºC. Murkovic et al. (2004) reported that when the oil emerges from the cellular structure, the vitamin E concentration increased, because of an increased extraction efficiency. Therefore, these results sugggested that changes of tocoherol contents in the oils were dependent on roasting temperature and processing time.

Crude oil contents may have changed depending on the lines for two years (Table 2, 3). It ranged from 37.41-45.74% in 2007 and 35.4641.45% in 2008. The total fat contents of pumpkin seeds were higher (40-50%). These results are confirmed by the findings of Loy 1990; Idouraine et al. 1996, Abak et al. 1999; Murkovic et al. 1999; El-Adawy and Taha 2001; Fruithwirth and Hermetter 2007; Yegül 2007). Results shown in Table 2-3 revealed that roasted pumpkin seed oil content was decreased or increased depending on the lines. Anjum et al. (2006) reported that roasting decreased the oil content of seeds significantly. However, Badifu (2001) reported that the contents of oils increased 3-7% in all *Cucurbitaceae* species. Due to the increase in the volume of intercellular spaces, oils were released from lipid bodies during heating as reported by Saklar (2002). As for Artık (2004), the increase of oil content in hazelnut with roasting, proceeded from increase of oil content concentration due to the loss in moisture.

The fatty acid composition of control and roasted pumpkin seed oils are in Table 2 and 3. Differences between the fatty acid contents of pumpkin lines were statistically significant at a 5% error limit. The dominant fatty acids that are found in pumpkin seed oil are palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. This observation closely agreed with the observation of Murkovic et al. (1999) and Younis et al. (2000).

The fatty acid composition of oil can be an indicator of its oxidative stability and nutritional quality. Generally, it is accepted that the higher degree of unsaturation of an oil, the more susceptible it is to oxidative deterioration. Total unsaturated fatty acids content ranging from 80-85 %, saturated fatty acids (palmitic and stearic acids) were determined in the rate of 10-15%. Total unsaturated fatty acids of the 7 pumpkin lines we studied was similar to other studies (Lazos 1986; Idouraine et al. 1996; Abak et al. 1999; El-Adawy and Taha 2001; Fruhwirth 2003; Alfawaz 2004; Glew et al. 2006; Nakic et al. 2006; Sabudak, 2007; Badr et al. 2010). The composition of oleic and linoleic acid was found to be in the range of 41.65-56.26%

and 26.02-43.35% respectively in raw seeds.

Significant differences ($p \leq 0.05$) also occurred in the fatty acid distribution between roasted lines of pumpkin. Oleic and linoleic acids were more affected by roasting than palmitic and stearic acids. Generally, after roasting oleic acid was decreased, whereas linoleic acid contents were increased in most of the lines. However in contrast to our results Anjum (2006) reported that microwave heating increased oleic acid and decreased linoleic acid. As for Murkovic et al. (2004), the contents of palmitic, stearic and oleic acids remained stable, only linoleic acid was decreased. Yoshida and Kojimoto (1994) and Kim et al. (2002), found no differences in fatty acids composition of rice germ and sesame seed oils prepared at different roasting temperatures and times. These results suggest that the differences in pumpkin cultivars could be appreciable, based on the fatty acid distribution.

The results of nutritionally valuable minerals are presented in Table 4-5. Pumpkin seeds contained significant concentrations of minerals. Although only fair amounts of iron (Fe) and zinc (Zn) were present, pumpkin seeds were good sources of some other minerals, especially phosphorus (P), potassium (K) and magnesium (Mg). Minerals are important in the diet because they serve as cofactors for many physiologic and metabolic functions and in their absence, clinical deficiencies may occur (Christian et al. 2007).

It was found that the concentrations of minerals depended on the line. Potassium (K) content of raw pumpkin seed was found to be 488.33-1340.00 mg/100 g in both years. Results obtained for potassium (K), naked pumpkin seeds contained higher than unnaked seeds. Varietal genetic diffrence may be the major factor. Similar values for potassium (K) contents of pumpkin seeds were reported by Giami and Wachuku (1997). Generally total K were significantly ($p \leq 0.05$) increased, but some lines decreased when the seeds were roasted. Both roasted and unroasted pumpkin seeds contained significantly high amount of phosphorus. Magnesium (Mg) content of pumpkin seeds ranged between 410.70-719.00 mg/100 g in both years. Increased or decreased Mg content of roasted samples may be due to the effect of heat treatment. The least abundant minerals were zinc (Zn) and Iron (Fe), which ranged between 3.93-10.0 mg/100 g and 5.34-10.67 mg/100 g respectively.

In conclusion, obtained results in this study indicated that pumpkin seeds are rich sources of nutrients. Processing affects the level of nutrients in pumpkin seeds. However, through roasting, the membrane layer surrounding the seed was removed, then it facilitated cracking and brought some characteristics such as colour, aroma and texture unique to the pumpkin seed, raw taste disappeared and it became more resistant to the proper storage conditions through reduction of humidity.

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Table 1. Effect of roasting on moisture, protein and vitamin E contents.

	Lines	Moisture		Protein		Vitamin E	
		Raw	Roasted	Raw	Roasted	Raw	Roasted
2007	3	6.23 ab	2.44 c	33.73 d	36.32 b	3.72 b	13.99 c
	5	6.15 ab	2.88 ab	35.53 b	35.37 b	3.75 b	13.68 cd
	7	6.12 ab	3.01 a	36.38 a	33.87 c	4.02 ab	15.41 b
	10	6.01 ab	2.53 c	34.77 c	37.47 a	4.14 ab	12.75 d
	14	6.51 a	2.06 d	33.80 d	38.03 a	4.17 ab	17.52 a
	3K	5.88 b	1.80 d	36.60 a	35.90 b	4.47 a	17.90 a
	7K	5.38 c	2.67 bc	34.90 c	36.27 b	3.79 b	13.93 c
	LSD (5%)	0.485	0.257	0.396	0.905	0.467	1.097
		Moisture		Protein		Vitamin E	
		Raw	Roasted	Raw	Roasted	Raw	Roasted
2008	3	7.24 a	1.74 d	33.55 c	34.17 d	2.85 c	14.12 d
	5	6.55 c	2.35 a	33.56 c	32.12 e	3.74 a	14.18 d
	7	6.73 bc	2.27 ab	34.79 b	32.82 e	3.41 b	16.95 bc
	10	7.06 ab	1.88 cd	35.00 b	35.75 c	3.75 a	11.81 e
	14	6.97 abc	2.32 a	31.02 d	32.50 e	3.03 c	17.97 b
	3K	5.85 d	1.72 d	36.48 a	39.99 a	3.87 a	23.46 a
	7K	6.13 d	2.03 bc	35.73 ab	38.80 b	3.48 b	16.25 c
	LSD (5%)	0.410	0.244	1.093	1.001	0.244	1.352

Values having different superscript letters in a column differ significantly at $p \leq 0.05$

Table 2. Effect of roasting on crude oil and fatty acid distrubution (2007).

Lines	Crude oil		Palmitic ($C_{16:0}$)		Stearic ($C_{18:0}$)		Oleic ($C_{18:1}$)		Linoleic ($C_{18::2}$)	
	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted
3	37.41 e	38.44 f	13.90 b	14.39 a	5.24 e	5.37 e	43.62 e	43.14 d	36.39 c	36.09 cd
5	39.37 d	38.06 f	12.11 e	11.70 e	6.21 a	6.26 a	47.67 c	44.44 c	33.13 e	36.69 c
7	39.60 d	48.07 a	14.62 a	13.95 b	5.47 d	4.86 f	41.65 f	39.49 f	37.43 b	40.83 as
10	41.11 c	40.38 e	12.47 d	13.86 b	6.08 b	5.93 b	47.01 d	41.05 e	33.54 d	38.10 b
14	42.59 b	42.26 d	13.88 b	12.87 cd	5.15 f	4.99 f	40.82 g	45.86 b	39.57 a	35.40 d
3K	45.74 a	47.05 b	12.81 c	13.07 c	5.58 c	5.73 c	49.26 b	47.65 a	31.12 f	31.97 f
7K	42.10 b	46.15 c	12.69 f	12.57 d	5.57 c	5.55 d	51.50 a	47.85 a	30.83 g	33.09 e
LSD (5%)	0.853	0.654	0.0568	0.300	0.0578	0.142	0.267	0.793	0.0858	1.079

Values having different superscript letters in a column differ significantly at $p \leq 0.05$

Table 3. Effect of roasting on crude oil and fatty acid distrubution (2008).

Lines	Crude oil		Palmitic ($C_{16:0}$)		Stearic ($C_{18:0}$)		Oleic ($C_{18:1}$)		Linoleic ($C_{18::2}$)	
	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted
3	35.46 d	39.86 c	12.62 a	12.49 a	5.67 d	5.84 e	44.29 g	46.05 g	37.42 a	35.63 a
5	37.85 c	41.27 b	11.40 c	11.81 c	5.11 f	5.62 g	51.37 e	49.38 d	32.12 c	33.20 d
7	35.63 d	37.86 b	11.32 c	11.84 c	5.93 c	6.33 b	51.82 d	48.46 e	30.94 d	33.36 c
10	35.41 d	37.21 c	11.42 c	11.64 d	5.52 e	6.16 d	52.72 c	51.74 c	30.35 e	30.46 e
14	34.53 e	38.56 d	12.29 b	12.41 b	5.70 d	5.67 f	48.52 f	48.07 f	33.48 b	33.86 b
3K	41.45 a	45.07 a	10.54 e	10.35 e	7.19 a	7.03 a	56.26 a	55.99 b	26.02 g	26.63 g
7K	39.48 b	45.27 a	11.30 d	10.33 e	6.50 b	6.19 c	54.34 b	56.68 a	28.46 f	26.80 f
LSD (5%)	0.619	0.655	0.115	0.0687	0.060	0.0289	0.178	0.104	0.128	0.0415

Values having different superscript letters in a column differ significantly at $p \leq 0.05$

Table 4. Effect of roasting on minerals on pumpkin seeds (2007).

Lines	Fe		Zn		Mg		K		P	
	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted
3	9.30 b	6.73 f	7.53 b	5.33 e	544.67 de	587.00 b	527.00 d	599.67 c	1.133.00 d	1.313.67 c
5	10.67 a	8.97 c	10.00 a	6.57 c	570.00 c	583.33 b	581.00 c	625.33 c	1.216.67 c	1.255.33 d
7	10.20 a	8.00 d	7.03 bc	6.30 d	536.67 e	587.33 b	595.33 c	521.33 d	1.127.67 d	1.263.00 d
10	6.01 e	7.27 e	4.80 d	7.23 b	556.67 cd	547.67 d	488.33 e	661.33 b	1.156.67 d	1.237.00 d
14	8.33 c	10.02 a	7.30 b	7.87 a	510.33 f	495.33 e	560.33 cd	530.00 d	1.174.00 cd	1.022.33 e
3K	7.90 c	9.53 b	6.27 c	6.37 d	613.67 b	567.33 c	995.67 a	730.00 a	1.663.00 b	1.393.67 b
7K	6.83 d	7.87 d	7.27 b	6.57 c	719.00 a	603.33 a	636.33 b	486.67 e	1.751.33 a	1.639.00 a
LSD (5%)	0.804	0.238	0.889	0.157	17.985	13.619	36.646	28.835	53.622	35.046

Values having different superscript letters in a column differ significantly at p≤0.05

Table 5. Effect of roasting on minerals on pumpkin seeds (2008).

Lines	Fe		Zn		Mg		K		P	
	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted
3	8.58 a	7.23 c	5.18 b	4.92 e	578.10 ab	490.83 bc	886.90 c	741.60 de	1.006.1 a	1.042.6 ab
5	6.40 b	8.65 b	4.34 bc	5.15 cde	410.70 d	471.27 cd	607.40 d	742.07 de	846.77 d	1.067.6 a
7	6.50 b	8.87 b	3.93 c	5.00 de	499.33 c	459.27 cd	777.20 c	660.80 e	871.63 cd	1.074.0 a
10	5.72 bc	8.96 b	4.60 bc	7.10 a	520.13 bc	539.27 b	784.03 c	824.30 d	913.20 b	943.07 c
14	5.34 c	10.56 a	6.11 a	5.72 c	633.13 a	625.13 a	852.60 c	1057.6 c	1.028.00 a	1.056.3 a
3K	6.14 bc	11.00 a	4.68 bc	5.63 cd	452.87 cd	503.33 bc	1.180.6 b	1480.3 a	927.40 b	969.30 bc
7K	8.52 a	11.56 a	4.76 bc	6.40 b	469.30 cd	432.43 d	1.340.0 a	1348.0 b	907.97 bc	994.27 abc
LSD (5%)	0.985	1.037	0.844	0.604	62.357	48.003	144.57	121.741	37.141	79.825

Values having different superscript letters in a column differ significantly at p≤0.05

Some morphological and fruit parameters of pumpkin genotypes

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Keywords: Cucurbita, genetic resources, characterization, variability

Abstract

In this research, 28 pumpkin genotypes collected from different regions of Turkey were used. In this genotypes, size of leaf, color of leaf blade, color of leaf petiole, length of leaf petiole, plant habitus, some flower and stem traits were determined. Average fruit length was 19.49 cm, average fruit diameter was 16.61 cm and average thickness of fruit skin was found 6.70 mm for genotypes.

INTRODUCTION

Cucurbitaceae family consists of about 118 genera and 825 species (Jeffrey 1990). Squashes (*Cucurbita* spp.) are members of the *Cucurbitaceae* family (Balkaya et al. 2010; Aliu et al. 2011; Sari et al, 2008; Aliu et al, 2012) and according to archaeological recordings, *C. pepo* is the first domesticated species in Cucurbitaceae (Aliu et al. 2011).

The most morphologicaly variable genera is Cucurbita (Esteras et al. 2012). *Cucurbita maxima* was the only cultivated species with a native range restricted to South America, in the warm temperate areas of Uruguay and Argentina. *Cucurbita moschata* was native to the low lands of tropical and sub-tropical America (Mexico and South America) (Bisognin 2002).

Turkey, China, India, Russian Federation, America, Iran, Egypt, Mexico, Ukraine and Italy are the major producers of Cucurbita (Faostat 2010). Turkey is the 10th largest Cucurbita producer with 430,402 tons.

Turkey is very rich in cucurbit genetic resources and is an important diversity center due to climatic conditions (Sari et al. 2008). There are wide variations in fruit characters especially skin colour and shape in Cucurbita (Whitaker and Davis 1962, İnan et al. 2012; Balkaya et al. 2010). Morphological characterization is very important to classify genetic resources (Balkaya et al. 2010).

The objective of this study was to characterize a collection of pumpkin populations from different geographical zones in Turkey (Eskişehir, Tekirdağ, Diyarbakır, Rize, Çanakkale, Ordu).

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MATERIAL AND METHODS

This study was carried out in open fields of Bati Akdeniz Agricultural Research Institute (BATEM) in 2011. In this study 37 different pumpkin genotypes were used. Ten seeds of each population were sown on March 18 and seedlings of pumpkin genotypes were planted at 2-3 leaf stage at a spacing of 2.8 m x 2.0 m on April 11 in 2011. Standard fertilization and cultural practices were applied during the growing season. Observations on vegetative morphological characteristics were evaluated second month after transplanting. Leaf blade size, leaf petiole lenght, male peduncle length were measured with ruler. Fruit traits (fruit diameter, fruit length, skin thickness, flesh thickness, length of seed cavity, fruit weight) were recorded at physiological maturity. Three plants per genotype were measured. Fruit traits except fruit weight were measured with digital caliper. The harvest period lasted from 15 June to 20 June. Vegetative morphological features (Leaf blade size, leaf petiole lenght, male peduncle length) and variability of fruit traits (fruit diameter, fruit length, skin thickness, flesh thickness, length of seed core, fruit weight) of Cucurbita L. species were given Table 1, 2 and 3. The mean value and standard deviation for leaf blade size, leaf petiole lenght, male peduncle length, fruit diameter, fruit length, skin thickness, flesh thickness, length of seed core, fruit weight were determined.

RESULTS AND DISCUSSION

Growth habit, leaf blade size, upper leaf surface color, petiole and stem color,, and male flower petiole color were examined in collected genotypes. Growth habit of the collected genotypes was mainly semi-bush and prostrate. In this study the green color of the upper leaf blade surface was generally dark green. Leaf petiole, stem, and male flower petiole color was generally light green. Ferriol et al. (2004) reported that the majority of characterized genotypes from Spain growth habit was semi-bush and prostrate. In our study we have obtained observation compatible with Ferrial et al. (2004).

There was variability in fruit characteristics. The highest value for fruit diameter was 37.4 cm and the lowest was 8.3 cm. Avarage fruit length was 19.49 cm. Average fruit weight was 2.17 kg and the heaviest fruit weight was from the genotype 24 with 5.70 kg. This genotype was also the longest. In this population average skin thickness was 6.71 mm and average flesh thickness was 21,87 mm. Aliu et al. (2011) reported that average flesh thickness ranged from 3.46 to 1.83 cm. In our study we have obtained similar results with Aliu et al. (2011).

Fundamental asis of plant breeding is identification of population features (Tsivelikas *et al.* 2009). The discussed features should be helpful in selection. The discussed characterized materials will be used in future rootstock breeding programs.

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Table 1. Vegetative morphological features of *Cucurbita* L. species.

Genotypes	Leaf blade size (cm)	Leaf petiole lenght (cm)	Male peduncle length (cm)
1	22	29	17
2	28,5	32	13,5
3	23,4	30	17
6	29	19	12
7	29	17	27,5
8	27	25	19
9	26	22	19
10	27	31	19
11	25,5	24	24,5
14	20	31	14
16	29	13	14,5
17	30,5	30	15
18	28,5	26	17
19	22	19	20
20	25	27	24
21	30,5	19	16
22	27	32	18
23	30	32	22
24	27	17	12
25	19	21	15
26	17	18	13
27	18	17	16
32	27	27	16
33	23	32	10
34	28	25	12
35	25	26	14
36	29	24	12
37	25	31	30
Standard deviation	3,81	5,77	4,88
Mean	25,64	24,86	17,11
CV(%)	14,85	23,22	28,54

Table 2. Frequency distribution of discrete description in *Cucurbita* genotypes.

Descriptor	Score code	Descriptor state	Frequency (%)
Growth Habit	1	Bush	14,28
	2	Semi-bush	46,42
	3	Prostrate	39,28
The Green Color of the Upper Surface of Leaf Blade	1	Light	25,00
	2	Medium	32,14
	3	Dark	42,85
The green color of the leaf petiole	1	Light	89,28
	2	Medium	10,71
The green color of stem	1	Light	60,71
	2	Dark	39,28
Male flower petiole color	1	Light green	89,28
	2	Green	10,71

Table 3. Variability of some fruit traits of *Cucurbita* species.

Genotypes	Fruit diameter (cm)	Fruit length (cm)	Skin thickness (mm)	Flesh thickness (mm)	Length of seed cavity (mm)	Fruit weight (kg)
1	12.70	19.00	1.76	22.55	72.35	1.300
2	12.40	21.20	5.62	20.06	65.02	1.050
3	13.60	14.70	5.60	19.06	60.12	1.500
6	12.50	19.50	9.37	8.90	7.12	0.880
7	14.40	21.50	3.23	21.19	101.72	1.350
8	15.80	13.50	11.13	13.44	89.89	2.030
9	10.40	10.22	8.10	12.63	68.54	1.080
10	8.30	15.40	9.83	7.19	85.69	1.380
11	9.00	14.30	12.31	2.23	91.77	1.300
14	14.00	17.00	4.59	20.21	119.98	1.680
16	11.60	24.40	6.20	10.68	64.95	2.790
17	11.00	15.00	2.35	27.89	99.00	2.010
18	15.50	16.00	12.70	2.36	95.07	1.310
19	13.90	14.12	5.24	12.22	84.01	0.820
20	29.70	34.20	6.12	71.29	84.73	3.770
21	33.30	36.80	5.01	21.49	96.65	4.390
22	31.00	36.50	5.55	24.35	80.34	3.460
23	37.40	38.00	4.90	65.78	52.27	2.370
24	25.00	34.30	13.67	33.23	156.09	5.700
25	8.00	15.00	7.40	22.63	101.62	1.250
26	11.50	17.00	6.39	10.46	74.65	1.200
27	17.50	19.90	6.73	20.64	128.00	2.880
32	12.00	19.00	11.18	16.58	137.49	1.380
33	14.50	8.90	11.36	10.21	100.50	1.150
34	20.60	11.80	3.19	24.09	110.31	4.390
35	11.00	15.00	2.35	27.89	99.00	2.010
36	18.80	10.00	3.32	24.64	88.77	2.500
37	19.80	13.50	2.57	38.55	72.10	3.700
Mean	16,61	19,49	6,71	21,87	88,85	2,17
Standard deviation	7,68	8,45	3,43	15,47	27,97	1,25
CV(%)	46,2	43,3	51,1	70,7	31,4	57,6

Determination of heterotic groups in summer squash (*Cucurbita pepo* L.) using morphological and molecular methods

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Keywords: Summer squash, morphologic-molecular characterization, SRAP

Abstract

Genetic distance of parental lines is crucial for heterosis breeding to be successful. Thus, it is important to do morphological and molecular characterization in order to determine differences and relationships of squash genotypes representing major gene pools.

A total of 59 summer squash genotypes (*Cucurbita pepo* L.) were used in this study. The genotypes were both characterized for 83 morphological traits according to UPOV and for molecular markers *via* SRAP markers. Genetic relationship was determined using NTSYS2.1 statistical software based correlation matrix and unweighted pair group method arithmetic average (UPGMA). The morphological and molecular data were used for Principal Coordinate Analysis. The six main groups included 3, 1, 8, 33, 1 and 13 genotypes, respectively. Fifty nine summer squash genotypes were evaluated by SRAP markers. The 23 SRAP primer combinations generated 166 bands, 155 of which (92%) were polymorphic. The summer squash genotypes used in this study showed a high level of polymorphism for SRAP and phenotypic traits. Principle Coordinate analysis resulted with two major heterotic groups. Hybrid combinations have been made and will be tested for predictive value of morphological and molecular markers.

INTRODUCTION

Total world squash production is 22 141 402 tons. China leads squash production with 6 506 966 tons followed by India with 4 108 510 tons, Russia with 1 123 360 tons, and United States with 749 879 tons. Turkey's total squash production is 411 942 tons, ranking the 10th in the world (FAOSTAT 2010). According to the data, 314 882 tons of summer squash were produced in Turkey in

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2010 (TUIK 2010). Summer squash cultivation is performed in different regions, including Mediterranean and Aegean areas.

The aim of this study was to estimate heterotic groups for use in F1 hybrid breeding programs. Most plant material was obtained from Alata Horticultural Research Station gene pool, local population of Sanliurfa and some genotypes from Spain (provided by the Cucurbits Breeding group at COMAV-UPV). The morphological and molecular differences were used to estimate heterotic groups. Crosses were made in order to make use of the results. Morphological and molecular data were used to create the dendrograms showing the genetic relatedness of the lines.

Morphologic-molecular characterization and determination of relationships among summer squash lines are important for breeding new hybrid cultivars. The objectives of this study are to characterize and study genetic relationships among summer squash lines in order to assist summer squash breeding programs. The summer squash lines were morphologically evaluated according to UPOV descriptors and were molecularly characterized *via* SRAP markers.

MATERIALS AND METHODS

Plant Material

A collection of 59 lines of different summer squash genotypes was used in this study. The plant material was a collection of 32 genotypes of Alata Horticultural Research Station, 8 local genotypes of Sanliurfa region, 8 genotypes of Spain and 11 genotypes belonged to commercial seed companies (Table 1). The study has been conducted at the Alata Horticultural Research Station's greenhouses and laboratories.

Morphological Characterization

Seeds of the 59 accessions were sown in plastic multipots, containing a mixture of peat and perlite (2:1 v/v) on March 8th (2012), under greenhouse conditions. Ten plants of each accession were transplanted in greenhouse on March 20th, at 3–4 true leaf stage with 1.2 m x 0.4 m spacing for morphological characterization.

Accessions were evaluated for a total of 69 phenotypic characters (*Plant*: shape of cotyledon, growth habit, branching, degree of branching, (bushiness; *Stem*: green color, tendrils; *Leaf blade*: size, incisions, green color of upper surface, marbling, area; *Petiole*: green color, length, thickness, shape of cross section, number of prickles; *Female flower*: ring at inner side of corolla, color of ring at inner side of corolla, intensity of color of green ring at inner side of corolla, length of sepals, color of pistil (before opening); *Male flower*: expression of colored ring at inner side of corolla, color of colored ring at inner side of corolla, length of pedicel, diameter of pedicel, color of pedicel, grooving of pedicel, hairiness of pedicel, length of sepal;

Young fruit: length, maximum diameter, ratio length/maximum diameter, neck, curving of neck (on normally set fruits), number of colors, major color, intensity of major color, shape of cross section, warts, glossiness, color of ribs compared to color of rest of fruit, color of linear bands on ribs, mottling, type of mottling, color of mottling, conspicuousness of mottling of skin, size of flower scar, protrusion of flower scar, length of peduncle, main color of peduncle, intensity of main color of peduncle, mottling of peduncle, conspicuousness of mottling of peduncle; *Fruit*: general shape, maximum length, maximum diameter, length/maximum diameter; *Fruit fully developed in size*: major color, intensity of major color, color of stripes on ribs, color of mottling of skin; *Ripe fruit*: major color intensity of major color, color of stripes on ribs, color of mottling of skin; *Skin*: size, shape, color) by using a modified UPOV descriptor (The International Union for the Protection of New Varieties of Plants).

In addition to morphological characters, 14 quantitative traits were also measured for all accessions. These were: *Seedling*: Hypocotyl length (cm), Cotyledon width (cm), Cotyledon length (cm); *Leaf*: Leaf blade width (cm), Leaf blade length (cm), Petiole length; *Plant*: Plant height length, Diameter of the main body of the plant, Number of nodes plant, *Young Fruit*: Length (cm), Maximum diameter (cm), Ratio length/maximum diameter; *Fruit*: maximum length (cm), maximum diameter (cm) were recorded. The lengths were determined by a meter, ruler or a digital compass.

DNA extraction and SRAP analysis

For molecular analysis, genomic DNA was extracted from young leaves by the CTAB method as described by Doyle and Doyle (1990). DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc., Vinooski, USA) and 10 ng/mL DNA templates were made using TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). A total 23 SRAP primers were used for all summer squash genotypes (Table 2). PCR reaction components and PCR cycling parameters were performed as described by Uzun et al. (2009). PCR products were separated on 2% agarose gel in 1 x TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 110 volt for 4 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder were used for SRAP analysis.

Molecular analysis was carried out as follows: each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf 2000). A similarity matrix was constructed using SRAP data based on Dice (1945) coefficient. Then, the similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the cultivars studied. The genetic similarity matrix

and ultrametric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test (Mantel 1967). The result of this test is a cophenetic correlation coefficient, *r* that indicates how well dendrogram represents similarity data. The Principal Coordinate analysis (PCoA) of the original binary data matrix was also performed using NTSYS-pc version 2.1.

RESULTS AND DISCUSSION

The 83 phenotypic traits were used in clustering for PCoA. The resulting cluster was presented in Fig. 1. The accessions were divided into five main clusters. Within these clusters, subclusters were identified including accessions from different regions. Correlation coefficients among pairs ranged from 0.60 to 0.99. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was $r = 0.89$, $P < 0.01$.The six main groups were detected at 0.86 of correlation coefficient. Group 1, 2, 3, 4, 5 and 6 included 3, 1, 8, 33, 1 and 13 genotypes, respectively (Fig. 1). Tsiversalis et al. (2009) reported two groups with 16 genotypes that belonged to *Cucurbita moschata, C. maxim,* and *C. pepo* species with PCoA analysis, using 38 morphological data. Méndez-López et al. (2010) selected 46 *C. pepo* genotypes among 5 000 genotypes where there were 3 groups clustered based on genotype and morphological characteristics. Nacar et al. (2011) characterized and determined relationships among squash lines aiming to assist squash breeding program in Alata Horticultural Research Station. Tree-hundred-sixty squash lines were characterized for 54 traits according to UPOV and the relationship of the lines was determined using NTSYS 2.1 statistical software based correlation matrix and unweighted pair group method arithmetic average (UPGMA). In that study correlation coefficients among pairs ranged from 0.40 to 0.97 and at the level of 0.71, 14 main groups were determined. The three main groups were detected at 0.40 of correlation coefficient. Group 1- to -14 included 247, 21, 10, 6, 1, 18, 2, 20, 6, 14, 11, 2, 1 and 1 genotypes, respectively. However, PCoA analysis in our study indicated presence of two major groups.

Fifty nine summer squash genotypes were also evaluated by SRAP markers. A total of 166 bands were generated from 23 SRAP primer combinations. The 92% of the markers were polymorphic among genotypes. Number of bands per primer combinations was 7.1 whereas polymorphic bands per primer combinations were 6.7. The primers Me2-Em14, Me4-Em2 and Me4-Em13 yielded the highest number of polymorphic bands (11). The lowest number of polymorphic bands was obtained using Me9-Em13 primers. The highest polymorphism ratio was with Me2-Em14, Me4-Em2 and Me4-Em13 primer combinations (100%) (Table 2). Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high ($r=0.84$, $P< 0.01$), suggesting that the cluster analysis strongly represents

the similarity matrix. Correlation coefficients among pairs ranged from 0.59 to 0.92. The seven main groups were detected at 0.74 of correlation coefficient. Group 1-to-7 included 4, 1, 2, 3, 39, 2 and 8 genotypes, respectively (Fig. 2).

The 83 phenotypic traits and SRAP markers were also combined for PCoA analysis. The resulting cluster was presented in Fig. 3 and Fig. 4. Of the thirteen genotypes in group I, eight were local (Şanlıurfa), and five belonged to genotypes obtained from COMAV. The second group included 33 genotypes from Alata Horticultural Research Institute, 10 from local open pollinated cultivars, and three from COMAV.

The accessions were divided into nine main clusters. Within this clusters, subclusters were identified including accessions from different regions. Correlation coefficients among pairs ranged from 0.64 to 0.95. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was $r = 0.83$, $P < 0.01$.The six main groups were detected at 0.83 of correlation coefficient. Group 1-to- 6 included 1, 1, 27, 8, 9, and 13 genotypes, respectively (Fig. 3 and Fig. 4). Ferriol et al. (2004) used SRAP and AFLP markers in 47 different *Cucurbita moschata* genotypes. In that study, SRAP analyses with 11 primer combinations generated 148 bands, and 62% were found to be polymorphic. The results of morphological characterization data were coherent. Inan (2008) used twenty-four genotypes, including *C. pepo*, *C. moschata* and *C. maxima* as plant material from which morphological and molecular characterization were carried out. Eight SRAP (ME1, ME2, ME6, ME8, EM1, EM2, EM3, and EM6) primer combinations were used and 71 bands were generated being all polymorphic. The summer squash genotypes used in this study showed a high level of polymorphism for SRAP markers and phenotypic traits. This is promising for maintaining diversity among cultivated genepool.

The results indicate the potential of the morphological and molecular techniques to discriminate among the summer squash genotypes, very useful in selecting the parental genotypes used in hybrid breeding programs. Crossed are to be made in order to test heterotic groups suggested by the results of the study.

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Table 1. List of summer squash accessions used in this study include species classification for each genotype.

Number	Variety	Number	Variety
1	Sanliurfa Merkez	31	Alata 3
2	Bozova	32	Alata 172
3	Yaslıca	33	Alata 302
4	Tutluca 1	34	Alata 189
5	Tutluca 2	35	Alata 62
6	Konak	36	Alata
7	Halfeti 2	37	Alata 73
8	Halfeti 1	38	Alata 108
9	Altın Rengi Kabak	39	Alata 226
10	Zucchini Kabak	40	Alata 360
11	Dolmalık Top Tipi Kabak	41	Alata 308
12	Sari Ince Boyunlu Kabak	42	Alata 26
13	Italyan Zucchini Cizgili Sari Kabak	43	Alata 297
14	Early Yellow Crockneck	44	Alata 262
15	Early Profect Straightneck	45	Alata 261
16	Costarta Romenesco	46	Alata 123
17	Caserta Squash	47	Alata 322
18	Cocozella Cizgili Kabak	48	Alata 167-A
19	Sakız	49	Alata 477
20	BGV004370 (COMAV, Spain)	50	Alata 120
21	BGV003837 (COMAV, Spain)	51	Alata 223
22	BGV005303 (COMAV, Spain)	52	Alata 260
23	BGV000080 (COMAV, Spain)	53	Alata 85
24	BGV005371 (COMAV, Spain)	54	Alata 193
25	BGV000848 (COMAV, Spain)	55	Alata 341
26	BGV009477 (COMAV, Spain)	56	Alata 36
27	BGV003465 (COMAV, Spain)	57	Alata 207
28	Alata 82	58	Alata 1
29	Alata 328	59	Alata 6
30	Alata 112		

Table 2. List of SRAP primer pairs used to study the genetic relatedness of 59 summer squash genotypes accessions.

Name	No. of bands	No. of polymorphic bands	Polymorphism (%)	Size (bp) (min–max)
Me3-Em3	4	4	100	180-620
Me3-Em14	7	7	100	130-880
Me4-Em14	11	9	81	120-930
Me5-Em2	6	6	100	290-970
Me7-Em4	10	8	80	110-830
Me2-Em14	11	11	100	140-740
Me3-Em13	7	7	100	200-900
Me10-Em12	5	4	80	340-800
Me10-Em14	5	5	100	190-910
Me3-Em9	7	7	100	110-950
Me5-Em3	5	5	100	210-620
Me12-Em15	5	5	100	130-420
Me4-Em2	11	11	100	130-790
Me7-Em2	3	2	66	120-320
Me7-Em3	6	6	100	100-690
Me4-Em13	11	11	100	120-700
Me2-Em6	9	9	100	120-900
Me2-Em5	5	5	100	230-700
Me3-Em6	9	7	77	170-790
Me4-Em8	8	8	100	130-950
Me5-Em7	8	8	100	280-900
Me2-Em3	9	9	100	180-950
Me9-Em13	3	1	33	250-430
Total	166	155	92	
Mean	7.2	6.7		

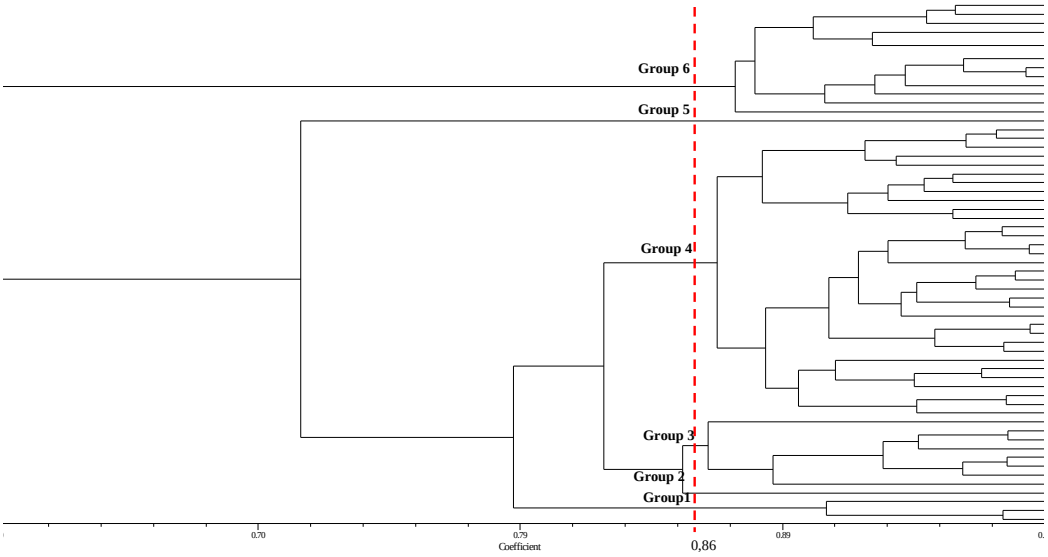

Fig. 1. Dendrogram of summer squash genotypes obtained from cluster analysis of 83 qualitative agromorphological traits using average method.

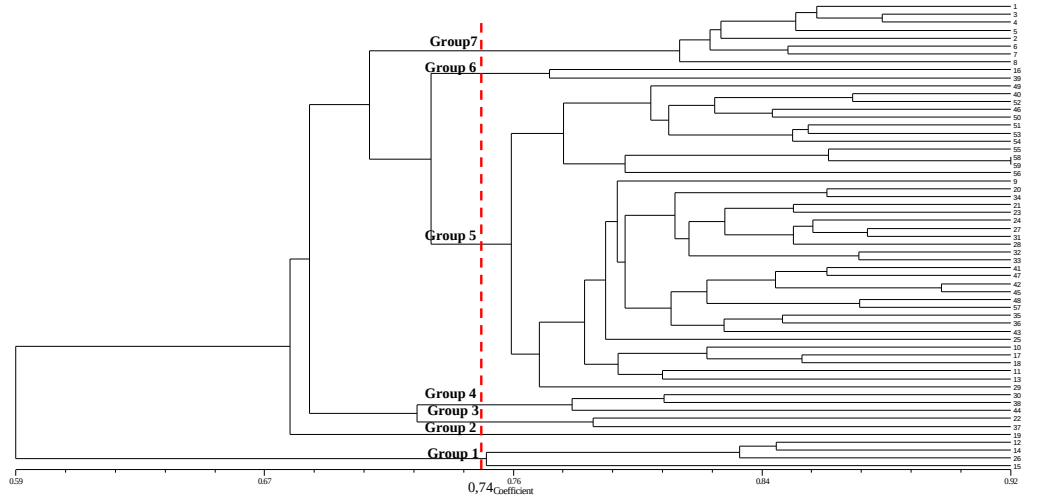

Fig. 2. Dendrogram of the 59 summer squash genotypes accessions using UPGMA method obtained from SRAP markers.

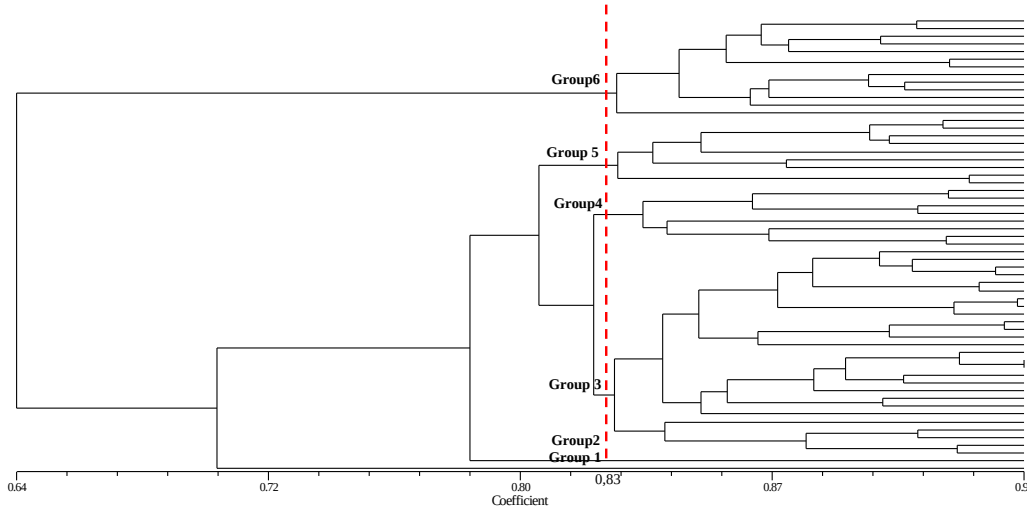

Fig. 3. Dendrogram of summer squash genotypes obtained from cluster analysis of agromorphological and SRAP data traits using average method.

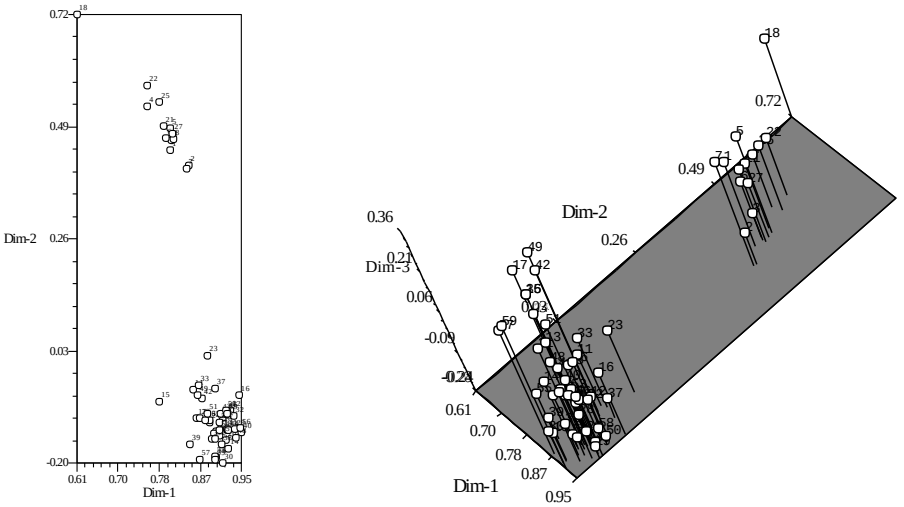

Fig. 4. Two and three dimensional plot of the principal coordinate analysis of agromorphological and SRAP data includes summer squash.

SRAP markers as a tool for the assessment of genetic diversity in *Cucurbita* spp.

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Keywords: Genetic diversity, SRAP, Cucurbits

Abstract

Cucurbita spp. is one of economically the most important genus in Cucurbitaceae. In this study, to determine the level of genetic diversity, a total of 48 genotypes belong four species have been collected from different parts of the world and were analyzed using Sequence-Related Amplified Polymorphism (SRAP) markers. Twenty-four primer pairs generated a total of 337 polymorphic bands. Average polymorphism information content (PIC) was 0.23. A dissimilarity matrix was prepared by using SRAP data based on Dice coefficient. Dissimilarity matrix was used to construct a dendrogram using the Neighbor-Joining cluster analysis to determine genetic relationships among the studied genotypes. Cophenetic correlation between similarities of tree and the dissimilarity matrix was $r = 0.99$, $P < 0.0001$. In the Neighbor-Joining dendrogram based on the Dice's coefficient the genotypes clustered into four major groups. This study revealed the utility of SRAP markers in detecting genetic diversity between the collected genotypes and provide basis for future efficient use of these molecular markers in the genetic analysis of other members of Cucurbitaceae.

INTRODUCTION

Cucurbitaceae containing 800 species in 130 genera are economically the most important families of plants, with many names dating back to Medieval or even Greek and Roman medical and horticultural treatises (Kocyan et al. 2007). The family is distinct morphologically and biochemically from other families and is therefore considered monophyletic. As the plants of this family produce unisexual flowers, cross pollination is a regular feature (Sikdar et al. 2010). The genus *Cucurbita* ($2n=2x=40$) contains five domesticated species; *C. pepo*, *C. maxima*,

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C. moschata, *C. mixta* and *C. ficifolia* (Inan et al. 2012). Information on genetic diversity and phylogenetic relationships within and among crop species and their wild relatives is necessary for the efficient utilization of plant genetic resource collections in the breeding programs (Sikdar et al. 2010). Among the many types of molecular markers, Sequence-Related Amplified Polymorphism (SRAP) has been demonstrated to be a useful tool for genetic studies (Feng et al. 2009). SRAP is a PCR-based marker system with two primers. It targets coding sequences in the genome. (Li and Quiros 2001). SRAP markers have been applied successfully in diversity analyses and molecular characterization of *C. pepo* (Ferriol et al. 2003) and *C. maxima* (Ferriol et al. 2004) and pumpkin (Zhi-Hong et al. 2009) and some *Cucurbita* genotypes including hull-less seed pumpkin (Inan et al. 2012). The objectives of this study aimed to identify the polymorphisms and determine genetic relationships among 48 genotypes belonging to 10 groups of 4 species of *Cucurbita* using the SRAP technique and to evaluate the efficiency of SRAP markers in them.

MATERIALS AND METHODS

Plant materials and DNA extraction

A total of 48 accessions of *C. pepo*, *C. moshata*, *C. maxima* and *C. ecuadorensis* with representatives of each in 11 morphotypes were subjected to SRAP analysis (Table 1). Genomic DNA was isolated from expanded young leaves using Wizard Genomic DNA Purification Kit (Promega Corp., Madison, USA). DNA concentration was measured with a spectrophotometer and diluted to a concentration of 8 ng/µl for PCR.

SRAP Analysis

SRAP was carried out based on the protocol described by Li and Quiros (2001) with minor modifications. PCR reactions were performed in a total volume of 10 µl as follows: 0.55 µl dNTP mix (2.5 mM), 1.0 µl PCR buffer (10X), 0.4 U Taq, 0.33 µl forward and reverse primer (10 Pmol), 25-30 ng genomic DNA template, and 4.41 µl ddH_2O. The SRAP markers were amplified using the following parameters: 5 cycles of 94°C for 1 min, 35°C for 1 min, 72°C for 1 min, and 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min. The PCR products were separated on a 10% polyacrylamide gel electrophoresis and stained using silver nitrate.

In SRAP analysis, Presence or absence of each SRAP fragment was coded as “1” and “0”. Polymorphism information content (PIC) values per locus was calculated using formula of $PIC = 1 - \sum f_i^2$ $PIC = 1 - \sum f_i^2$. A dissimilarity matrix was constructed by using SRAP data based on Dice coefficient. Dissimilarity matrix was used to construct a dendrogram using the Neighbor-Joining cluster analysis to determine genetic relationships among the studied accessions. The

distance matrix and dendrogram were constructed using the DARwin5 and the MEGA5 softwares respectively. The representativeness of dendrograms was evaluated by estimating cophenetic correlation for the dendrogram and comparing it with the dissimilarity matrix, using Mantel's matrix test. The result of this test is a cophenetic correlation coefficient, r. Cophenetic correlation between similarities of tree and the dissimilarity matrix was generated using Mantel's matrix test and was performed by the XLSTAT, Version 13.3.2.0 software. Principal coordinate analysis (PCA) was performed to better understand relationships among accessions using the DARwin5 software.

RESULTS AND DISCUSSION

Of the 337 polymorphic fragments obtained from 24 SRAP primer combinations, number of polymorphic bands varied from 3 (Me1-Em5) to 35 (Me1-Em10) with an average of 14.04. The PIC values ranged from 0.10 (Me1-Em8) to 0.43 (Me1-Em2), with a mean of 0.23 (Table 2). Based on the analysis by DARwin 5 software, the coefficient between 48 *Cucurbita* genotypes ranged from 0.1013 to 0.9459. The min. coefficient detected between Max199 and Gleisdorfer Oil, the max. coefficient occurred between Early White Bush and Galeux. The data obtained from SRAP analyses were used to perform genetic similarity analysis in the whole genotypes. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was $r = 0.98$, $P < 0.0001$. All of genotypes used in this assay were distinguished. Based on the unweighted pair group method of arithmetic averages (UPGMA) cluster diagram and Dice similarity coefficient, the 48 genotypes may be divided with the genetic distance of 0.3 into four groups. Group I consisted 3 genotypes of Maxima and Group II included a genotypes of Ecuadorensis. Group III and Group IV included 5 and 39 genotypes corresponded to ssp *moschata* and *pepo* respectively (Fig. 1). A dendrogram indicated the genetic relationships among the genotypes were highly associated with the morphotypes in which germplasms were classified. PCA was performed based on the genetic similarity matrix in order to better understand the relationships between the genotypes. The first and 2nd vectors of components accounted for 35.03% and 22.18% of variation, respectively (Fig. 2).

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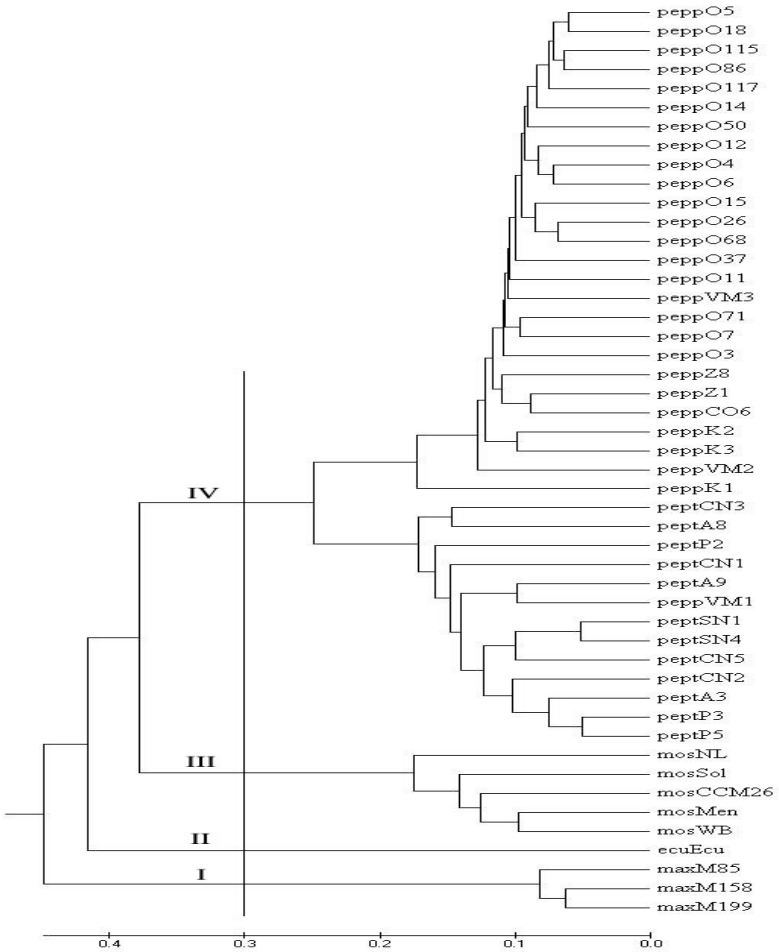

Fig. 1. Dendrogram of *Cucurbita* genotypes using UPGMA method.

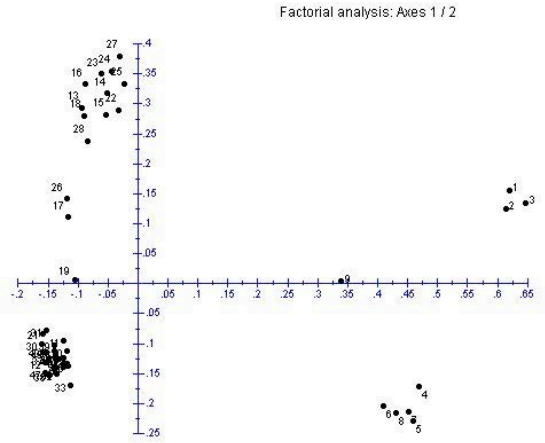

Fig. 2. Principal component analysis score plot of 48 *Cucurbita* genotypes isolates based on 24 SRAP markers. PC1: first principal component; PC2: second principal component.

Table 1. List of genotype used in this study.

Local Name	Type Name	Abbr. Name	Local Name	Type Name	Abbr. Name
1. Maxima	Max 85	maxM85	25. Acorn	Tay Belle	peptA3
2.	Max 158	maxM158	26.	Thelma Sanders	peptA8
3.	Max 199	maxM199	27.	Yugoslavian finger	peptA9
4. Moschata	Soler	mosSol	28. Vegetable Marrow	Without Name	peppVM1
5.	Menina	mosMen	29.	Bulgarian Summer	peppVM2
6.	Nigerian. Local	mosNL	30.	Alba	peppVM3
7.	Waltham Buttern	mosWB	31. Oilkurbis	Retzer Gold	peppO11
8.	CCM26	mosCCM26	32.	Gleisdorfer	peppO12
9. Ecuadorensis	Ecuadorensis	ecuEcu	33.	Sepp	peppO14
10.	True French	peppZ1	34.	Markant	peppO15
11.	Striato di Italia	peppCO6	35.	Chinesischer	peppO3
12.	Erken	peppZ8	36.	S-Afrika	peppO4
13. Straightneck	General Patton	peptSN1	37.	Lady Godiva	peppO5
14.	Sunary	peptSN4	38.	Estancia Bugar	peppO6
15. Crookneck	Bianco Friulano	peptCN1	39.	Georgica	peppO71
16.	Courge Cou Tours	peptCN2	40.	Miranda	peppO37
17.	Without Name (CN3)	peptCN3	41.	Slovenska Golica	peppO50
18.	Sundance	peptCN5	42.	Kakai	peppO18
19. Pumpkin	Pomme d`Or	peppK1	43.	Lu`s Oilkurbis	peppO26
20.	Tondo di Padana	peppK2	44.	Szentesi Oliva	peppO7
21.	Chinese Miniature	peppK3	45.	09H4 CZ	peppO115
22. Patisson	Without Name	peptP2	46.	PM 18	peppO117
23.	Early White Bush	peptP3	47.	Anton Berger	peppO68
24.	Galeux	peptP5	48.	PI 285611	peppO86

Table 2. List of SRAP primers used in this study, polymorphic fragments and polymorphism information contents (Li and Quiros 2001).

Primer combination	Polymorphic bands	PIC	Primer combination	Polymorphic bands	PIC
Me1- Em1	9	0.30	Me2- Em3	19	0.29
Me1- Em2	4	0.43	Me2- Em4	13	0.12
Me1- Em3	18	0.21	Me2- Em5	9	0.21
Me1- Em4	17	0.19	Me2- Em6	20	0.26
Me1- Em5	3	0.31	Me2- Em7	21	0.20
Me1- Em6	16	0.18	Me2- Em8	11	0.32
Me1- Em7	19	0.26	Me2- Em9	9	0.11
Me1- Em8	14	0.10	Me2- Em10	23	0.22
Me1- Em9	12	0.25	Me3- Em1	6	0.27
Me1- Em10	35	0.21	Me3- Em2	8	0.25
Me2- Em1	18	0.25	Me3- Em3	13	0.25
Me2- Em2	13	0.21	Me3- Em4	7	0.13
Mean				14.04	0.23

Determination of some morphological characteristics of edible seed pumpkin (*Cucurbita pepo* L.) genotypes

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Keywords: Breeding, Inbred line, edible pumpkin seeds, S4

Abstract

Some morphological characteristics such as seed yield per fruit, fruit quantity per plant, average fruit weight and size, seed width/length rate and thousand seed weight, seed color and hint easiness of the pumpkin lines for edible seeds in S4 level were determined according to inbred and UPOV parameters. In S4 level of the study, 320 g seed yield were taken per plant on average. 99 of genotypes had maximum seed yield with 1753 g/plant. Generally, seed color was cream. 39 genotypes were elliptical while 72 genotypes were wide–elliptical and 3 genotypes were narrow–elliptical. 90 genotypes were found as easy to hint.

INTRODUCTION

Pumpkin has been taken its place and its value increasing day by day due to usage in human feeding with many utilization purposes. The varieties of squash and pumpkin having commercial importance are including many differences in terms of their fruit length, shape, out and inside colour of the fruit (Paris et al. 2006). Because of those differences, there are different types for its classification and they are usually known as *Cucurbita pepo, C. moschata, C. mixta* and *C. maxima* species.

As in many parts of the world for the production of pumpkin, the fruit is produced in the seeds. Pumpkin seeds are used in human nutrition value as well as different industries. Pumpkin cultivation has some advantages such as less irrigation need or well growing under drought conditions, being a good species to rotation, easiness of harvest, applicable to mechanization in cultural processes, fewer problems in diseases and insects in edible pumpkin growing for seed compared to the other edible pumpkin growing. The common type in edible seed pumpkin growing is *C. pepo* in Turkey. The variety of *C. moschata* is being used less (Yanmaz and Düzeltir 2003). But, the rate of allogame pollination is quite high because of monoic characteristic of the flower. Therefore, it is possible to

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occurance of different heterozygous landraceses instead of the original seeds at the beginning. Hence, the main problem of edible seed pumpkin growing is limitation in commercial variety (Toprakkarıştıran 1997).

Seeds of pumpkin are being used in many areas due to their richness in terms of mineral, vitamin and oil content. Its oil is also known a good food source in hormone balance, brain function and skin health whereby its Omega 3 and Omega 6 essential oils (Anonim 2008).

This study was undertaken to develop for edible seed pumpkin cultivar. For this purpose, the edible pumpkin seeds were collected from different regions of Turkey. The aim of the research was to determine promising cultitvar by investigation of some morphological characteristics.

MATERIALS AND METHODS

This research was conducted in the trial field of Selcuk University, Agricultural Faculty Department of Horticulture in 2010. A total of 124 edible pumpkin seed genotypes which were collected from Konya, Eskişehir, Nevşehir, Tekirdağ, Sakarya, Kayseri, Kırşehir and Afyonkarahisar provinces and at the stage of S4 lines (inbred line) were used as plant material. Seeds were sown on 14th of May 2010 by distance of 80x150 cm and 2-3 seeds were sown in each pit and 15 seeds/ plant from each genotype. The plants were fertilized with doses of 6 kg N, 10 kg P ve 10 kg K/da after elimination. The hoeing and filling the bottom of plant during the seedlings had 3-5 leaves. Selfing was made according to Bassett (1986) and Düzeltir (2004).

The plant characterics such as seed yield (g/fruit), fruit per plant, 1000 seed weight, seed colour-shape, fruit index (length/diameter),fruit weight, apparence of plant, branch prolongation and degree, stem and leaf colour, lobe of leaf, apparence and colour of ring in the bottom of corolla, density and colour of spot on the fruit, fruit colour and easiness of seed coat crackling were determined according to the UPOV criteria.

The statistical analysis was conducted using the package program. Data were subjected to analysis of variance (ANOVA). The differences between the means were compared using Duncan test. The means parametric values were calculated by Microsoft Excel computerized program and they were converted as numeric values.

RESULTS

As it is seen on the Table 1, mean seed yield per fruit was 44.20 g and the genotype 95 was the promising with 139.43 g fruit^{-1} yield. It was followed by the genotypes 87, 116, 47 and 61 with 80.36, 66.61, 66.59 and 62.33 g fruit^{-1} yield, respectively. The genotypes 71, 89, 4, 88 and 39 showed the lowest seed yield per fruit as 23.69, 23.33, 22.76, 18.61, 12.58 g fruit^{-1} yield, respectively. Average

fruit number per plant was 1.2 fruit plant^{-1}. While the genotype 19 had the highest number of fruit with 3.2 fruit /plant, 34, 48, 71 and 75-30 genotyeps took place in the first five with 3, 2.9, 2.4 ve 2.1 fruit /plant, respectively.

Mean of 1000 seed weight of all genotypes was 225.3 g. The genotype 42 reached the highest value (421.85 g) and it was followed by the genotypes 40, 20, 53 and 34 having 410.87, 375.83, 358.73 and 352.03 g, respectively. Seventy seven (64%) genotypes seed coat color was cream colour while 23 (% 19) of the seeds showed light cream and 13 (% 11) of the seeds showed dark cream colour (Table 1).

Mean fruit length was 22.15 cm. The genotype 65 had the longest fruit (42 cm) and it was followed by the genotypes 23, 92, 55 and 29 with 36.67, 35.67ve 31.67 cm, respectively. The genotypes 113, 124 and 101 showed the shortest fruit (11, 13.33 and 14 cm, respectively). The mean of fruit diameter was 16.97 cm. The genotype 121 had the highest fruit diameter (25 cm) and it was fallowed by the genotypes 57, 7 and 123 having 24, 22.67 and 22.40 cm, respectively. The genotypes 69, 92 and 107 had the smallest fruit diameter (11, 10.33 and 9 cm, respectively) (Table 1).

The mean of fruit index (length/diameter ratio) was 1.36. The genotype 92 showed the highest fruit index (3.55) and it was fallowed by the genotypes 65, 107, 23 and 69 with 3.50, 3.22, 2.53 and 2.48 respectively. The genotypes 53, 110, 7 and 124 showed the lowest fruit index value (0.78, 0.76, 0.69 and 0.68, respectively) (Table 1).

The mean fruit weight was 2837 g. The genotype 99 had the heaviest fruit with 7012 g and it was fallowed by the genotypes 57, 55, 123 and 36 having 6509, 5943, 5441 and 4930 g, respectively. The genotypes 77, 16, 67, 96 and 78 had the smallest fruit with 1547, 1473, 1424, 1380 and 1207 g, respectively. The mean of seed length/width index was 1.85. The genotype 66 showed the highest ratio (2.78) and it was fallowed by the genotypes 101, 100, 106 and 1 having 2.69, 2.66, 2.16 and 2.13, respectively (Table 1).

According to the Table 2, twenty nine (23%) genotypes were perpendicular while 58 (47 %) of the genotypes were semi-wrapped and 16 (12 %) of the genotypes showed a wrapped shape. Thirty three (28 %) of the genotypes did not show branch prolongation while 77 (62 %) of the genotypes showed, and 33 (28 %) of the genotypes had a medium level of branch elongation. The stem color change from light green to dark green and 44 (37 %) of the genotypes showed light green while 36 (32 %) of the genotypes were dark green and 40 (29 %) of the genotypes had green stem colour. The colour of the leaf was light green in 27 (22 %) of the genotypes while dark green in 52 (42 %) of the genotypes and green in 41 (33 %) of the genotypes. Twenty five 25 (20 %) of the genotypes showed a lower level of leaf lobenss, 5 (4%) of the genotypes were slightly lobed, 36 (29 %) were medium level lobed, 26 (21 %) were excess level lobed, and 27 (22 %) of the genotypes showed

excessively lobed leaves.

As shown in the Table 2, 4 (3 %) ring in the bottom of corolla was absent in the 4 genotypes while 82 (66%) of the genotypes had. The colour of the rings was yellow in 27 (33 %) of the genotypes while green in 36 (44 %) and green-yellow colored in the 19 (23 %) of the genotypes. The colour of leaf spot was green in 25 (21 %) genotypes and orange in 11 (9 %) genotypes, and there was no leaf spot in 88 (70 %) genotypes. From those 36 (30 %) of the genotypes with spotted leaves separated into three groups.Seven genotypes showed little spots, 17 genotypes were intensively spotted and 12genotypes were over intensively spotted. The colours of matured fruit were highly variable. It was as following: The following mature fruit color were observed; yellow (20) , light yellow (36), dark yellow (9), dark yellow-green mealy (13), orange (9), green (1), green-yellow mealy (10) and cream (15). In 90 genotypes seed coat cracling was easy while it was hard in 30 genotypes. Seed coat crackling test could not been determined in 4 genotypes because of the shortage of the seeds.

DISCUSSION

In the present research, the mean seed yield per fruit in the S4 lines was 44.2 g. The highest yield per fruit was taken from the genotype 95 with a yield of 139.43 g. These results are agrement with Abak et al. (1990), Murkoviç et al. (1997), Toprakkarıştıran (1997), Düzeltir (2004) findings.

The average number of fruit per plant was1.2 fruit /plant, the genotype 19 produced the the highest number of fruit per plant with 3.2 fruit /plant. Researchers reported that the number of fruits per plant was between 1 and 9 (Abak et al. 1990; Warid et al. 1993; Toprakkarıştıran 1997; Düzeltir 2004). In our research, reducing the number of fruit was because fruits of the open-pollinated cutting off from the plant.

The mean weight of 1000 seed was 225.3 g. The colour of seed was cream in 77 genotypes and light cream in 23 genotypes, and dark cream in 13 genotypes. In previous studies, Joshi et al. (1993), Toprakkarıştıran (1997), Düzeltir (2004) reported that 200 g for 1000 seed weight which means more filled seeds.

The mean fruit length was 22.15 cm. The genotype 42 showed the highest value (42.00 cm). The mean fruit diameter was found as 16.97 cm and the genotype 121 had the highest value (25 cm). The mean fruit index was 1.36 and the genotype 92 reached the highest value (3.55). The characteristics such as fruit diameter and fruit length showed great variation because genotypes had different fruit shape. The similar results were also shared by the other researchers (Paris et al. 2006).

Mean fruit weight in the S4 stage was 2837 g. The genotype 99 had the highest value (7012 g). The variation in mean fruit weight was also higher. The studies in the different environments were found that fruit weight was ranged from 2 kg to 5

kg in the edible pumpkins (Abak et al. 1990; Murkovic et al. 1997; Toprakkarıştıran 1997; Düzeltir 2004).

Mean of seed length/width index was 1.85. The genotype 66 had the highest ratio (2.78). A wide range of variation for seed shape, flower structure and fruit characteristics were observed in the investigated characteristics. This outcome looks as an advantage to developing different shaped seeds which are demanded by markets.

As a result, the collected edible pumpkin seeds genotypes from different provinces of Turkey showed promising result as regarded to investigated parameters, and it is possible to develop edible seed pumpkin varieties with different characteristics for different ecological conditions.

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Table 1. Yield, fruit and seed characteriscs in the S4 stage of edible seed pumpkin.

Genotype No	Seed yield (g/ fruit)	Fruit/ plant	1000 seed weight (g)	Seed Colour	Seed index (mm/mm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Fruit index (mm/mm)
1	43,71	0,9	177,85	Cream	2,13	15,50	16,50	2400	0,94
2	38,50	0,8	157,10	Cream	1,76	19,00	11,50	1606	1,65
3	27,61	2,0	183,65	Cream	1,81	20,00	13,00	1790	1,54
4	22,76	0,9	180,85	Cream	1,91	18,25	15,50	1619	1,18
6	39,77	1,3	241,57	Cream	1,73	20,00	14,33	1701	1,40
7	45,12	1,3	239,30	Cream	1,75	15,67	22,67	3116	0,69
8	22,77	0,5	235,15	Cream	1,87	21,50	19,50	3436	1,10
9	32,78	0,8	227,50	Cream	1,93	25,00	18,00	3834	1,39
10	45,90	0,7	183,10	Light Cream	1,89	19,00	17,00	2542	1,12
11	44,33	1,4	227,15	Light Cream	1,80	18,00	15,00	1751	1,20
12	33,47	1,2	268,33	Dark Cream	1,86	19,33	12,00	1502	1,61
13	52,83	1,5	271,60	Cream	1,54	22,00	17,50	2788	1,26
14	26,67	1,3	207,77	Cream	1,94	19,33	15,33	1898	1,26
15	41,52	0,9	179,95	Light Cream	1,77	15,50	18,00	2027	0,86
16	36,86	1,7	177,20	Dark Cream	1,91	19,00	14,25	1473	1,33
17	32,98	1,3	204,97	Cream	2,02	30,33	12,67	2613	2,39
18	33,99	1,2	147,88	Cream	1,90	22,00	18,00	2577	1,22
19	36,75	3,2	179,87	Cream	1,89	20,67	21,00	3981	0,98
20	43,98	1,9	375,83	Light Cream	1,95	22,86	15,71	2847	1,45
21	35,11	1,1	176,84	Cream	1,64	25,40	12,40	1883	2,05
22	35,74	1,2	223,87	Light Cream	1,69	22,33	14,67	3719	1,52
23	27,18	0,3	217,65	Light Cream	1,90	38,00	15,00	5408	2,53
24	52,36	1,1	206,40	Cream	1,88	27,00	14,00	2530	1,93
25	37,45	1,5	208,33	Dark Cream	1,97	15,67	15,67	1706	1,00
26	30,66	1,1	233,23	Cream	1,87	19,33	20,33	2929	0,95
27	46,55	1,0	295,54	Cream	1,66	27,50	17,25	3259	1,59
28	49,34	1,4	214,68	Cream	1,78	26,50	15,50	2743	1,71
29	42,39	0,9	195,53	Dark Cream	1,94	31,67	16,00	3503	1,98
30	49,48	2,1	189,83	-	-	29,00	14,67	2486	1,98
31	36,44	1,7	66,70	Cream	1,74	21,00	21,00	2420	1,00
32	38,80	2,0	273,98	Cream	1,83	26,00	17,40	2563	1,49
33	37,97	1,3	183,32	Cream	1,87	19,80	19,20	2539	1,03
34	23,42	3,0	352,03	Cream	1,74	17,33	17,33	2812	1,00
35	36,94	1,5	254,62	Cream	2,01	26,60	15,20	3451	1,75
36	38,15	1,1	242,53	Light Cream	1,90	27,00	19,33	4930	1,40
37	58,42	0,2	262,65	Light Cream	1,07	22,00	17,00	3084	1,29
38	51,60	1,3	217,80	Cream	1,64	23,33	13,00	2159	1,79
39	12,58	1,0	196,55	Cream	1,66	16,50	17,00	2245	0,97
40	60,55	1,0	410,87	Cream	2,02	24,00	21,00	4258	1,14
41	53,81	0,9	169,20	Cream	1,95	17,00	16,00	1690	1,06
42	53,74	1,1	421,85	Light Cream	1,88	23,00	15,50	2386	1,48
43	60,03	1,2	217,15	Light Cream	1,92	24,75	16,25	3320	1,52
45	59,35	1,9	235,30	Cream	2,02	22,00	17,00	2637	1,29
47	66,59	0,1	220,70	Cream	1,88	22,00	14,00	1618	1,57
48	41,31	2,9	215,93	Cream	1,88	24,83	21,67	3852	1,15
50	36,75	1,3	152,50	Dark Cream	1,99	28,00	18,00	3388	1,56
51	51,71	1,4	237,14	Cream	1,73	23,20	19,20	2787	1,21

Table 1 continued

Genotype No	Seed yield (g/ fruit)	Fruit/ plant	1000 seed weight (g)	Seed Colour	Seed index (mm/mm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Fruit index (mm/mm)
52	52,87	1,3	254,85	Light Cream	1,60	28,50	21,00	4824	1,36
53	47,17	1,5	358,73	Cream	1,91	14,75	19,00	1783	0,78
54	44,15	1,8	247,53	Cream	1,95	27,50	13,75	2511	2,00
55	55,14	0,8	261,57	Cream	1,75	35,67	19,67	5943	1,81
56	50,61	1,1	245,20	Cream	1,76	20,14	13,71	1708	1,47
57	53,24	1,2	259,55	Cream	1,57	31,50	24,00	6509	1,31
59	40,14	1,4	214,38	Cream	1,75	20,60	19,20	3204	1,07
60	51,77	1,4	222,50	Light Cream	1,82	21,00	18,33	3457	1,15
61	-	-	-	-	1,83	27,50	15,00	3190	1,83
62	46,53	1,0	209,92	Light Cream	1,75	24,60	12,80	1888	1,92
64	35,93	1,1	223,07	Cream	1,80	20,67	19,33	2468	1,07
65	36,01	1,1	250,20	Cream	2,06	42,00	12,00	2082	3,50
66	39,47	1,5	163,37	Cream	2,78	24,00	13,00	1768	1,85
67	27,86	1,1	124,50	Cream	2,09	24,50	11,50	1424	2,13
68	36,63	0,9	143,72	Cream	1,93	22,40	13,60	1629	1,65
69	36,37	0,9	151,80	Dark Cream	2,04	27,33	11,00	2396	2,48
70	49,80	0,4	253,80	Cream	1,88	22,00	20,00	2892	1,10
71	23,69	2,4	225,05	Cream	1,83	18,43	14,86	1709	1,24
72	48,94	1,5	273,52	Cream	1,97	21,60	19,00	2568	1,14
73	43,41	0,7	233,88	Cream	1,69	21,50	15,50	2047	1,39
74	61,73	1,4	271,85	Light Cream	1,66	27,80	17,57	3323	1,58
75	49,19	2,1	244,20	Light Cream	1,73	18,33	15,33	1795	1,20
76	46,50	1,0	243,38	Dark Cream	1,64	21,75	15,25	2360	1,43
77	47,28	1,1	230,75	Dark Cream	2,01	15,00	16,00	1547	0,94
78	59,12	0,3	270,10	Cream	1,82	15,00	15,25	1207	0,98
79	52,97	0,9	191,8,7	Cream	2,00	19,33	16,00	2009	1,21
81	33,79	0,5	184,50	Cream	2,10	20,00	20,50	2927	0,98
82	39,90	1,7	168,58	Light Cream	1,74	20,80	14,60	1656	1,42
83	41,65	0,7	174,17	Cream	1,97	26,00	21,33	4590	1,22
84	58,13	0,7	217,40	Cream	1,73	17,00	16,00	1658	1,06
85	35,56	1,0	193,10	Light Cream	1,91	20,00	17,00	2598	1,18
86	54,81	1,2	244,95	Light Cream	1,88	20,83	18,83	3459	1,11
87	80,36	1,1	272,33	Cream	1,81	24,00	21,33	4562	1,13
88	18,61	0,8	181,60	Cream	1,82	20,00	15,00	1994	1,33
89	23,33	0,9	199,47	Cream	1,74	20,00	17,00	2457	1,18
90	50,93	1,3	275,70	Light Cream	1,85	22,00	18,00	2298	1,22
92	43,78	0,7	238,27	Cream	1,88	36,67	10,33	3411	3,55
94	32,03	1,0	245,60	Cream	1,78	24,00	20,50	3762	1,17
95	139,43	0,4	199,10	Light Cream	1,56	21,00	22,00	3740	0,95
96	38,00	0,1	-	Cream	1,65	16,00	18,00	1380	0,89
98	19,31	1,5	152,05	Dark Cream	1,69	17,50	21,00	2998	0,83
99	35,26	1,5	318,90	Cream	1,85	28,00	22,00	7012	1,27
100	8,59	0,8	280,60	Cream	2,66	19,50	17,00	2193	1,15
101	39,64	1,1	228,97	Cream	2,69	14,00	16,00	1827	0,88
102	30,95	1,1	202,03	Cream	2,01	18,00	14,00	1807	1,29
103	51,84	1,2	250,26	Light Cream	1,97	22,80	17,80	3498	1,28
104	50,98	1,0	162,45	Cream	1,94	14,50	14,00	1553	1,04
105	43,71	1,2	168,27	Cream	1,83	23,00	14,25	2197	1,61

Table 1 continued

Genotype No	Seed yield (g/ fruit)	Fruit/ plant	1000 seed weight (g)	Seed Colour	Seed index (mm/mm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Fruit index (mm/mm)
106	51,98	1,1	161,00	Cream	2,16	17,00	18,00	2564	0,94
107	43,42	1,5	172,80	Cream	1,69	29,00	9,00	1950	3,22
109	56,18	1,4	310,83	Cream	2,04	22,67	18,33	3865	1,24
110	42,03	1,3	263,10	Cream	1,47	14,50	19,00	2242	0,76
111	32,86	1,3	111,07	Cream	1,81	20,00	20,00	3665	1,00
112	40,30	1,0	208,85	Cream	1,92	23,00	16,00	2577	1,44
113	39,30	1,1	232,05	Cream	1,77	11,00	18,60	2198	0,59
114	36,09	1,3	207,95	Cream	1,95	21,67	19,67	4649	1,10
116	66,61	1,0	248,53	Cream	2,01	20,33	16,33	3059	1,24
117	47,83	1,1	195,72	Cream	1,70	16,80	18,80	2506	0,89
118	61,38	1,2	281,05	Cream	1,73	26,00	21,50	5685	1,21
120	53,25	0,9	173,00	Cream	1,86	19,33	19,67	3499	0,98
121	51,45	0,6	280,40	Dark Cream	1,71	20,00	25,00	4304	0,80
122	60,23	1,2	203,30	Dark Cream	2,02	29,00	15,00	4010	1,93
123	61,32	1,0	225,76	Cream	1,99	24,20	22,40	5441	1,08
124	67,41	0,9	238,23	Cream	1,08	13,33	19,67	2077	0,68
125	44,42	1,2	205,16	Cream	2,07	16,00	17,00	1652	0,94
126	47,92	0,7	265,00	Light Cream	1,63	20,67	16,33	3201	1,27
127	57,24	1,2	229,83	Cream	1,86	22,00	16,33	2825	1,35
128	44,24	0,8	325,90	Dark Cream	1,87	28,00	19,00	4750	1,47
Mean	44.20	1.2	225.3		1.85	22.15	16.97	2837	1.36

Table 2. Plant, leaf, flower and mature fruit characteristics in the S4 stage of edible pumpkin.

Gen.	A	B	C	D	E	F	G	H	I	J	K	L
1	2	1	1	3	2	4	1	4	4	3	5	2
2	1	2	Absent	1	2	5	2	1	Absent	Absent	2	1
3	2	1	2	2	3	2	1	2	Absent	Absent	6	1
4	2	1	1	1	2	3	1	4	Absent	Absent	6	1
5	2	1	2	2	1	1	1	3	Absent	Absent	-	1
6	3	1	3	1	2	2	1	2	Absent	Absent	6	2
7	2	1	2	1	1	4	1	2	Absent	Absent	1	1
8	3	1	2	3	3	3	1	2	4	1	4	1
9	3	1	3	1	2	3	2	1	Absent	Absent	2	1
10	2	2	Absent	2	2	2	2	1	Absent	Absent	6	1
11	3	1	3	1	2	2	1	2	Absent	Absent	2	1
12	2	2	1	2	3	3	2	1	Absent	Absent	2	2
13	3	1	2	1	3	2	1	2	Absent	Absent	6	2
14	3	1	1	2	1	2	2	1	4	3	5	1
15	2	1	2	1	1	3	1	4	3	1	6	1
16	2	1	2	2	1	5	1	3	Absent	Absent	4	1
17	2	1	2	2	2	5	2	1	Absent	Absent	1	1
18	2	2	Absent	2	2	4	1	2	Absent	Absent	2	1
19	2	1	2	2	2	2	1	4	3	3	8	1
20	2	1	1	1	2	2	1	2	Absent	Absent	6	1
21	2	1	2	1	3	4	2	1	Absent	Absent	2	1

Table 2 continued

Gen.	A	B	C	D	E	F	G	H	I	J	K	L
22	2	1	1	1	3	2	2	1	Absent	Absent	2	1
23	2	2	Absent	1	2	2	2	1	Absent	Absent	6	2
24	2	1	2	3	2	5	2	1	Absent	Absent	7	1
25	2	1	2	1	3	5	1	3	4	1	4	2
26	2	1	1	1	3	5	2	1	Absent	Absent	8	1
27	1	2	Absent	2	3	5	1	3	4	2	6	1
28	2	1	1	1	1	5	1	2	Absent	Absent	6	1
29	2	1	2	3	2	4	2	1	Absent	Absent	2	1
30	2	2	Absent	2	3	3	2	1	Absent	Absent	7	-
31	2	1	2	2	2	3	1	2	Absent	Absent	6	2
32	2	1	1	2	3	4	2	1	4	2	5	1
33	2	1	1	2	3	3	1	3	Absent	Absent	2	1
34	3	1	3	1	2	2	1	2	Absent	Absent	7	2
35	1	2	Absent	1	2	3	1	2	Absent	Absent	6	1
36	2	1	1	3	3	3	1	3	Absent	Absent	2	1
37	2	1	1	2	3	3	1	2	Absent	Absent	6	1
38	1	2	Absent	3	3	2	1	2	Absent	Absent	1	1
39	1	2	Absent	2	2	3	1	3	Absent	Absent	6	1
40	2	2	Absent	1	2	4	1	2	Absent	Absent	1	1
41	3	1	3	3	1	5	2	1	Absent	Absent	7	1
42	2	2	Absent	3	2	4	1	3	4	2	7	1
43	1	2	Absent	2	3	5	2	1	Absent	Absent	1	1
45	2	2	Absent	2	2	4	1	4	3	1	2	1
46	3	1	2	2	2	4	1	2	Absent	Absent	-	-
47	3	1	3	3	2	2	1	4	3	2	-	1
48	1	2	Absent	3	3	4	1	3	3	2	8	1
50	2	1	3	1	3	5	1	4	Absent	Absent	6	1
51	3	1	2	2	2	3	2	1	Absent	Absent	6	2
52	2	2	Absent	2	2	5	1	2	Absent	Absent	6	2
53	3	1	3	2	1	5	1	4	Absent	Absent	8	1
54	2	1	1	3	3	3	2	1	Absent	Absent	8	1
55	2	1	3	3	3	3	1	3	4	1	8	1
56	3	1	3	1	2	4	1	2	Absent	Absent	1	1
57	2	2	Absent	3	3	2	1	3	Absent	Absent	2	2
58	2	-	Absent	3	3	3	2	1	3	2	-	-
59	3	2	Absent	2	1	3	1	2	4	2	6	1
60	1	2	Absent	1	2	4	1	2	4	1	6	1
61	2	2	Absent	3	2	3	2	1	Absent	Absent	5	1
62	1	2	Absent	2	2	5	2	1	4	1	6	1
64	3	1	2	2	3	5	2	1	Absent	Absent	6	1
65	1	1	3	1	3	5	1	3	3	2	4	1
66	1	2	Absent	1	3	3	1	3	Absent	Absent	6	1
67	1	1	1	2	2	1	1	3	Absent	Absent	2	2
68	3	1	2	1	2	4	1	4	3	3	4	2
69	2	1	2	1	1	3	1	3	Absent	Absent	7	2
70	1	2	Absent	1	1	5	2	1	4	3	5	2
71	2	1	1	1	3	4	2	1	Absent	Absent	6	2
72	2	1	2	3	3	3	1	4	Absent	Absent	6	2
73	2	2	Absent	3	1	3	1	2	Absent	Absent	2	2

Table 2 continued

Gen.	A	B	C	D	E	F	G	H	I	J	K	L
74	2	1	1	3	3	5	2	1	Absent	Absent	6	2
75	1	2	Absent	2	3	3	1	2	Absent	Absent	6	1
76	2	2	Absent	2	2	5	2	1	Absent	Absent	7	1
77	2	1	1	3	3	5	1	4	4	2	5	1
78	3	1	2	1	2	2	1	2	Absent	Absent	1	1
79	2	2	Absent	3	3	3	2	1	Absent	Absent	7	1
81	1	2	Absent	3	1	4	1	2	4	3	5	2
82	2	2	Absent	3	3	3	1	3	Absent	Absent	6	2
83	2	1	1	3	3	2	1	4	4	3	4	1
84	1	2	Absent	2	2	2	1	3	3	3	3	1
85	1	2	Absent	1	3	3	1	4	Absent	Absent	2	-
86	2	2	Absent	2	1	3	1	2	Absent	Absent	6	1
87	3	1	2	3	2	3	1	3	4	2	5	1
88	2	1	1	3	3	4	1	3	Absent	Absent	2	1
89	2	2	Absent	1	1	3	2	1	Absent	Absent	2	1
90	3	1	2	1	2	1	1	2	Absent	Absent	6	1
91	-	-	Absent	-	2	1	2	1	Absent	Absent	-	1
92	1	1	2	1	1	4	1	3	4	2	5	2
93	3	1	3	1	1	2	1	3	Absent	Absent	-	2
94	3	1	2	2	2	2	1	3	4	2	4	2
95	2	2	Absent	2	3	3	1	4	4	2	1	1
96	2	2	Absent	3	2	4	2	1	Absent	Absent	1	1
97	-	2	Absent	-	1	1	2	1	Absent	Absent	-	1
98	1	2	Absent	3	2	2	1	3	Absent	Absent	7	1
99	1	-	-	1	1	5	1	2	Absent	Absent	6	2
100-a	3	1	2	1	-	5	1	2	Absent	Absent	2	1
100-b	1	1	1	3	1	2	1	2	Absent	Absent	6	2
101	1	-	-	1	1	3	2	1	Absent	Absent	1	1
102	3	-	-	1	2	3	1	2	Absent	Absent	1	1
103	2	1	1	2	2	3	2	1	Absent	Absent	6	1
104	3	1	2	1	-	5	2	1	Absent	Absent	6	2
105	2	-	-	1	1	2	1	4	Absent	Absent	6	1
106	3	1	1	3	1	4	2	1	3	2	4	1
107	3	1	3	2	3	5	2	1	Absent	Absent	1	1
108	-	-	-	-	2	1	1	4	4	2	8	1
109	2	1	1	1	2	4	1	2	3	3	4	1
110	3	1	1	3	2	3	2	1	Absent	Absent	6	1
111	3	1	2	3	2	2	2	1	4	3	5	1
112	1	1	1	1	2	5	1	2	Absent	Absent	2	1
113	2	2	Absent	2	3	4	2	1	Absent	Absent	-	1
114	3	1	3	3	3	4	1	3	Absent	Absent	1	1
116	2	1	2	1	3	3	1	2	4	3	4	1
117	1	1	1	3	2	5	2	1	4	2	5	1
118	1	2	Absent	3	3	5	2	1	Absent	Absent	-	2
120	3	1	3	1	1	4	1	3	4	3	4	1
121	3	1	2	2	1	4	1	3	Absent	Absent	8	1
122	3	1	2	2	2	1	1	4	Absent	Absent	8	1
123	1	1	2	3	3	4	1	2	Absent	Absent	7	1
124	1	-	-	3	1	1	1	2	Absent	Absent	1	1

Table 2 continued

Gen.	A	B	C	D	E	F	G	H	I	J	K	L
125	2	1	2	2	2	5	1	3	4	2	5	2
126	1	-	-	2	2	2	1	4	4	2	5	1
127	1	1	1	1	1	2	1	2	Absent	Absent	6	2
128	3	1	1	2	2	3	1	4	Absent	Absent	1	1

Note: A=Appearance of plant: perpendicular-1, semi-wrapping-2, wrapping-3; B=Brack prolongation: presence-1, absence-2; C= Degree of branc prolongation: weak-1, medium-2, excess-3; D= Stem colour : light green-1, green-2, dark green-3; E=Leaf colour: light green-1, green-2, dark green-3; F=Slice of leaf: presence or very few-1, slight-2, medium-3, excess-4, excessive-5; G= Presence of ring in the bottom of corolla: presence-1, absence-2; H=Colour of the ring in the bottom of corolla: absence-1, green-2, yellow-3, green-yellow-4; I=Spot colour on the matured fruit: cream-1, yellow-2, orange-3, green-4; J=Density of spot: slight-1, intense-2, excess-3; K=Colour on matured fruit: cream-1, yellow-2, green-3, green-yellow-mealy-4, dark yellow-green mealy-5, light yellow-6, dark yellow-7, orange-8; L=Easines of seed coat crackling : simple-1, difficult-2

Expression of H^+ phosphatse from *Arabidopis* enhances the resistance of drought stress in transgenic bottle gourd (*Lagenaria siceraria* Standl.)

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Keywords : Bottle gourd, water deficit, transformation, root, recovery

Abstract

Drought and salt accumulation are the most important problems of continues cropping in vegetables production. Bottle gourd (*Lagenaria siceraria* Standl.) has been used as a rootstock for watermelon to promote the root system under stress condition. *AVP1* encoded a vacuolar H^+-PPase of *Arabidopsis* which showed resistance to salt and drought stress. In this study, to develop the bottle gourd with salt and drought tolerant, we introduced *AVP1* integrated with *bar* in bottle gourd cotyledon plants. We successfully generated transgenic bottle gourd over-expressing *AVP1* integrated with *bar* under CaMV 35S promoter by *Agrobacterium*-mediated transformation. The transgenic plants over-expressing AVP1 were selected by PCR with *AVP1*-specific primer. Southern and northern blot analysis showed transgenic lines 28-16-3 and 31-17-2 has one copy of *AVP1*. The transgenic plants were also resistance to herbicide. Wild type and *AVP1* transgenic plants were tested for drought tolerance by growing the plants under conditions of water deprivation. After 16 days of water deprivation, both wild type and transgenic plants started to wither and each plants were re-watered. After 20 days of re-watering, transgenic plants continued normal growth, but wild type stopped growing. Root of transgenic plant expressing *AVP1* showed higher root length than those of wild type. However the root length did not show significant difference between wild type and transgenic plant under 150 mM NaCl treatment.We suggest that the transgenic bottle gourd expressing *AVP1* can be used as rootstock to produce watermelon under drought stress.

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INTRODUCTION

Salinization of soil and drought are the most significant problems of continues cropping vegetables, such as watermelon in greenhouse. To reduce these problems, the bottle gourd (*Lagenaria siceraria* Standl.) has been used as a rootstock for watermelon. Vigor of root system of bottle gourd is required for grafting with other cucurbit. However, genetically modified crops can be useful to improve stress resistance. Recently, *Agrobacterium*–mediated transformation system was successfully established to introduce useful gene into bottle gourd which can be provided to produce the non-GM watermelon under environmental stress condition (Han et al. 2004). *AVP1* encoded a vacuolar H^+-PPase of *Arabidopsis* is resistance to salt and water stress (Gaxiola et al. 2001). Transgenic tomato expressing *AVP1* increased root biomass and enhanced recovery of plant from soil water deficient (Park et al. 2005).

In this study, to develop the bottle gourd with salt and drought tolerant, we introduced *AVP1* integrated with *bar* in bottle gourd cotyledon plants. We characterized the transgenic bottle gourd expressing *AVP1*especially tolerant to drought and salinity.

MATERIALS AND METHODS

Plant material and transformation

Bottle gourd (*Lagenaria siceraria* Standl) inbred line G5 (National Horticultural Herbal research Institute, Korea) was used for transformation and control line. The pRG521 plasmid was constructed with cloning of *AVP1,* gain of function mutant (AVP1D) of *Arabidopsis,* integrated with *bar* gene downstream of a tandem repeat of the *35S* promoter. pRG521 was introduced into bottle gourd by *Agrobacterium*-mediated transformation methods using cotyledon explants of G5 inbred lines as described (Han et al. 2004).*Agrobacterium tumefaciens* strain GV3101 or LBA4404 with the pRG521 (CaMV35S::AVP1) were used for this study. T_1*AVP1* plants were selected with 2 mg $L^{-1}$of $_{DL}$-phosphinothricin (ppt) selection medium and transferred the soil. T2 *AVP1* plants are segregated 3:1 with basta selection and PCR analysis. Homozygous of *AVP1* were confirmed with PCR and southern blot analysis.

PCR and Southern blot analysis

For PCR analysis, genomic DNA was extracted from leaf tissue using DNeasy plant mini kit (Qiagen, USA). The *AVP*1-specific primers were as follows: forward sequence (F) 5-TGCTGGTGGTATTGCTGAAATGGC-3; reverse sequence (R), 5-GATCTGAACACCGGATACAA-3. PCR products amplified from putatively transformed tissues were visualized by electrophoresis on a 0.8% agarose gel stained with ethidium bromide. For Southern blot analysis, DNA was extracted from

leaf tissue of PCR-positive lines using CTAB methods (Doyle and Dickson 1987). 30μg of DNA was digested with XbaI and was separated by electrophoresis in 1% agarose gel, and blotted onto a nylone Hybond^{TM}-N^{+}membrane (Amersham Life science, UK). AVP1 probe was made by RedyprimeII^{TM} Random Prime labeling System (Amersham Life science, UK). Hybridization were followed standard method (Sambrook et al. 1989)

Soil water deficit experiment

Seed of homozygous AVP1 expressing lines and wild type were germinated with distilled water and transferred on soil pot. Fifteen plants were selected from wild-type, vector control and homozygous AVP1 transformants and have been tested for soil water deficit experiment. The plants regularly were watered to field capacity for 20 days and stop watering until plants showed severe drought symptom. In this experiment the plant were re-watered after 16 days of water deprivation

Root bio assay

To evaluate whether AVP1 expression effect on root growth under salt stress, non-transformed controls and homozygous AVP1 transformants were grown on 1/2MS medium vertically under 16hr/ 8hr (light/dark) at 25°C. The root tips were cut into 15mm segments from 8 days old seedlings. The segments were incubated with 0, 150 and 300 mM NaCl in 1/2 MS containing 0.6% MES (pH 6.7) for 60 hr at 25°C under 16hr/ 8hr (light/dark) and measured the root length.

RESULTS AND DISCUSSION

We obtained the two lines with one copy of transferred gene, 28-16-3 and 31-17-2 expressing *AVP1*(Fig. 1). These transgenic lines showed also resistance to herbicide *(data not shown*) Homozygous transgenic lines were obtained through the segregation analysis with PCR(segregation pattern of 3:1) with T2 seeds from self pollinated T1 plants expressing *AVP1*.

Under condition of water deprivation, wild type, vector-transformed containing *bar* gene showed water-deficit symptom such as wither after 16 days of water deprivation (Fig. 2A). However, homozygous *AVP1* transgenic plants, 28-16-3 and 31-17-2 demonstrated recovery after re-watering that was not evident in controls. After 20 days, of re-watering, transgenic plants showed better recovery and resumed faster growth than the control plants. As shown Fig. 2, transgenic line 28-16-3 and 31-17-2 were less damaged by water stress like normal growth, but control plant showed poor recovery and stop growing. Root and leaf of transgenic plants showed more vigor than those of controls after re-watering. Similar result has been reported that AVP1 overexpression results in increased cell division at the one set of organ formation and enhanced root growth and dry weight (Li et al.

2005). The recovery of water deficit phenotype may be due to in part to root growth in the AVP1expressing tomato (Park et al. 2005). Results presented in Fig. 3 were consistent with this explanation for enhanced performance shown by transgenic bottle guard under water deficit stress. The root growth of transgenic bottle gourd expressing AVP1 under 1/2MS medium showed better than those of controls. However the root length did not show significant difference between controls and transgenic plants under 150mM NaCl treatments (Fig. 3).These result support the hypothesis that expression of AVP1 increase root growth in bottle guard to help the improvement of water deficient recovery. We suggest that overexpression of H^+-PPase in bottle gourd can help the engineering of root growth in agriculturally important crops for enhanced performance under soil water deficit condition. And transgenic bottle gourd expressing *AVP1* can be used as rootstock to produce watermelon under drought stress.

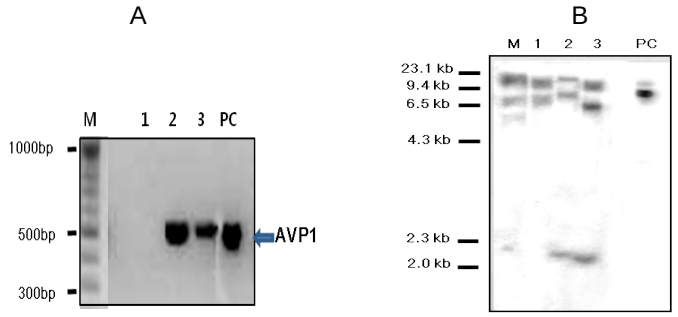

Fig. 1. Molecular characterization of *AVP1*-expressing transgenic bottle gourd. A. PCR analysis of *AVP1*-expressing lines. Lanes M, 100bp ladder marker, 1, Negative control (G5, wild type), 2 and 3, transgenic plants (T0 28-16-3, 31-17-2), PC, Positive control (Plasmid). B. Southern blot analysis of transgenic bottle gourd. Lanes: M, 100bp ladder marker, 1,control (G5, wild type), 2 and 3, transgenic plants (T0 28-16-3,31-17-2), PC, Positive control.

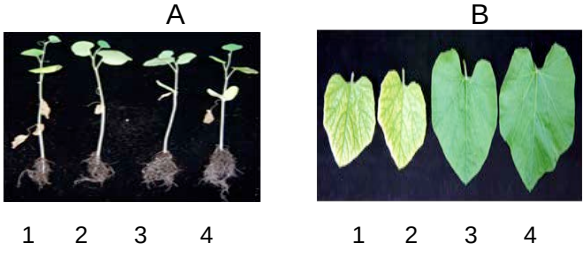

Fig. 2. Comparison of transgenic plants and control plants grown under drought stress. A. Plant under water deficit stress. Picture was taken16 days after imposed soil water deficit. B. Plant recovered from drought stress. Picture was taken 20 days after re-watering. Lanes: 1,G5(wild type), 2, B7001(vector control), 3 and 4, transgenic plants expressing *AVP1* (T2 28-16-3,31-17-2)

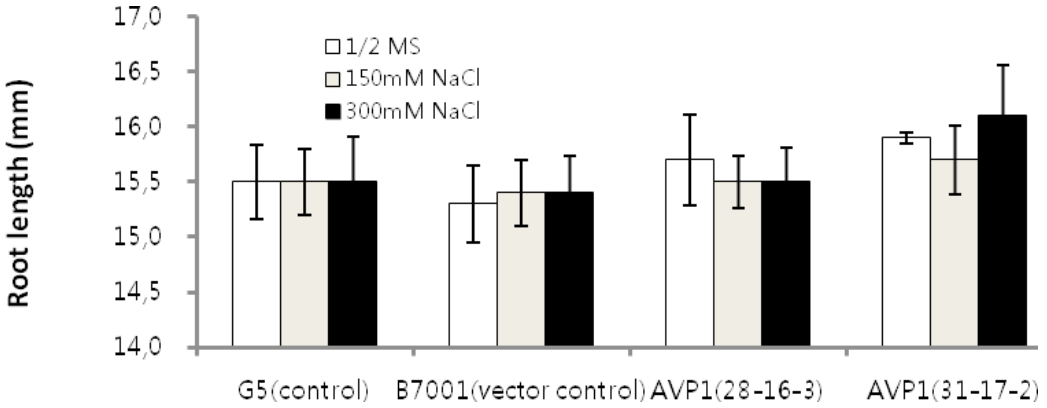

Fig. 3. Root growth of bottle gourd seedlings in medium under salt stress.

Data indicated the mean of root length after 60hr incubation on medium with/ without NaCl. Var means the standard error (n=30).

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Determination of fungal and bacterial pathogens greenhouse cucurbit crops in the province of Antalya in Turkey

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Keywords: Bacterial and fungal diseases, Cucurbitaceae, Greenhouse, Vegetables

Abstract

In this work, the causal agents of fungal and bacterial diseases in cucurbit plants grown in greenhouse were investigated in eleven different locations of Antalya. Based on the surveys done in different greenhouses, causal agents were determined extensively in the stages of seedling and fruiting of Cucurbitaceae plants (melons, watermelon, cucumbers, squashes). Melons (*Cucumis melo* L.) and watermelons (*Citrullus lanatus* (Thunb.) Matsum.&Nakai) are eaten as fruits, whereas summer and winter squashes (*Cucurbita pepo* L.), cucumber (*Cucumis sativus* L.) are eaten as vegetables. Fungal and bacterial pathogens of Cucurbitaceae plants grown in the province of Antalya were determined by symptomatological, pathological and microscopic tests. Initial symptoms of bacterial infection occurred on leaves and consist of small, irregularly shaped, water-soaked lesions, they become angular in shape and delimited by leaf veins. Leaf spots and necrotic lesions, fruit spots and lesions also occurred. Damping off and root rots (*Fusarium* spp.*, Phytophthora* spp.*, Pythium* spp.*, Rhizoctonia solani* Kühn.) were determined in seedlings. Downy mildew (*Pseudoperonospora cubensis* (Berk and Curt.) Rostow) and powdery mildew (*Golovinomyces cichoracearum*) were determined in melon, squashes and cucumber. Powder mildew of cucumber is very common in nearly all cucumber-growing areas. *Botrytis cinerea* ((De Bary) Whetzel) and *Sclerotinia sclerotiorum* ((Lib.) De Bary) were determined in squash. The bacterial pathogens of *Pseudomonas syringae* pv. *lachrymans* ((S.&B.) Y.D.&W.) on cucumber, *Xanthomonas* spp. on melon, *Acidovorax avenae* subsp. *citrulli* on watermelon.Management practices effective for these diseases include rotation, fumigation, solarization, pathogen-free seed, treated seed, host plant resistance, sanitation, adjusting soil pH, controlling weeds and insects, and using chemical and biological fungicides and bactericides.

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INTRODUCTION

Cucurbitaceae is an important family consisting of approximately 125 genera and 825 species like various squashes, melons,cucumber, pumpkins and watermelon (Perveen and Qaiser 2008). Cucurbits are a family of healthy foods. Cucumbers are prime dieting food. They are 96 percent water, with a little fiber and only a few calories (Deyo and Malley 2008).

Vegetable production is extensive in the province of Antalya because of appropriate climatic factors, soil and irrigation water conditions. The Turkish vegetable export increased from 628 kg in 2006 to 958 kg in 2010. About half of the vegetable export consists of tomatoes. Cucumbers are second in the list of main export vegetables. The most important export destination for Turkish vegetables are Bulgaria, Romania, Germany, Ukraine and Iraq (Tuik 2012). According to the Turkstat total fresh fruit and vegetable production is up 4.9% from 42.5 millions tons to 44.7 million in 2011 mainly due to significant increase in tomato production (Tuik 2012). Cucumbers are one of the traditional vegetable from in greenhouses. Watermelons and melons, having as here of 12.3% in overall fresh fruit and vegetables production ranked second. Production of these vegetables increased by 3.5% from 5.3 million to 5.5 million tons in 2011 (Tuik 2012). The Cucurbitaceae has a lot of pests and diseases. Diseases are major causes of the vegetable plants for the reduction of quantity and quality of the production. Especially, fungal pathogens cause both quantitative and qualitative losses (Watson 2009).

The main objective of this work is to determine fungal and bacterial pathogens of greenhouse cucurbit crops and to suggest their control in the province of Antalya in Turkey.

MATERIALS AND METHODS

Survey areas

The infected cucurbit plants were collected in the following survey areas: Aksu, Gaziler, Boztepe, Topçular, Kemerağzı, Koyunlar, Çalkaya, Serik, Çakırlar, Habipler, Kurşunlu which are the potential vegetable cultivation areas. Surveys were made in the years 2010 and 2011. Infected foliage, fruit or whole plants were taken out and put into polyethylene bags, labelled and brought to the laboratory immediately for symptomatological, pathological and microscopic tests. The plants were kept in refrigerator until using for analysis.

Identification and isolation of fungal and bacterial pathogens

The diseased plant samples of Cucurbitaceae were randomly collected from melons, watermelon, cucumbers and squashes. Inıitial symptoms occur on leaves and consist of small, irregulary-shaped, water-soaked expand, the become angular in shape as lesion edges become delimited by leaf veins. Leaf spots and necrotic lesion also fruit spot and lesions were analyzed by identification tests. The fungal

isolations from the samples were done based on the methods described by Agrios (1997). The surface of roots, stems, leaf and fruit materials were rinsed with tap-water prior the disinfection with 70% alcohol. The plant materials were sterilized by 1% hypochloride and washed by sterile tap water and inoculated on Potato Dextrose Agar (PDA) (Difco) and Water Agar (WA). Inoculated Petri dishes were incubated at 27^0C. Microscopic observations of fungi grown on Petri dishes were done based on colony color and morphological structure of the fungi. The bacterial isolations from the samples were done based on the methods of Agrios (1997) and Schaad (2001).

RESULTS AND DISCUSSION

Damping off and root rots (*Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*) were determined in seedling. Downy mildew (*Pseudoperonospora cubensis* (Berk and Curt.) Rostow) and powdery mildew (*Golovinomyces cichoracearum*) were determined in melon, squashes and cucumber. Powder mildew of cucumber is very common on all cucurbits in studied areas. *Botrytis cinerea* ((De Bary) Whetzel) and *Sclerotinia sclerotiorum* ((Lib.) De Bary) were determined in squash and cucumber. The bacterial pathogens of *Pseudomonas syringae* pv. *lachrymans* ((S.&B.) Y.D.&W.) on cucumber, *Xanthomonas* spp. on melon, *Acidovorax avenae* subsp. *citrulli* on watermelon. Based on surveys done to 10 different Cucurbitaceae plant production areas, *Sclerotinia sclerotiorum*, *Botrytis cinerea* , *Pythium* spp, *Phytophthora* spp. and *Golovinomyces cichoracearum* were detected at the rate of 19.6 %,17.8 %, 16.1%,14.9% and 14.6%, respectively (Table 1**).** Plant pathogenic bacteria were less common than plant pathogenic fungi. Bacteria (*Pseudomonas syringae* pv. *lachrymans,* *Xanthomonas* spp. and *Acidovorax avenae* subsp. *citrulli*) were detected at the rate 2.4%, 0.9% and 0.5%, respectively (Table 1). Fungal pathogens of Cucurbitaceae in Turkey were previously studied (Sagır 1988; Yücel and Güncü 1991;Yücel 1994; Kırbag and Turan, 2005). Based on our study, certified seed and seedlings should be used for the early protection of the diseases determined in this work. Managing diseases is a very important component of production for melons, cucumbers, squashes and other Cucurbit crops. Management practices effective for these diseases include rotation, fumigation, solarization, pathogen-free seed, treated seed, host plant resistance, sanitation, adjusting soil pH, controlling weeds and insects, and using chemical and biological fungicides and bactericides.

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Table 1. Distribution of fungi and bacteria on cucurbit crops in Antalya province.

			Antalya (Survey Locations/number of isolates)											
Host	**Fungi** ****	**Bacteria*****	A***	B	C	D	E	F	G	H	I	J	**Total***	**(%)****
Squash	*Sc*	-	8	10	12	15	5	12	6	12	13	12	105	19.7
Cucumber	*Bc*	-	10	7	8	15	18	3	2	10	7	15	95	17.8
Cucumber Squash, melon	*Pyt.*	-	4	8	6	12	9	8	6	12	6	15	86	16.1
Cucumber Squash, melon	*Phy*	-	5	4	10	8	6	4	15	10	10	8	80	14.9
Cucumber Melon	*Gc*	-	7	9	6	11	7	9	2	9	6	12	78	14.6
Squash Melon, Cucumber	*Pc*	-	-	2	1	2	2	-	1	2	-	2	12	2.2
Cucumber Squash	*Rs*	-	6	6	3	4	8	2	2	5	8	11	55	10.3
Melon Watermelon	*F*	-	-	-	-	-	1	-	-	1	-	-	2	0.4
Cucumber	-	*Psl*	1	1	-	2	4	-	-	3	1	1	13	2.4
Melon	-	*X*	1	-	-	-	1	-	-	-	1	2	5	0.9
Watermelon	-	*Aac*	-	-	-	-	1	-	-	1	1	-	3	0.6
													534	100

*Total number of isolates
** Presence of pathogen (%)
***A:Aksu, B:Kemerağzı, C:Koyunlar, D: Topçular; E:Boztepe; F:Çalkaya; G:Çakırlar; H:Habipler; I:Kurşunlu; J:Gaziler
****Ss: Sclerotinia sclerotiorum,Bc: Botrytis cinerea,Pyt: Pythium* spp.,*Phy: Phytophthora* spp.,*Gc: Golovinomyces cichoracearum, Pc: Pseudoperonospora cubensis,Rs: Rhizoctonia solani,F: Fusarium* spp.
*****Psl: Pseudomonas syringae* pv. *lachrymans, X: Xanthomonas* spp., *Aac: Acidovorax avenae* subsp. *citrulli*

Current status of virus infections in cucurbit plantations in Ankara and Antalya provinces

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Keywords: Cucurbitaceae, virus diseases, serological testing

Abstract

Virus diseases are the major cause of economic losses in commercial cucurbit production in Turkey. Recently, symptomatic cucurbit plants were collected for detection of virus incidance in cucurbit plantations in Ankara and Antalya provinces. Collected samples were subjected to double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with specific antisera for *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus*-2 (WMV-2), *Cucumber green mottle mosaic virus* (CGMMV), *Papaya ringspot virus*-W (PRSV-W) and *Squash mosaic virus* (SqMV). According to the results, all tested viruses were present in Ankara whereas ZYMV, CMV and WMV-2 were common in Antalya province. WMV-2 and ZYMV were detected as prevalent virus infectionsof both provinces.

INTRODUCTION

Cucurbitaceae species are widely grown in different provinces of Turkey. The most cultivated species are watermelon (*Citrullus lanatus* (Thunb.) (3.864,489 t), melon (*Cucumis melo* L.) (1.647,988t) , cucumber (*Cucumis sativus* L.) (1.605,319 t) and squash *Cucurbita pepo* L.) (317,705 t) (TUIK 2012).

Ankara and Antalya provinces are the main vegetables growing areas of Turkey. However, the production of Cucurbitaceae is restricted by some biotic agents in these provinces. Viral pathogens cause sistemic infections mosaic and deformation symptoms on foliage, the formation of small and abnormal fruit, and economical losses on both quality and quantity of the production. Absence of chemicals aganist viral pathogens makes difficult to control these pathogens. Also it was hypothesized that WMV and ZYMV have more common vectors and are transmitted more efficiently from natural weed hosts to cucurbits by aphids. Non-persistently transmitted viruses are difficult to prevent by insecticide application.

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Cucurbits family are affected by more than 35 viruses all around the world (Provvidenti, 1996). Some of viral pathogens in cucurbits are *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* -2 (WMV-2), *Cucumber green mottle mosaic virus* (CGMMV), *Papaya ringspot virus*-W (PRSV-W) and *Squash mosaic virus* (SqMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Cucumber vein yellowing Ipomovirus* (CVYV), *Melon mosaic virus* (MMV)(Lisa and Lecoq, 1984; Purcifull et al, 1984).

The aim of this study was to determined current status of virus infections observed on cucurbit growing areas in Ankara and Antalya provinces of Turkey.

MATERIALS AND METHODS

Surveys and collection

Surveys were performed on different cucurbiateceae fields located in Antalya and Ankara provinces in between 2011 and 2012. The samples were obtained from melon, pumpkin, squash and watermelon plants. The symptoms included deformation, curling, mosaic on leaf, fruit deformation and plant stunting.

ELISA tests

All samples obtained were tested by DAS-ELISA (Double antibody sandwich enzyme-linked immunosorbent assay) to ZYMV, CMV, WMV-2, CGMMV, PRSV-W and SqMV (Clark and Adams 1977). Antiserum kits were obtained from Loewe (Biochemica GmbH, Germany). All experiments were replicated three times. Test was considered as positive when the mean absorbance value of tested sample was greater than twice of healthy control (Abou-Jawdah et al., 2000; Cradock et al., 2001; Ertunç, 1992; Şevik and Arlı-Sökmen, 2003).

RESULTS AND DISCUSSION

During the surveys 2011 to 2012, samples were collected in cucurbit fields including mostly squash, pumpkin, watermelon and melon plants in Ankara and Antalya provinces of Turkey. A total 197 plant samples were randomly collected during the surveys. These plant samples were subjected to DAS-ELISA.

Serological tests showed that three out of the six tested viruses were present in the Antalya province. According to results of serological tests, cucurbit plants were infected with ZYMV, CMV, WMV-2 and no infection of CGMMV, PRSV-W and SqMV was found in cucurbit fields in Antalya. In these province, 79 pumpkin plants were collected and 17 plant (21.5%) were effected with ZYMV and 47 plant (59.4%) with WMV-2 and 8 plant (10.1%). Double virus infection was detected with ZYMV and WMV-2 in 17 (21.5%) of 79 samples. Triple infections with ZYMV, WMV-2 and CMV were found in only 10 (12.6%) samples.

In Ankara province, 118 samples were collected from cucurbit plants during

the surveys and were determined by DAS-ELISA. All viruses were found on the samples: ZYMV (50.8%), WMV-2 (65.2 %), CMV (21.1%), PRSV-W (20.3%), SqMV (2.5%) and CGMMV (11.6%).

According to our results ZYMV and WMV-2 are the most wide spread cucurbit viruses in the Antalya and Ankara provinces. The ZYMV +WMV-2 mixed infection type was the most common both in Antalya and Ankara provinces. In our study, WMV-2 were determined the most widespread virus in Antalya and Ankara provinces. Similarly, these viruses were reported as the most common viruses in Samsun province, and ZYMV and WMV-2 are determined as prevalent on melon plants in the Mediterranean coast of Turkey (Sevik and Arli-Sokmen, 2003; Yılmaz et al., 1992; Erdiller and Ertunç, 1988). The mixed infection was also found in mostly of pumpkin species. Also, in the survey conducted in the Thrace region of Turkey, Serological tests showed that rates of incidence of tested viruses on watermelon were found: ZYMV (45.5%), WMV-2 (34.2%), CMV (19.9%), PRSV-W (2.1%), SqMV (1.8%) and MNSV (0.4%), while the incidence on melon were ZYMV (40.3%), WMV-2 (31.2%), CMV (7.2%), PRSV-W (2.3%), SqMV (0.5%) and MNSV (1.8%). The WMV-2+ZYMV mixed infection type was the most widespread both on melon and watermelon samples at 16.7% and 11.4%, respectively (Köklü and Yılmaz, 2006).

The most damaging viruses on Cucurbitaceae were detected as ZYMV, CMV, WMV-2 around the world (Lisa and Lecoq 1984, Zitter et al., 1996). To date, the presence of these viruses on cucurbit plants have been studied in different parts of Turkey. The incidence of ZYMV, WMV-2, CMV, PRSV-W and SqMV has been reported by different researchers (Çağlar et al., 2004; Davis and Yılmaz, 1984; Erdiller and Ertunç, 1988; Fidan, 1995; Köklü and Yılmaz, 2006; Kurcman, 1977; Nogay and Yorgancı, 1984; Özaslan et al., 2006; Yılmaz and Davis, 1985; Yılmaz et al., 1991; Yılmaz et al., 1995; Şevik and Arlı-Sökmen, 2003). CGMMV reported fistly this work.

ZYMV and WMV-2 are the most destructive viruses infecting cucurbit-growing areas. These viruses are easily transmitted by vectors and widely disseminated in fields. Absence of chemicals aganist viral pathogens makes difficult to control these pathogens.Control of the vectors may restrict virus transmission at early growing stages for field-grown cucurbits.

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Outbreak of bacterial fruit blotch disease on melon and watermelon in Cukurova region, Turkey

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Keywords: *Acidovorax citrulli*, melon, watermelon, fruit blotch

Abstract

Acidovorax citrulli is the causal agent of bacterial fruit blotch disease of watermelon and melon. The disease was first reported in 2005 in Cukurova Region, Turkey. In summer of 2009-2011, severe disease symptoms and significant economic losses were observed on watermelon (*Citrullus lanatus* cv. Blade, Zeugma, Crisby and Starburst) and melon (*Cucumis melo* cv. Surmeli) in some villages of Adana and Osmaniye in the region. Pathogen is seedborne and first inoculum sources are infected seeds or seedlings. Initial symptoms in nurseries observed water soaked small lesions on cotyledons. Reddish brown to dark brown, irregular and rarely greasy symptoms can be observed on watermelon true leaves. Pathogen can get through true leaves to fruit. Mostly, dark oil green lesions are observed on pericarp of fruit. Those lesions surround the entire fruit surface within 5-8 days; brown cracks, amber colored and foaming like stream appears on diseased fruit. Symptoms on melon are light brown, water soaked and irregular spots on true leaves, brown, receding lesions and cracks on surface and finally soften and rotten of melon observed. The disease caused significant economical losses and nearly 5000 da watermelon fields were quarantined by the authorities and banned to grow watermelon or other cucurbits for 4 years in the region. Many watermelon and melon seedlings were destroyed in the nurseries because of the disease. In the PhD study, of 85 bacterial strains, 70 strains from watermelon and 15 strains from melon were isolated from diseased watermelon and melon plants. The strains were identified as *Acidovorax citrulli* according to the morphological, physiological, biochemical, pathogenicity and PCR tests. Based on the studies, occurrence and outbreak of bacterial fruit blotch of melon and watermelon, causal agent of *Acidovorax citrulli* were determined in Cukurova Region.

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INTRODUCTION

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* (Schaad et al. 1978; Schaad et al. 2009) is the most destructive disease, which has been responsible for significant economic losses of cucurbit crops worldwide since the late 1980s. Due to the disease, most losses have been reported in watermelon and melon (Zitter et al. 1996). In Turkey, the pathogen was first reported in 1996 in Edirne, European part of Turkey, and the infected watermelon field was eradicated (Demir 1996). The bacterium was reported in 2005 in Cukurova Region, where watermelon and melon are important commercial crops (Mirik et al. 2006).

The bacterium is seedborne and the first inoculum resources are contaminated cucurbit seeds. In nurseries, the bacterium can spread rapidly under warm and humid environments. The bacterium can contaminate other healthy seedlings via overhead irrigation in nursery house. Pathogen can affect cotyledons, true leaves or fruit. If the inoculum source is contaminated seeds, first symptoms are observed as water-soaked areas or brown spots on cotyledons. Meanwhile, the symptoms do not appear so often in the field, reddish brown to dark brown, irregular and rarely greasy symptoms can be observed on watermelon true leaves. Pathogen can get through true leaves to fruit. Mostly, small, irregular, smaller than 1mm in diameter dark oil green lesions, brown cracks, amber colored and foaming like stream are observed on whole fruit. Symptoms on melon are light brown, water soaked and irregular spots on true leaves, brown, receding lesions and cracks on surface and finally soften and rotten of melon.

In Cukurova Region during 2009-2011, the BFB disease caused significant economically losses on watermelon and melon and nearly 5000 da watermelon fields were quarantined by the authorities and banned to grow watermelon or other cucurbits for 4 years. Furthermore, many watermelon and melon seedlings were destroyed in the nurseries because of the disease.

The objectives of this study were (1) to survey and collect diseased leaves or fruit of melon and watermelon during 2009 to 2011 (2) to determine the occurrence and outbreak of BFB on watermelon and melon in Cukurova Region.

MATERIALS AND METHODS

Source of strains

During 2009 to 2011, surveys were conducted in many watermelon and melon fields or nurseries. Symptomatic seedlings, true leaves or fruit were collected for detecting the presence of *Acidovorax citrulli (Ac)*. Bacteria were isolated from symptomatic lesions on seedlings, true leaves or fruit. Bacterial strains were grown for 48 hours at 25 ºC on King's Medium B (KB) and creamy colored, circular, smooth and non-florescent type bacterial colonies were purified.

Biochemical Assays

All isolates were tested for their biochemical features, using gram reaction, arginine dyhidrolase, pectolytic activity on potato slices, oxidase reaction and infiltration to tobacco leaves (*Nicotiana tabacum* cv. Samsun) for hypersensitive reaction.

Seedling pathogenicity assays

All strains were grown on King's Medium B at 25 ºC, and, for inoculation, cultures were washed from KB plates using 5 ml of sterile distilled water. Bacterial suspensions were adjusted to an OD_{600} of 0.2, using a spectrophotometer and diluted to final concentrations of $1.44x10^8$ colony forming units (cfu) ml^{-1}, which were used for inoculation. Pathogenicity tests were carried out on 3 week-old healthy melon (*Cucumis melo* cv 'Napolyon') and watermelon (*Citrullus lanatus* cv 'Blade') seedlings and prepared suspensions were sprayed and injected to the healthy leaves. Inoculated seedlings were kept in a growth chamber room at 28 ºC.

Serological and molecular assays

ELISA was performed with monoclonal (Agdia, SRA 14800) antibodies against *Acidovorax citrulli*. Test was followed manufacturer's instruction, optical densities (OD) were measured at 405 nm with a microplate reader, their true values were calculated by comparing the mean negative control value. Values of higher 2 times or more of mean negative control were regarded as positive (+). Specific primers (WFB1: 5'-GAC CAG CCA CAC TGG GAC-3'/ WFB2: 5'-CTG CCG TAC TCC AGC GAT-3')designed and produced from the DNA sequence of the 16S ribosomal RNA (rRNA) gene from a standard strain of *Ac* were used in classical polymerase chain reaction for specific DNA amplification. DNA purified and kept in 30 µl deionized water at -20 ºC. PCR products were determined by electrophoresis on 2% agarose gels.

RESULTS AND DISCUSSION

According to the conducted surveys in Cukurova Region of the diseased materials from Adana (Ceyhan, Karatas, Kozan and Yuregir) and Osmaniye (Kadirli) provinces, creamy colored, circular, smooth and non-florescent type 70 bacterial strains from watermelon and 15 bacterial strains from melon were isolated. Of the gathered symptomatic materials, watermelon cultivars 'Blade', 'Zeugma', 'Crisby' and 'Starburst' and melon cultivar 'Surmeli' were contaminated by the pathogen.

Bacterial fruit blotch has great potential to cause significant economic losses to cucurbit production, threating watermelon and melon industry. After the first report in Cukurova Region (Mirik et al. 2006), it is started to gain importance due to severe outbreaks during 2009 to 2011. Since most losses have been reported in watermelon and melon worldwide, our surveys also confirmed the significance of the disease in Cukurova Region.

All isolates tested were gram, arginine dyhidrolase and pectolytic activity negative, oxidase and hypersensitive reaction positive on tobacco leaves (*Nicotiana tabacum* cv. Samsun) in 24-48 h after infiltration.

In pathogenicity assays, Walcott et al. (2004) observed visible BFB symptoms, including water soaking and coalescing reddish-brown lesions on cotyledons, symptoms on true leaves included reddish-brown lesions that developed along the venation after 10 days. In this study, all seedlings used were susceptible to BFB strains. Furthermore, seedling pathogenicity assays showed that injection method is more reliable than spraying to the leaves in order to observe water soaked lesions on cotyledons and true leaves after 7-10 days.

ELISA test was performed with monoclonal (Agdia, SRA 14800) antibodies against *Acidovorax citrulli*. Optical densities (OD) were measured at 405 nm with a microplate reader, absorbance values of 85 isolates were between 1.88 to 2.02. All isolates were regarded as positive (+).

According to the specific primers, all the strains were identified as *Ac* and the identification was confirmed by amplification of 360 bp bands in classical PCR tests. Walcott and Gitaitis (2000) detected 100% of *A. citrulli* strains with ELISA tests using polyclonal antibodies and WFB1 and WFB2 specific primers. Willems et al. (1992) found that the bacteria that yielded amplicons with WFB 1 and 2 by PCR were closely related to *Ac.* These results were also observed in our tests.

Bacterial fruit blotch (BFB) is still threating watermelon and melon production in the region. Even in 2012, we observed disease symptoms in fields. Since the pathogen is seedborne, first step has to be using healthy seeds. Our study also advises that more test techniques must be validated for seed health testing. Since the pathogen can remain in the soil, infected plant debris, voluntarily germinated cucurbits or volunteer cucurbit plants, and control strategies are also crucial at these stages to minimize the spread of this disease in the region.

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Genetic characterization of isolates of *Pseudoperonospora cubensis* causing downy mildew in different countries using molecular markers

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Keywords: Cucumber, downy mildew, ISSR, SSR, SRAP, genetic diversity

Abstract

Downy mildew caused by Pseudoperonospora cubensis is a major disease of cucurbits worldwide. In cucumber it causes serious economic yield losses. P. cubensis has a high degree of evolution potential. The recent appearance of new genotypes of the pathogen in the USA, EU and Israel resulted in the breakdown of genetic resistance and expansion of host range. Therefore, revealing the pathogen diversity is required. In this study, pathogen isolates were collected during 2008-2010 from cucumber fields in different regions of Turkey, Israel and The Czech Republic and genetically compared using SSR, ISSR and SRAP molecular markers. The data showed remarkable genetic diversity among and between isolates recovered in Israel, Turkey and The Czech Republic. The isolates from Israel were clearly distinguished from those recovered in Turkey or The Czech Republic. The isolates from Turkey and The Czech Republic were highly variable. These findings mayt indicate sexual recombinations in nature and support the notion that selected molecular markers can be used to follow genetic differentiation in P. cubensis isolates.

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New hosts of *Pseudoperonospora cubensis* in the Czech Republic and pathogen virulence variation

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Keywords: *Cucumis sativus, C. melo, Cucurbita* spp., *Citrullus lanatus*, cucurbit downy mildew, disease prevalence and severity, pathotypes, virulence factors

Abstract

During the 2009, 2010, and 2011 growing seasons, disease prevalence and severity and the host range of *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew, were evaluated at more than 70 locations in two main regions of the Czech Republic. Infection by *P. cubensis* was observed primarily on *Cucumis sativus*, rarely on other cucurbits. Medium to high disease-severity levels were most frequently recorded on *C. sativus.* During the years 2010 and 2011, *P. cubensis* infection was also recorded on *Cucumis melo*, *Citrullus lanatus* and *Cucurbita moschata*. Occurrence of *P. cubensis* on *C. melo* and *C. lanatus* has been formerly reported from the Czech Republic, however, infection on *C. moschata* was observed for the first time in the Czech Republic in 2009. In the years 2010 and 2011, four new hosts (*Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita ficifolia* and *Lagenaria siceraria*) of *P. cubensis* were found in the Czech Republic.

Virulence structure and its temporal changes (2010 to 2011) were studied in populations of *P. cubensis* in the Czech Republic. Seventy *P. cubensis* isolates, collected from *Cucumis sativus* and *melo, Cucurbita maxima, pepo,* and *moschata*, and *Citrullus lanatus*, were analyzed for virulence variation. The variation of pathogen populations was expressed by the designation of pathotypes using tetrad numerical codes. The most susceptible group of differentials was *Cucumis* species; in contrast, the lowest frequency of pathogenicity was recorded on *Cucurbita pepo* subsp. *pepo*, *Citrullus lanatus* and *Luffa cylindrica*. A high proportion of Czech *P. cubensis* isolates were able to infect two cucurbit species, *Benincasa hispida* and *Lagenaria siceraria*, that are not commonly cultivated in the Czech Republic or elsewhere in Central Europe. In this study period (2009-2011), there were substantial changes to the pathogen virulence structure in comparison with the period 2001-2008.

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INTRODUCTION

The Cucurbitaceae, a large family and heterogeneous group of plants are most diverse in America, Africa and Asia (Bates et al. 1990; Lebeda et al. 2007) but are now grown in most countries of the world, primarily in the warmer and temperate regions. It is a family of many economically important species, particularly those with edible fruits (Robinson and Decker-Walters 1997). In the Czech Republic, cucumber (*Cucumis sativus*) is among the traditional, favorite and most frequently grown vegetable crops (Moravec et al. 2004). However, since 1984 cucumber production has been seriously limited by the occurrence of epidemics of cucurbit downy mildew caused by the oomycete, *Pseudoperonospora cubensis* (Lebeda 1986). These outbreaks cause significant crop damage and lead to death of the plants at the adult stage (Lebeda and Schwinn 1994). During last decade in the Czech Republic, other cucurbits, such as *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata* have been grown more frequently (Lebeda et al. 2011).

Cucurbit downy mildew is the most important foliar disease of cucurbit crops worldwide. Currently, the pathogen is very destructive in all humid areas of the world as well as some temperate areas, and the disease, therefore, plays a crucial role in determining the quantity and quality of cucurbit production. Recently, more than 60 species were reported to be affected by *P. cubensis* (Lebeda and Cohen 2011). In the Czech Republic, disease prevalence, host range and disease severity were evaluated from 2001-2009. The geographical distribution of *P. cubensis* was assessed on ca. 80 -100 locations per year in two main regions of the Czech Republic (central and southern Moravia, and eastern, northern, and central Bohemia) (Lebeda et al. 2011). Infection by *P. cubensis* was observed primarily on cucumber (*C. sativus*) but only on the leaves. The majority of *C. sativus* crops were heavily infected at the end of growing season (second half of August). Generally, *P. cubensis* was present at high or very high disease severity across the whole area of the Czech Republic studied. The loss of foliage from such severe infections results in the reduction of the quality and quantity of marketable fruits. Very rarely, in past years, we had also recorded infections on muskmelon (*Cucumis melo*) and *C. moschata* (Lebeda et al. 2011). However, beginning in 2009, the pathogen population changed dramatically, and new hosts were recognized by new pathotypes that were able to establish serious infection of *Cucurbita* spp. (Pavelková et al. 2011) and watermelon (*Citrullus lanatus*), not observed in 2001 to 2008 (Lebeda et al. 2011).

The objectives of this study were: 1) to address the existence of new host species for *P. cubensis*; 2) to measure variation in virulence (at the level of a pathotype) of *P. cubensis* in the Czech Republic in the period 2009-2011.

MATERIALS AND METHODS

The distribution, disease prevalence and severity caused by *P. cubensis* on cucurbitaceous vegetables were evaluated in 2009-2011 in the Czech Republic (Table 1), in a similar fashion to our earlier surveys (Lebeda et al. 2011). Three surveys were made per year (late July to late August) in two main areas of the Czech Republic (Moravia, central and southern parts; and Bohemia, eastern and central parts) from 2009 to 2011 (Lebeda et al. 2011). The main cucurbitaceous vegetable production areas were visited (e.g., South and Central Moravia, East Bohemia and Polabí). However, some marginal areas for cucurbit cultivation (e.g., areas of Jeseníky, Beskydy, Českomoravská Vrchovina, Podkrkonoší) were also surveyed. The occurrence of *P. cubensis* was monitored during the main harvest period in hobby gardens, small private fields and large production fields. Disease prevalence and severity were evaluated annually at more than 70 locations following the methodology of Lebeda et al. (2011).

The virulence of 70 isolates (collected in 2010 /37/ and 2011 /33/) was screened on a differential set of 12 cucurbit taxa (Lebeda and Widrlechner 2003, Table 2). A leaf-disc method was used (Lebeda and Urban 2010), with a visual 0-4 scale (Lebeda 1991) used to evaluate sporulation intensity over a two-day period from 6 to 14 days after inoculation. The sporulation intensity was expressed as the percentage of maximum sporulation intensity (Lebeda and Urban 2010). Leaf discs with no, or only a low level of sporulation ($\leq 35\%$), were considered to show an incompatible response; those with a medium or high level of sporulation were considered compatible genotypes (Lebeda and Urban 2010). The virulence level of isolates was determined on the basis of the number of virulence factors, i.e. number of compatible reactions within the differential set of cucurbitaceous taxa. Pathotypes were designated with tetrad numerical codes (Lebeda and Widrlechner 2003).

RESULTS AND DISCUSSION

Infection was observed primarily on cucumber, rarely on other cucurbits. Disease prevalence of *P. cubensis* on *C. sativus* ranged from 91-97%. The low (infection degree ID = 1), or medium to high (ID = 2-3) severity levels were most frequently recorded on *C. sativus*. During the years 2010 and 2011, *P. cubensis* infection was also recorded on *C. melo* (2011), *C. lanatus* (both years) and *C. moschata* (2010) (Table 1). The occurrence of *P. cubensis* on *C. melo* and *C. lanatus* has been formerly reported from the Czech Republic (Lebeda et al, 2011), however, infection on *C. moschata* was only reported for the first time in the Czech Republic in 2009 (Pavelková et al, 2011). During the years 2010 and 2011, four new hosts (*C. pepo* and *C. maxima* /2010-2011/, *Cucurbita ficifolia* /2010/ and *Lagenaria siceraria* /2011/) were found (Table 1).

The structure of, and temporal changes in, virulence in *P. cubensis* have been studied in the Czech Republic from 2001 to 2010 (Lebeda et al. in press). Nearly 400 *P. cubensis* isolates collected mostly from *C. sativus* (ca 96%), but also from *C. melo, C. maxima, C. pepo, C. moschata*, and *C. lanatus*, were analyzed for variation in virulence (at the pathotype level). The structure of, and changes in, virulence in the pathogen populations were expressed by the designation of pathotypes using tetrad numerical codes, based on a differential set of 12 genotypes of cucurbitaceous taxa (Lebeda and Widrlechner 2003, Table 2). The differential set identified 67 different pathotypes of *P. cubensis*. In these pathogen populations, 70 to 100% of the isolates expressed a high number (9-12) of virulence factors. "Super pathotype" 15.15.15 was often observed in our study and was one of the four most frequently recorded pathotypes (Lebeda et al. in press). Recently two new pathotypes were determined (15.7.9., 14.0.0.) from the new host plants (Table 3), however, we are expecting more new pathotypes from cucumbers (data not elaborated yet).

The most susceptible group of differentials was *Cucumis* species, in contrast the lowest frequencies of virulence were recorded on *C. pepo* subsp. *pepo*, *C. lanatus* and *Luffa cylindrica*. Notably, many Czech *P. cubensis* isolates were also able to infect two cucurbit species (*Benincasa hispida* and *L. siceraria*; Table 3) that are not commonly cultivated in the Czech Republic or elsewhere in Central Europe.

Over time, clearly *P. cubensis* populations have been evolving toward higher levels of virulence, with substantial changes when compared to the period 2001-2009 (Lebeda et al. 2010). Since 2009, the pathogen population has changed dramatically, and new pathotypes are now able to establish serious infections on *Cucurbita* spp. and *C. lanatus* (Lebeda et al. 2011; Table 3), which was not observed between 2001 and 2008 (Lebeda et al. in press).

Previous studies have shown that *P. cubensis* is a highly variable pathogen from the viewpoints of host-specificity, race-specificity and virulence (Lebeda et al. 2006, in press, Fig. 1.; Lebeda and Cohen 2011), findings also supported by recent molecular studies (Sarris et al. 2009; Mitchell et al. 2011; Quesada-Ocampo et al. in press; Runge et al. 2011). The comparison of *P. cubensis* isolates collected from 2001 to 2010 in the Czech Republic confirms that the virulence structure of Czech pathogen populations is very broad and dynamic in time and space (Lebeda et al. in press). However, from this study it is clearly evident that during these most recent surveys the host range and variation in virulence changed substantially (Tables 1 and 3). This supports previous hypotheses (Lebeda et al. 2006; Lebeda and Cohen 2011) that higher genetic diversity of resistance in *Cucurbita* spp. and some other Cucurbitaceae may indirectly, but significantly contribute to the selection of new *P. cubensis* pathotypes (Lebeda et al. in press). These changes must be seriously considered in the development of effective disease management, including the evolutionary dynamics of fungicide resistance (Lebeda and Cohen 2012).

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Table 1. *Pseudoperonospora cubensis* infection of cucurbitaceaous vegetables, other than cucumber (*Cucumis sativus*), recorded in the Czech Republic in 2009-2011.

Year Host species	Number of monitored localities	DI (degree of infection)* Number of monitored localities (frequency of localities %)				
		0	1	2	3	4
2009						
Cucumis melo	2	1(50)	1(50)	-	-	-
Cucurbita moschata	3	2(75)	1(25)	-	-	-
2010						
Cucurbita pepo	66	56(85)	8(12)	2(3)	-	-
Cucurbita maxima	38	32(85)	4(10)	2(5)	-	-
Cucurbita moschata	2	-	2(100)	-	-	-
Cucurbita ficifolia	1	-	1(100)	-	-	-
Citrullus lanatus	6	5(83)	-	-	1(17)	-
2011						
Cucumis melo	2	1(50)	-	-	-	1(50)
Cucurbita pepo	58	48(83)	7(12)	3(5)	-	-
Cucurbita maxima	29	18(62)	9(31)	2(7)	-	-
Cucurbita moschata	2	1(50)	1(50)	-	-	-
Citrullus lanatus	10	8(80)	-	1(10)	1(10)	-
Lagenaria siceraria	2	-	2(100)	-	-	-

*Degrees of infection (DI) measured on a 0-4 scale (Lebeda and Křístková, 1994)

Table 2. The Cucurbitaceae differential set for the determination of pathogenic variability in *P. cubensis* (Lebeda and Widrlechner 2003), EVIGEZ – Czech genebank number.

No.	Taxon	Accession number		Cultivar name
		Donor	EVIGEZ	
1	*Cucumis sativus*		H39-0121	Marketer 430
2	*Cucumis melo* subsp. *melo*	PI 292008	H40-1117	Ananas Yoqne´am
3	*Cucumis melo* subsp. *agrestis* var. *conomon*	CUM 238/1974	H40-0625	Baj-Gua
4	*Cucumis melo* subsp. *agrestis* var. *acidulus*	PI 200819	H40-0611	
5	*Cucurbita pepo* subsp. *pepo*	PI 171622	H42-0117	Dolmalik
6	*Cucurbita pepo* subsp. *texana*	PI 614687	H42-0130	
7	*Cucurbita fraterna*	PI 532355	H42-0136	
8	*Cucurbita maxima*		H42-0137	Goliáš
9	*Citrullus lanatus*		H37-0008	Malali
10	*Benincasa hispida*	BEN 485	H15-0001	
11	*Luffa cylindrica*		H63-0010	
12	*Lagenaria siceraria*		H59-0009	

Table 3. Characterization of virulence of *P. cubensis* isolates originating from a new host species (sampled in the period 2009-2011) (added and adapted according to Lebeda et al., in press).

Host plant[z]	Isolate	Origin Region/District/Location	Pathotype	No. of differential genotype[y]												VF[x]
				1	2	3	4	5	6	7	8	9	10	11	12	
CMe	89/09	OL/OL/ Olomouc-Holice	15.14.11	+[w]	+	+	+	-	+	+	+	+	+	-	+	10
CMe	19/11	OL/OL/Olomouc-Holice	15.14.15	+	+	+	+	-	+	+	+	+	+	+	+	11
CMe	20/11	OL/OL/Olomouc-Holice	15.7.9	+	+	+	+	+	+	+	-	+	-	-	+	9
CP	58/10	JM/HO/Mutěnice	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CP	61/10	JM/HO/Ratiškovice	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11
CP	72/10	ZL/ZL/Napajedla	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CP	73/10	ZL/ZL/Napajedla	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CP	59/11	ZL/KM/Postoupky	15.15.10	+	+	+	+	+	+	+	+	-	+	-	+	10
CP	66/11	ZL/KM/Napajedla	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11
CP	87/11	MS/NJ/Nový Jičín-Kojetín	14.0.0.	-	+	+	+	-	-	-	-	-	-	-	-	3
CP	89/11	MS/NJ/Nový Jičín-Kojetín	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CM	12/10	JM/BO/Moravské Bránice	15.15.7	+	+	+	+	+	+	+	+	+	+	+	-	11
CM	67/10	JM/HO/Veselí nad Moravou	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11
CM	81/10	OL/OL/Olomouc-Holice	15.6.0	+	+	+	+	-	+	+	-	-	-	-	-	6
CM	45/11	OL/OL/Olomouc-Holice	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CM	54/11	OL/PR/Polokovice	15.15.10	+	+	+	+	+	+	+	+	-	+	-	+	10
CMo	86/10	MS/NJ/Nový Jičín-Kojetín	15.15.15	+	+	+	+	+	+	+	+	+	+	+	+	12
CMo	88/09	MS/NJ/Nový Jičín-Kojetín	4.15.0	-	-	+	-	+	+	+	+	-	-	-	-	5
CL	83/10	OL/OL/Olomouc-Holice	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11

[z] CMe - *Cucumis melo;* CP - *Cucurbita pepo;* CM - *Cucurbita maxima;* CMo - *Cucurbita moschata;* CL - *Citrullus lanatus*
[y] 1 – 12, for details see Table 2.
[x] VF, number of virulence factors.
[w] +, compatible reaction of *P. cubensis* isolates on cucurbit differential genotypes; -, incompatible reaction of *P. cubensis* isolates on cucurbit differential genotypes.

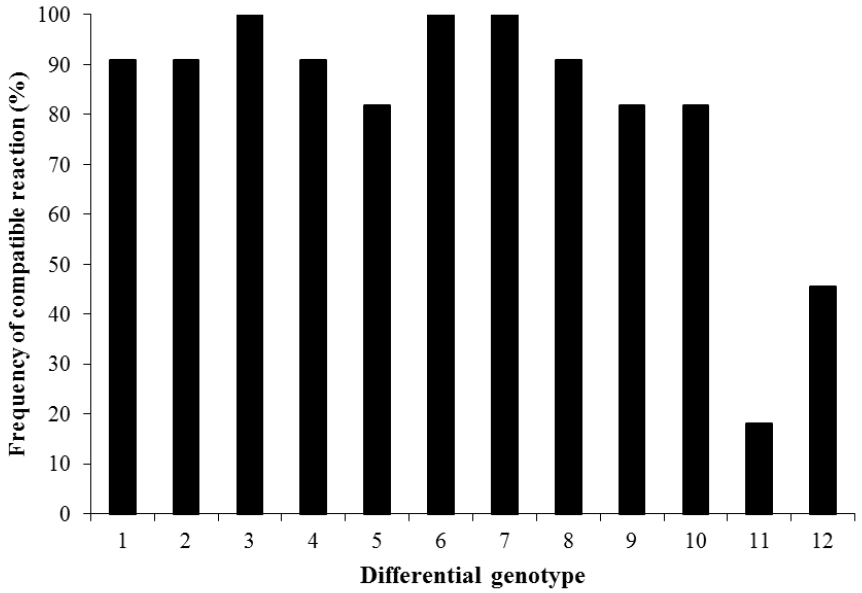

Fig. 1. Variation in susceptibility of cucurbit differential genotypes (1-12, see Lebeda and Widrlechner (2003)) to *P. cubensis* isolates (N=11) from new host species (origin from the years 2009 and 2010, see Tables 1 and 2) (adapted according to Lebeda et al. in press).

Morphological characteristics evaluation in Cucurbitaceae plant for breeding improvement

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Abstract

Cucurbitaceae wild germplasm are important genetic resources for breeding program. The various morphological traits and multi-resistance characteristics were useful for quality and field improvement on cucurbitaceous plant. Four wild cucurbitaceous plant *Coccinia grandis* (L.) Voigt, *Cucumis bisexualis* A.M.Lu et G.C.Wang., *Trichosanthes* L., and *Citrullus lanatus* ssp.*vulgaris* var. *megalaspermus* Lin et Chao. Which from China were compared with a watermelon accession (*Citrullus lanatus* (Thunb.) Manf.) and a melon accession (*Cucumis melo* ssp. *conomon* (Thunb.) Greb) for 19 botanical characteristics using multivariate methods, including one way difference significant test, principal component and cluster analysis to investigate the morphological variation and relationship among wild germplasm and cultivars.

Length and width for cotyledon, true leaf, hypocotyledonous stem, ovary, main stem, fruit and seed were calculated. Furthermore, corolla of female flower, length of stigma, seed number per fruit, seed weight and thickness of seed were also detected. One way significant test results indicated all these characteristics were moderated significant different. Morphological data were analyzed collectively with Principal Component Analysis (PCA), 97% of the observed variation was explained (47, 29, 12 and 8% in principal components 1, 2, 3 and 4 respectively). Length of cotyledon, Length of stem, length of stigma and length of hypocotyledonous stem were primary contributors to principal components 1, 2, 3 and 4 respectively. Cluster analysis showed that the *Citrullus lanatus* ssp.*vulgaris* var. *megalaspermus* Lin et Chao and *Citrullus lanatus* (Thunb.) Manf were similar to each other. *Coccinia grandis* (L.) Voigt was more similar to *Cucumis melo* ssp. *conomon* (Thunb.) Greb than *Cucumis bisexualis* A.M.Lu et G.C.Wang. In contrast, *Trichosanthes* L. was separated in relationship analyses.

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Cucurbitaceae 2012, Proceedings of the X[th] EUCARPIA meeting on genetics and breeding of Cucurbitaceae (eds. Sari, Solmaz and Aras) Antalya (Turkey), October 15-18[th], 2012

Cucurbit Yellow Stunting Disorders (CYSDV) and *Cucumber Vein Yellowing Virus* (CVYV) diseases on melon and cucumber in Turkey

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Keywords: CYSDV, CVYV, *Bemisia tabaci*, biotype, cucumber, melon

Abstract

The aim of this research was to determine the causal organisms of yellowing and vein clearing type of symptoms of melon and cucumber plants growing under greenhouse conditions, and which *Bemisia tabaci* biotypes are involved in transmission of viruses in Adana, Mersin and Antalya provinces. For this purposes, the diseases surveys were periodically made in 2010 and 2011, and whitefly vectors collected on the young leaves of the hosts from different district of cucurbits growing area. The virus infected plants were tested against to cucurbit viruses by PCR. The result of work suggested that CVYV and CYSDV are responsible for the disorders of the yellowing type of symptoms. The infected plants showed mainly interveinal chlorosis and yellowing type of nutrition deficiency symptoms on the leaves. Among the collected 134 melon samples, 63 and 24 plants were found to be infected by CYSDV and CVYV. In the case of cucumber samples, CYSDV and CYVV were detected on the 82 and 12 plants out of 110 cucumber plants. The collected whitefly-vector from the infected cucumber and melon plants were tested by PCR. The assay showed that *B. tabaci* Biotype B (92%) efficiently transmits the viruses but Q biotype (8%) poorly transmitted the viruses. The study of PCR clearly pointed out that individual whitefly is a harbour of viruses and transmits the viruses. DNA fragments were directly sequenced and compared with CYSDV and CVYV isolates with that of Israel, USA and Spain isolates. It was concluded that 96% of CYSDV (AY580985, FJ492808) and 95% of CYVV isolates nucleotide identity with the coat protein gene with Israel, USA and Spanish isolates.

INTRODUCTION

Cucurbitaceae 2012, Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (eds. Sari, Solmaz and Aras) Antalya (Turkey), October 15-18th, 2012

Virus diseases are a worldwide problem of (*Cucurbitaceae*) and a major limiting factor for cucurbit production. Cucurbit viruses can infect many plants belonging to different genera and families, and only a few viruses are restricted to cucurbits species. Cucurbit viruses reduce the plant vigour, number of flowers, and fruits (Luis-Arteaga et al. 1998). Among approximately 35 viruses infecting cucurbits worldwide, more than ten viruses have been reported in Turkey (Provvidenti 1996) (Yilmaz et al. 1989). The important cucurbit viruses are the aphid-borne *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV-W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Cucurbit aphid-borne yellows virus* (CABYV), the whitefly-borne *Cucumber vein yellowing virus* (CVYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Beet pseudo-yellows virus* (BPYV), the beetle-transmitted *Squash mosaic virus* (SqMV), and *Melon necrotic spot virus* (MNSV, genus Carmovirus, family Tombusviridae) (Zitter et al. (1996), Fidan et al. (2010)) During earlier studies whitefly-borne cucumber viruses were biologically detected but the distribution and incidence of the viruses were not studied.

During survey, leaf mottling, mosaic, vein banding, vein clearing; leaf and fruit malformation symptoms are most frequently noticed in field-grown cucumber and melon. In greenhouse conditions however yellowing type of symptoms were observed on cucumber which often attributed to Mg++, Fe and Mn++ deficiencies (Papyiannis et al. 2005).

Cucumber vein yellowing virus (CVYV) being responsible for important economical losses in several crops, causes a whitefly-transmitted disease in cucurbits. The virus has been taxonomically included in the genus Ipomovirus, family of Potyviridae (Lecoq et al. 2000). First, CVYV is transmitted by *Bemisia tabaci* in a semi-persistent manner (Mansour and Musa 1993), a feature shared with other members of the genus (Jones, 2003). Finally, molecular data derived from comparison of a partial sequence of the 3' region of the viral genome of the Israelian isolate CVYV-Isr have confirmed the closest relationship of CVYV with Ipomoviruses (Lecoq et al. 2000). CVYV naturally infects cucumber (Cohen & Nitzany 1960), melon (Yilmaz et al. 1989), watermelon (Janssen and Cuadrado 2002) and courgette. Wild cucurbits are also reported as hosts in Jordan (Mansour and Al-Musa 1993) CVYV is a positive-sense single-stranded RNA virus member of the family Potyviridae. The genomic RNA of potyviruses is translated into a single polyprotein that is proteolytically processed by three virus encoded proteases (Sweiss et al 2007).

B. tabaci became viruliferous after an acquisition period of 30 min and transmission reached 51% after a 4-h feeding period. Extending the acquisition time had little effect on the transmission rate. CVYV was retained by its vector for no longer than 4–6 h (Harpaz & Cohen, 1965) and the latent period was at least 75

min. Transmission by viruliferous whiteflies occurred after a 15-min inoculation feeding period. The highest level of transmission (57.5%) was reached after a 4-h inoculation period. The aphids *Aphis gossypii* and *Myzus persicae* are not vectors of CYSDV and CVYV (Cohen and Nitzany 1960).

CYSDV is a member of the genus *Crinivirus* (family Closteroviridae) efficiently transmitted by *B. tabaci*, biotypes-B (*B. argentifolii*) and Q (Berdiales et al. 1999). The natural host range of CYSDV is limited to the family of Cucurbitiacea mainly on cucumber, melon, watermelon and squash. The severe yellowing induced by CYSDV drastically affects fruit number and weight leading to a 30–50% yield reduction (Abou-Jawdah et al. 2000). First detection of CYSDV was in the United Arab Emirates in 1982 (Hassan and Duffus 1991) where it remains in epidemic proportions (Duffus 1995). CYSDV has since spread throughout the Mediterranean region (Celix et al. 1996) including Egypt, Israel, Jordan, Spain, Turkey, Lebanon, Portugal, and Morroco where it has caused major economic damage to cucurbit crops (Papyiannis et al. 2005). CYSDV has also been introduced to North America, specifically the Rio Grande Valley of southern Texas and northern Mexico (Kao et al. 2000).

MATERIALS AND METHODS

The survey and sample collection were carried out Mediterranean region during 2010–2012 in the major cucumber and melon growing area of Adana, Mersin and Antalya provinces (Table 1).

The survey and sample collection were carried out in Mediterranean region of Turkey during 2010–2012 in the major cucumber and melon growing area of Adana, Mersin and Antalya provinces (Table 1). A total of 244 plant samples showing virus-like symptoms (cucumber from 66 greenhouses and melon from 55 greenhouses) were collected from 34 different locations. At the same time a total of 340 adult whitefly individuals were collected on infected cucumber and melon plants inwhich same greenhouses.

Each sample consisted of the youngest fully developed leaf from plants exhibiting chlorosis, vein clearing or yellowing, and fruit discolorations and deformation (Fig. 2A, B). All samples were placed in plastic bags, stored at 4ºC, and tested within 1–3 days for virus infection either serologically [double-antibody sandwich enzyme-linked immunosorbent assay (DASELISA)] or by reverse transcription polymerase chain reaction (RT–PCR).

All plant samples (244) were tested by DAS-ELISA for the presence of CYSDV. The 244 samples that exhibited yellowing or vein clearing symptoms and originating from cucumber crops were also tested by RT–PCR for the presence of CYSDV, BPYV, CABYV and CVYV. A sample was considered virus-positive if its A_{405} nm value was greater than three times that of healthy plant extract.

Plant genomic RNA was isolated by freezing leaf in liquid nitrogen and grinding the tissue to a fine powder using a minipestle in an Eppendorf tube. After addition od 566 µl of extraction buffer (100mM Tris8.0, 50 mM EDTA, 500mM NaCl, 10 mM mercapto-ethanol and 1% sodium dodecyl sulfate (SDS)) the tubes were vortexed and incubated at 65 ºC for 10 min; then 160 µl of 5 M M potassium acetate was added to each tube, followed by vortexing and centrifugation for 15 min. The supernatant was removed and RNA precipitated with isoprooanol. The pellet was washed twice in 70% EtOH and resuspended in 400 µl of sterile water. One hundred nanograms of RNA was used for the each polymerase chain reaction (PCR)

Whitefly DNA isolation: Kill insects by freezing in microfuge tube. Add 10uL 0.5M NaOH to each tube. Crush insect with pipette or Kontes pestle for 30 seconds. Add 30uL 1M Tris-HCl buffer (containing 1% SDS and 20mM EDTA, pH8.0), mix well. Incubate solution at 65 C for 15 minutes, centrifuge at 12,000 rpm for 10 minutes. Transfer 30uL of supernatant to new tube. Add 30uL 95% ethanol, mix, and freeze for 30 minutes at -20ºC. Precipitate DNA by centrifugation at 12,000 rpm for 10 minutes. Dry pellet at 72 C and resuspend in 30uL 1X TE (Potter et al. 2003; Kadhi et al. 2008).

Reverse transcription polymerase chain reaction (RT-PCR) Primers used in this study and the expected length of each amplicon are presented in Table 2.

First-strand cDNA synthesis was carried out Maloney Murine Leukemia Virus (M-MLV) reverse transcriptase (FERMANTAS) using the antisense primers (Table 2). Two-step RT-PCR was performed according to the procedures described previously (Papyiannis et al. 2005). PCRs were performed using Taq Polymerase (Fermantas DreamTaq™ Green DNA Polymerase), according to manufacturer's recommendations and 0.2 µM of each forwards primers and Reverse specific primers respectively. The temperature regime for amplification reactions was as follows: initial denaturation for 5 min at 94°C, followed by 35 cycles of 95°C for 30 s, 54°C (CYSDV and BPYV), 55°C for CVYV, 62°C CABYV for 30 s and 72°C for 30 seconds. The final extension was at 72°C for 7 min. A GeneAmp 9700 thermocycler (Eppendorf Mastercycler Gradient PCR Thermocyler) was used for RT-PCR amplifications.

PCR reaction for whitefly: The basic mixture consists of 10.4 µl sterile distilled water (milli Q), 2 µl of 10Xpcr buffer, 1.4 µl of 50x MgCl2 d), 1 µl of 2.5 µl mM dNTPs, 1 µl of 10 µM Bem 23F, 1 µl of 10 µM Bem 23R, and 0.25 µl dreamTaq (5 uds/µl) in a final volume of 17 µl. The parameters of the thermal cycle are as follows: firstly at 94°C for 5 min, secondly at 94°C for 30 sec, thirdly at 55°C for 1 min, fourthly at 72°C for 1 min, fifthly repeating the cycle steps second through fourth, sixthly at 72°C 40 times for 10 min and lastly storing at 4°C until electrophoresis.

PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. The amplicons were then directed sequenced by SENTROMER Company. Sequence analysis was compared by using BioEdit v 7.0.5 programs.

RESULTS AND DISCUSSION

Plant samples were tested by ELISA and RT-PCR in order to determine which of the viruses (CVYV, CYSDV, CABYV and BPYV) showing similar symptoms infected the plants.

A total of 244 melon and cucumber were tested by ELISA for CYSDV. Among the tested samples 24 cucumber (22%) and 63 melon (47%) were found to be infected by CYSDV. On the other hand, CABYV and BPYV were not detected on the samples.

Cause of not detecting CABYV could be possible that absence of the vectors (aphids). Difficulties of entering into greenhouses and good pest management against to aphids cause this result. BPYV which is transmitted by *Trialeurodes vaporarium* have not been reported in Mediterranean region.

The coat protein specific for CYSDV cDNA band at 364 bp were obtained from 63 out of 143 melon samples and 12 out of 134 cucumber samples.

By using of ELISA and RT-PCR methods, CYSDV were quite often detected on melon growing in greenhouse. In the RT-PCR tests with primers specific to CVYV, 450 bp cDNA bands were successfully obtained from cucumber samples (82 out of 110) showing CVYV symptoms (Fig. 1B). The virus was detected among 74% of collected samples. On the other hand, infection rate in the melons was found to be 9 %. Among the collected 340 whitefly from the samples, *B tabaci* Biotype B (92%) were found in the using of PCR tests with co dominant primer Bem23. However, *B tabaci* Biotype Q were found 8 % at the same hosts by using of the Bem23 primer (Fig.1C). In order to investigate the presence of virus in the collected whiteflies samples, DNA was isolated from 1, 5 and 10 individuals. CYVY and CYSDV were detected even in a single white fly by using of RT-PCR however if the number of whitefly increased to the 10 individuals, the success of isolation of DNA also increased.

PCR the assay showed that *B.tabaci* Biotype B (92 %) efficiently transmits the viruses but Q biotype (8%) poorly transmitted the viruses. The study of PCR clearly pointed out that individual whitefly is harbour of viruses and transmits the viruses. DNA fragments were directly sequenced and compared with CYSDV and CVYV isolates with that of Israel, USA and Spain isolates. It was concluded that 96 % of CYSDV (AY580985, FJ492808) and 95 % of CYVV isolates nucleotide identity with the coat protein gene from Israel, USA and Spanish isolates.

These results are important for the management of the yellowing diseases

(CYSDV and CYVV) on cucurbits in Turkey. Chemical control of vectors (whiteflies) has not been effective to prevent to spread of these transmitted viruses by whiteflies therefore genetic resistance varieties has to develop in melon and cucumber germplasm as a means of controlling these diseases.

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Table 1. Survey and collection of samples at major cucurbits growing areas in Mediterranean region.

	CYSDV		CVYV	
	Cucumber	Melon	Cucumber	Melon
Adana	6[a]/27[b]	12[a] /41[b]	22[a] /27[b]	2[a] /41[b]
Mersin	7/39	22/48	29/39	7/48
Antalya	11/44	29/45	31/44	3/45

[a], Number of infected plants.

[b], Number of collected samples

Table 2. Primers used in RT-PCR, and predicted amplification size for detection of CVYV, CYSDV, BPYV and CABYV

Primer	Sequences	Amplicon size bp
CYSDVF	AGTGACATGCCTAACTGTTACTT	364bp
CYSDVR	ATAGCTGCTGCAGATGGTTC	
CVYVF	AGCTAGCGCGTATGGGGTGAC	450 bp
CVYVR	GCGCCGCAAGTGCAAATAAAT	
CABYVF	GAATACGGTCGCGGCTAGAAATC	600bp
CABYVR	CTATTTCGGGTTCTGGACCTGGC	
BPYVF	TCGAAAGTCCAACAAGACGT	251bp
BPYVR	CTGATGGTGCGCGAGTG	
Bem 23F	CGGAGCTTGCGCCTTAGTC	biotype”B” 220bp
Bem 23R	CGGCTTTATCATAGCTCTCGT	biotype”Q”410bp

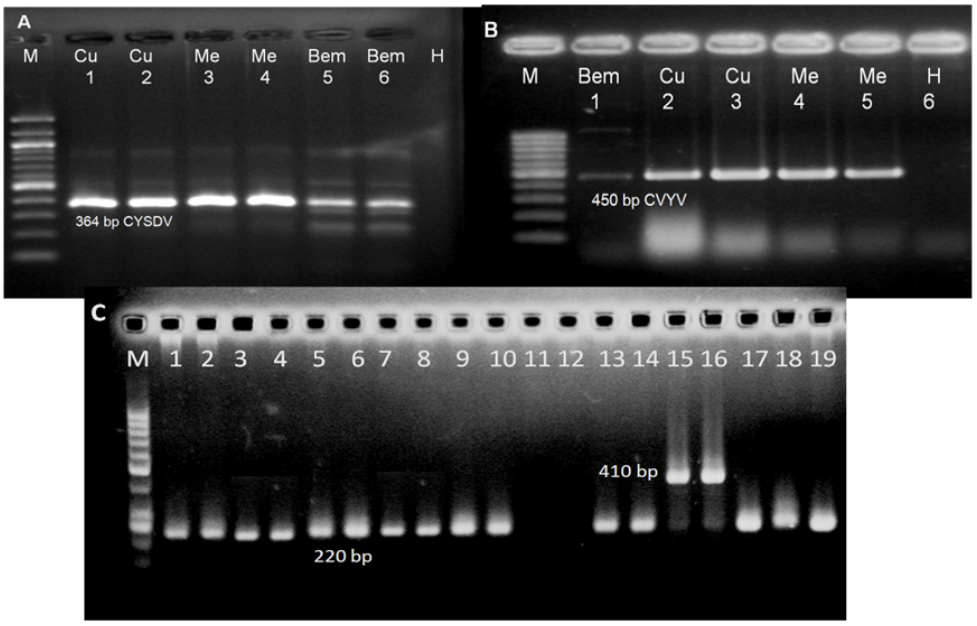

Fig. 1. A: Reverse-transcription polymerase chain reaction (RT-PCR) using primer set CYSDVF-R for detecting CYSDV in composite Line 1-2; Cucumber plants Line 3-4; Melon plants Line 5-6; Individual B tabaci PCR amplicons of 364 bp are indicated H: Negative Control.
B: RT-PCR using primer set CVYVF-R for detecting CYSDV in composite. Line1**;** Individual B tabaci PCR amplicons of 364 bp are indicated Line 2-3; Cucumber plants Line 4- 5; Melon plants Line 6 H; Negative Control.
C Line M: molecular weight marker (50 bp DNA ladder). Lines 1-10; Adult of biotype B of *Bemisia tabaci*, showing a band of 220 bp. Lines11–12; DNA free control Lines15-16: Adult of biotype Q of *Bemisia tabaci*, showing a band of 410 bp.

Fig. 2. A: Cucumber plant infected with CVYV showing vein clearing and yellowing symptoms. B: yellowing symptoms on Melon C: cucumber plant infected with CYSDV showing typical symptoms on the older leaves.

Potyviridae as a major challenge to growing cucurbits in Puerto Rico

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Keywords: *Cucurbita*, plant breeding, molecular variability, disease, potyvirus, ZYMV, PRSV.

Abstract

Potyviridae causes substantial yield losses in watermelon, pumpkin, zucchini, melon and squash, and are the most frequent family of viruses reported in cucurbits in Puerto Rico. Sampling conducted between 2006 and 2011 showed that PRSV (*Papaya ringspot virus*) and ZYMV *(Zucchini yellow mosaic virus*), both transmitted by aphids, are the most frequent virus species infecting various cucurbit species and the invasive species SqVYV (*Squash Vein Yellow Virus*), transmitted by whitefly, is severely affecting watermelons. The presence of wild cucurbit species provides a constant source of inoculum to crops and vice-versa. The most common wild cucurbit species in Puerto Rico, balsam pear (*Momordica charantia* L.) can be found everywhere on the island. West Indian gherkin (*Cucumis anguria* L.) and hedgehog gourd (*Cucumis dipsaseus* Ehrenb. ex Spach), are more frequently found at lower elevations. ELISA, immunostrip tests, RT-PCR and sequencing of a coat protein gene fragment were conducted to identify and characterize viruses affecting cucurbits. Mechanical transmission to *Cucurbita moschata* 'Waltham' was conducted and symptoms were evaluated in order to select virus isolates to challenge resistant lines of *C. moschata*. The different virus isolates induced a broad range of symptoms to inoculated *C. moschata*, indicating their high biological variability, which was confirmed by the genetic diversity of their sequences. More than one virus or strain usually infects cucurbits and their potential interaction is an additional challenge to cucurbit breeding programs.

INTRODUCTION

Cucurbits provide important basic ingredients for the Caribbean diet and pumpkins are the second most important vegetable crop in terms of revenue generated in Puerto Rico. Virus and severe virus vector outbreaks are a frequent and major cause of low yields and phytosanitary limitations to growing cucurbits

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in Puerto Rico. Continuous growing throughout the year and overlapping of susceptible crops makes Puerto Rico, a highly diverse island, an excellent and dynamic environment for plant viruses to evolve. Those aspects are also a major challenge for the development of control strategies. A survey was conducted to assess the types and prevalence of viruses infecting cucurbits. The virus isolates were mechanically transmitted, serological assays were conducted to identify the various viruses and RT-PCR followed by sequencing was done for the potyviruses, which were the most frequently encountered viruses in this survey.

MATERIALS AND METHODS

A total of ninety-nine cucurbit plants (mainly pumpkin and watermelon) showing virus-like symptoms were sampled in 14 municipalities of Puerto Rico (Adjuntas, Barranquitas, Caguas, Coamo, Corozal, Isabela, Juana Diaz, Mayagüez, Morovis, Orocovis, Santa Isabel, Vega Alta, Villalba, Yauco). Assays were conducted at the Rio Piedras Experimental Station under greenhouse and laboratory conditions. ELISA and 'immunostrips' assays (Agdia Inc.) were conducted to identify the occurrence and predominance of the following virus tests: potyvirus, PRSV, ZYMV, CMV, and SqMV. Viral extracts from field samples were inoculated to *Cucurbita moschata* 'Walthan' using phosphate buffer and carborundum. Inoculated plants were observed for 21 days for development of symptoms. Infected tissues were freeze-dried and added to the UPR/AES viral collection for preservation.

A core portion of the coat protein subunit gene was amplified in a PCR reaction using the degenerate primers MJ1 (5'-ATGGTHTGGTGYATHGARAAYGG-3') and MJ2 (5'-TGCTGCKGCYTTCATYTG-3') (Grisoni et al. 2006). Bands were cut from the gel, cleaned, and sequenced with ABI Dye Terminator kit (2 times per sample) (Sambrook and Russell 2001). Sequences were edited and aligned using Codon Aligner Software. Sequences of known PRSV and ZYMV isolates deposited at GenBank (http://www.ncbi.nlm.nih.gov/genbank/) were used as control for taxonomical information. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Ney 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 5). The analysis involved 32 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 209 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

RESULTS AND DISCUSSION

One hundred percent of the samples were positive for at least one of the tested viruses. Serological tests showed more than 90 percent of the samples to be positive for potyvirus, with 45% positive for PRSV and 40% positive for ZYMV. Ten percent of the samples were from an unknown potyvirus. WMV, SqMV and CMV were reported in less than 2 percent of the samples. Occurrence of co-infection with 2 or more viruses was common.

Following mechanical transmission to *Cucurbita moschata* 'Waltham' a wide range of symptoms was observed 2 weeks after inoculation with the various isolates, indicating a high degree of variability. Some isolates produced noticeably stronger symptoms than others.

RNA was extracted from some of the field samples and inoculated plants. RT-PCR was conducted with potyvirus degenerate primers and the amplicons were sequenced to identify more specifically the potyviruses. Sample sequences were highly diverse (Table 1), confirming the biological variability observed in the greenhouse assays. A phylogenetic tree produced by Neighboring Join (NJ) Method with Bootstrap values showed a diversity of virus isolates (Fig. 1).

The results of this research have been used to select virus strains to challenge resistant pumpkin and watermelon lines in our breeding program at the University of Puerto Rico Virus diversity, frequent co-infection events of related viruses, and abundance of natural reservoirs (weed species) are major forces challenging the successful breeding of tropical cucurbit species for virus resistance.

ACKNOWLEDGMENTS

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Table 1. Summary of the BLAST results from potyvirus coat protein (CP) gene fragments amplified from cucurbit plant samples collected in Puerto Rico.

		Closest match at GenBank						
ID	Host plant	Virus species	Accession	Max score	Query coverage	E value	Max. identity within species (%)	pb
809	*Melothia sp.*	PRSV	AB127935.1	280	82%	4.00E-72	81-77	376
570	*Cucumis sp.*	PRSV	AB127935.1	248	94%	2.00E-62	81-77	282
804	*C. pepo*	PRSV	AB127935.1	315	99%	2.00E-82	83-81	305
862	*C. pepo*	PRSV	AB127935.1	257	78%	3.00E-65	86-78	284
866	*Melothia sp.*	PRSV	AB127935.1	320	96%	4.00E-84	83-77	315
829	*C. pepo*	PRSV	AB127935.1	352	99%	6.00E-94	86-78	308
836	*Melothia sp.*	PRSV	AB127935.1	307	99%	2.00E-80	87-82	254
841	*Luffa sp.*	PRSV	AB127935.1	289	99%	5.00E-75	85-83	262
1031	*C. moschata*	PRSV	AB127935.1	205	88%	2.00E-49	82-74	245
1F	*C. lanatus*	ZYMV	JN561294.1	471	95%	9.00E-130	98-94	291
2F	*C. lanatus*	ZYMV	JN561294.1	479	96%	6.00E-132	98-95	287
3i	*C. lanatus*	PRSV	AB127935.1	385	99%	1.00E-103	87-80	314
3ii	*C. lanatus*	ZYMV	JF317296.1	547	97%	2.00E-152	97-94	336
4i	*C. lanatus*	ZYMV	JF317296.1	527	99%	2.33E-156	98-94	308
4	*C. lanatus*	PRSV	AB127935.1	284	96%	2.00E-73	83-78	285
4ii	*C. lanatus*	PRSV	AB127935.1	320	98%	3.00E-84	87-80	269
3	*C. lanatus*	ZYMV	JF792444.1	489	91%	4.00E-135	96-92	331
6	*C. lanatus*	ZYMV	JF792444.1	547	97%	2.00E-152	97-94	333
7	*C. lanatus*	PRSV	FJ467933.1	547	99%	2.00E-152	97-95	328
8	*C. lanatus*	PRSV	FJ467933.1	520	100%	2.00E-144	98-95	306
13	*C. lanatus*	PRSV	JN132457.1	535	95%	1.00E-148	97-95	333
18	*C. lanatus*	PRSV	JN132457.1	545	97%	6.00E-152	97-95	335
19	*C. lanatus*	PRSV	JN132457.1	542	98%	7.00E-151	97-95	330
20	*C. lanatus*	PRSV	FJ467933.1	549	97%	5.00E-153	97-95	337
21	*C. lanatus*	PRSV	FJ467933.1	551	99%	1.00E-153	98-95	328
22	*C. lanatus*	PRSV	FJ467933.1	468	95%	1.00E-128	97-95	294
23	*C. lanatus*	PRSV	JN132428.1	416	90%	6.00E-113	95-93	290

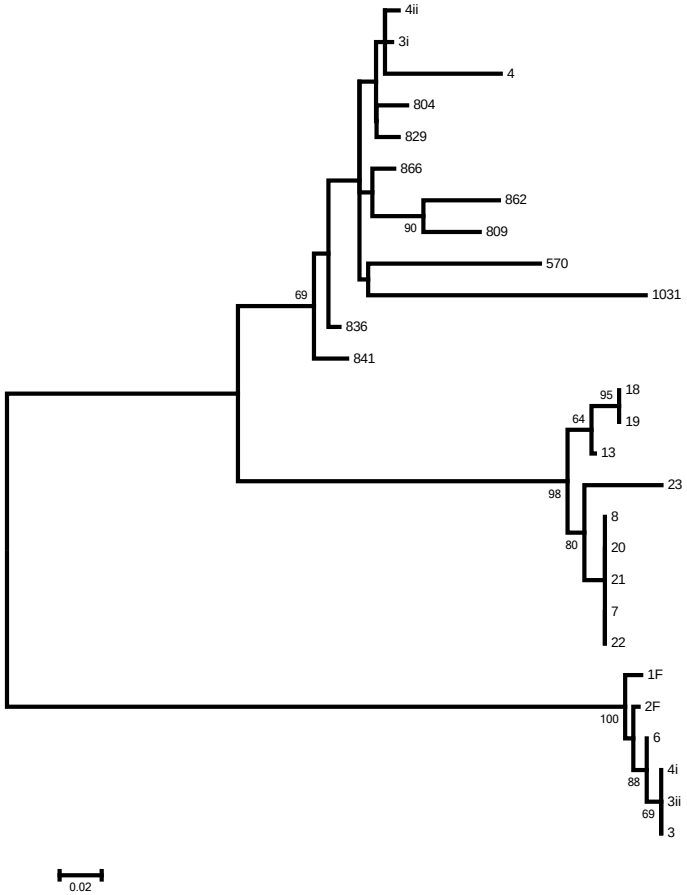

Fig. 1. A phylogenetic tree based on coat protein (CP) gene fragments sequences was produced by using the Neighbor-Joining method with Bootstrap (1000 replicates) on nodes.

Virus diseases of cucurbits in Konya province

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Keywords: Cucurbits, plant viruses, DAS-ELISA, Konya

Abstract

Virus diseases are very destructive especially on squash (*Cucurbita pepo* L.), which is grown for seeds in Konya province, therefore this research was conducted in order to determine the virus infections in major cucurbit growing areas in Konya province. In total, 423 plant samples with common symptoms of virus infections like mosaic, inward curling, blistering, mottling, distortion, shoestring, stunting and vine decline were collected from squash, zucchini, melon, watermelon, snake melon, cucumber and pumpkin plants during 2009 and 2010. The viruses were identified by DAS-ELISA and the results showed that 86.5% of plant samples were infected with *Zucchini yellow mosaic Potyvirus* (ZYMV), *Watermelon mosaic Potyvirus-2* (WMV-2), *Cucumber mosaic Cucumovirus* (CMV), *Papaya ringspot Potyvirus*-watermelon strain (PRSV-W) or *Squash mosaic Comovirus* (SqMV). ZYMV was predominant (55.1%) in the research area and occurred in squash, pumpkin, watermelon, snake melon, melon and cucumber plants. WMV-2 was detected in snake melon at the ratio of 75% and it was followed by pumpkin (51.5%), watermelon (46.7%), squash (45.4%), melon (39.1%) and cucumber (20%). Also mixed infections were common in squash, melon and pumpkin. *Cucumber green mottle mosaic Tobamovirus* (CGMMV) was not present in the research area.

INTRODUCTION

According to the different systems of evaluations, 3-5% of overall vegetable production is lost due to virus infections, but losses can be occasionally higher where pest control is insufficient especially in developing countries (Caciagli 2010). Virus diseases are a worldwide problem for cucurbit production and cause serious economic losses. Indeed, more than 35 different viruses have been isolated from cucurbits (Provvidenti 1996). These viruses constitute complex and dynamically changing problems as described by Nameth et al. (1986). Cucurbits are among the major vegetables grown in Konya province of Turkey. They occupied 5952 ha in the province during 2009-2010, with an estimated production of 118.051

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tons (Anonymous 2012). From different parts of Turkey, several virus diseases inducing mosaic symptoms were previously reported including *Cucumber mosaic Cucumovirus* (CMV) (Kurcman 1977), *Watermelon mosaic Potyvirus*-2 (WMV-2) (Nogay and Yorgancı 1984), *Zucchini yellow mosaic Potyvirus* (ZYMV) (Davis and Yılmaz 1984), *Papaya ringspot Potyvirus*-watermelon strain (PRSV-W) (Erdiller and Ertunç 1988), *Cucumber vein yellowing Ipomovirus* (CVYV) (Yılmaz et al. 1991), *Cucurbit aphidborne yellows Polerovirus* (Yılmaz et al. 1992), *Melon mosaic virus* (MMV) (Yılmaz et al. 1995), *Tomato ringspot Nepovirus* (TRSV) and *Tomato black ring Nepovirus* (TBRV) only in cucumber (Fidan 1995), *Squash mosaic Comovirus* only in melon (SqMV) (Çağlar et al. 2004).

In the present research, two years of surveys were undertaken in order to evaluate the incidence and distribution of viruses (WMV-2, ZYMV, CMV, SqMV, PRSV-W, and CGMMV) infecting cucurbit crops grown in the Konya province.

MATERIALS AND METHODS

Collection of infected plant material

Symptomatic leaf samples were collected from July through October during 2009 and 2010 in the main cucurbit-growing areas of Konya province. Each sample consisted of the youngest fully developed leaf from plants exhibiting symptoms such as mosaic, mottling, vein clearing, blistering distortion, shoestring, stunting or yellowing and fruit discoloration and deformation. All collected samples were placed in plastic bags, and stored in a freezer (-20°C) until use.

Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA)

All collected samples were tested for the presence of ZYMV, WMV-2, PRSV-W, CMV, SqMV and CGMMV using the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) tests as described by Clark and Adams (1977). The antisera and conjugates were purchased from BIOREBA AG (Reinach, Switzerland) (ZYMV, WMV-2, SqMV, CMV, and PRSV-W), and ADGEN Phytodiagnostics (Neogen Europe Ltd., Scotland, UK) (CGMMV) and used according to the instructions of the companies. Tests were considered positive when the mean absorbance values of tested samples were greater than twice the healthy control (Abou-Jawdah et al. 2000; Cradock et al. 2001; Ertunç 1992; Şevik and Arlı-Sökmen 2003).

RESULTS AND DISCUSSION

In this study, 423 plant samples, such as 251 squash (snack), 64 melon, 25 cucumber, 15 watermelon, 8 snake melon, 27 zucchini squash and 33 pumpkin samples were tested by DAS-ELISA. The relative frequencies of the different viruses infecting the cucurbits are reported in Tables 1 and 2. The data clearly showed that ZYMV and WMV-2 are the most wide spread cucurbit viruses in

the research area. Their incidence reached 70.5 and 44.6% in squash, and 40 and 46.7% in watermelon, respectively. They are followed by CMV, PRSV-W and SqMV, 25.3, 6.4 and 5.9% in all tested samples, respectively. CGMMV were not detected in any of the tested cucurbit samples. Double virus infection was detected in 112 of the samples. Among double infected plants, 47.3 % were infected with ZYMV+WMV-2, the most frequently detected viruses in the samples. Double infections with CMV+WMV-2 and CMV+ZYMV were detected at 14.3 and 13.4%, respectively. Triple infections involving different combinations with all viruses were found in only 11 samples. Quadruple infections were detected in only three samples. Two and only one samples of squash (snack) were simultaneously infected with ZYMV+WMV-2+CMV+PRSV-W and ZYMV+WMV-2+SqMV+PRSV-W, respectively. Especially in cucumber and zucchini squash, a significant percentage of samples which showed virus-like symptoms didn't react with the antisera against any of the six viruses (Table 1). The absence of positive reactions may be due to an unidentified virus present in Turkey or low concentration of virus in the sample.

In Konya province, cucurbits are economically important but have a high incidence of virus-like symptoms. Viruses causing mosaic, leaf deformation, fruit deformation and reduced growth were observed in cucurbit plants in the province. Diseases symptoms were similar to the symptoms previously reported from virus-infected cucurbits fields worldwide (Abou-Jawdah et al. 2000; Alonso-Prados et al. 1997; Davis et al. 2002; Dodds et al. 1984; Lecoq et al. 1981; Luis-Arteaga et al. 1998; Makkouk and Lesemann 1980; Massumi et al. 2007; Provvidenti 1996; Sammons et al. 1989; Yuki et al. 2000). From different parts of Turkey, the presence of ZYMV, WMV-2, CMV, PRSV-W and SqMV has been reported by different researchers (Çağlar et al. 2004; Davis and Yılmaz, 1984; Erdiller and Ertunç, 1988; Fidan 1995; Köklü and Yılmaz 2006; Kurcman 1977; Nogay and Yorgancı 1984; Özaslan et al. 2006; Yılmaz and Davis 1985; Yılmaz et al. 1991; Yılmaz et al. 1995). ZYMV and WMV-2 were the most widespread viruses in our study. Similarly, Yılmaz et al. (1992) found that they were the most common viruses among five viruses in different provinces of Turkey. Also, a survey was performed by Şevik and Arlı-Sökmen (2003) on 165 cucurbits in Samsun province, 53.9% WMV-2, 38.8% ZYMV and 20.6 CMV of 165 samples were determined. In the survey conducted in 33 fields in the Gaziantep province of Turkey for viruses infecting cucurbits, ZYMV was found in higher incidence than two other viruses, CMV and *Potato Potyvirus Y* (PVY) (Özaslan et al. 2006).

CONCLUSION

For the first time, viruses of cucurbit plants in Konya province were detected with this study. The presences of ZYMV, WMV-2, CMV, PRSV-W and SqMV on cucurbits were determined in the province. The results revealed that cucurbits grown

in commercial fields commonly were infected with viruses in Konya. According to the results obtained, the following recommendations should be considered for Konya Province

1. Certified and virus-free seed must be used.
2. Except for SqMV, all viruses detected were spread efficiently by aphids and mechanical inoculation (Hollings et al. 1981; Kaper and Waterworth 1981). Unfortunately, growers in the province are not aware of spreading the viruses from plant to plant and don't know about control measures for virus dissemination.
3. Weeds play an important role on virus epidemiology and are a common problem in vegetable-growing areas in the province. In order to control the virus infection, weeds should be controlled.
4. Cultural practices are very important in cucurbits. For this purpose, cultural practices such as sowing, fertilizing and irrigation should be performed properly.
5. Virus infected plants should be destroyed promptly to prevent them from serving as sources of further infections.

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Table 1. Number of plants infected by one of the following viruses: ZYMV, WMV-2, CMV, PRSV-W, SqMV or CGMMV as determined by DAS-ELISA (Single infections).

Crop	No. Tested	Healthy	ZYMV	WMV-2	CMV	PRSV-W	SqMV	CGMMV
Cucumber	25	4	2	3	12	0	1	0
Melon	64	17	13	11	5	0	0	0
Watermelon	15	2	2	3	1	0	2	0
Squash (Snack)	251	21	73	38	7	0	1	0
Zucchini Squash	27	4	1	11	4	0	0	0
Pumpkin	33	8	5	6	0	1	0	0
Snake Melon	8	2	0	2	0	0	0	
Total	423	58	96	74	29	0	4	0

Table 2. Number of samples infected by two or more of the following viruses: ZYMV, WMV-2, CMV, PRSV-W or SqMV as determined by DAS-ELISA.

Crop	Double virus infection						Multiple virus infections
	CMV+ WMV-2	ZYMV+ WMV-2	SqMV+ ZYMV	SqMV+ CMV	PRSV-W + ZYMV	CMV+ ZYMV	
Cucumber	0	1	0	1	0	1	0
Melon	5	5	1	1	0	1	4
Watermelon	2	2	0	0	0	1	0
Squash (Snack)	3	34	6	0	14	12	11
Zucchini squash	3	4	0	0	0	0	0
Pumpkin	1	7	2	0	0	0	2
Snake Melon	3	0	0	0	0	0	0
Total	17	53	9	2	14	15	17

Evaluation of some native cucurbits (watermelon, melon and cucumber) for resistance to *Acidovorax avenae* subsp. *citrulli* in Turkey

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Keywords: *Acidovorax avenae* subsp. *Citrulli,* BFB, resistance screening, watermelon, melon, cucumber

Abstract

Bacterial fruit blotch (BFB), caused by *Acidovorax avenae* subsp. *citrulli* (Aac), is a serious disease of cucurbit plants. In 1965, It was first reported in watermelon seedlings of several plant introduction lines in Georgia. But this pathogen attacked all of the world from the United States. In Turkey, this pathogen has been observed for the first time in 1996. There are a few studies with related to Aac resistance some cucurbits in the world, especially watermelon and melon. But, there is no data on resistance of native cucurbits in Turkey. The objective of this study is determining to resistance reactions of some cucurbits for Aac. Totally 62 genotypes (35 melons, 23 cucumbers and 7 watermelon) and highly virulence Aac 26/11 isolate were used for resistance screening. As a result, 7 genotypes (4 Cucumber, 3 melon) were obtained resistant and other genotypes were fined susceptible for Aac.

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Functional analysis of *luxR/luxI* in *Acidovorax citrulli*

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Keywords: *Acidovorax citrulli*; QS system; *luxI*; *luxR*

Abstract

Bacterial fruit blotch of melons, caused by *Acidovorax citrulli*, is a seed borne disease that inflicts serious damages to cucurbit crops worldwide. Like most gram-negative bacteria, the *Acidovorax citrulli* pathogen has luxR / luxI quorum sensing (QS) system to regulate its biological functions.

A method of homologous recombination to construct three mutant allele combinations associated with the QS signal systemic functional genes was employed. The QS system tested contained *luxR* and *luxI* single gene deletion mutants and *luxR/luxI* double gene deletion mutant. The *luxR/luxI* QS system function was studied by comparing the difference between the wild type and mutant type strains.

The results are as follows:

(1) Detecting the signaling molecule Acyl homoserine lactones (AHLs): Compared with the wild-type strain, signal substances cannot be detected in luxI and luxR/luxI because of the deletion of luxI, while the luxR could still display a blue chromogenic reaction due to the presence of luxI. This result is consistent with the conclusion that luxI is the gene producing signal substances and luxR is the gene generating the carrier of the signal substances.

(2) *Pathogenicity determination*: the mutant strains displayed reduced virulence, indicating that QS system play an important role in *Acidovorax citrulli* pathogenicity.

(3) *Physiology and biochemistry assay*: growth ability of mutant lines was: *luxR*/*luxI* >*luxI* >*luxR*. The Mutant strains increased in adherent ability of biofilm. Thereis no obvious difference in motility ability of extracellular polysaccharide secretion.

(4) *Real-Time PCR*: The expression of five genes related to virulence and two gene including *luxI* and *luxR* related to quorum sensing were quantitatively detected by Real-Time PCR. The expression of the seven genes decreased in mutant strains, displaying positive regulation. And the quorum sensing system related gene *luxI* and *luxR* were expressed at a lower level in *luxR* and *luxI*, respectively.

(5) The AHLs of bacterial fruit blotch of melons were extracted, and OHHL (3-oxo-hexanoyl homoserine lactone) was identified quantitatively by liquid chromatography–mass spectrometry (UPLC-MS/MS). The pathogenicity of *luxI* to melon seedlings was recovered after adding the OHHL.

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In vitro ovule and ovarium culture in watermelon

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Keywords: Watermelon, ovule-ovary culture, TDZ, SPM

Abstract

Unfertilizated ovaries of *Halep karasi* watermelon genotype were picked on the day of anthesis, one day before anthesis and one day after anthesis. Ovaries were sliced for ovary culture and ovules were extracted from ovaries for ovule culture in sterile conditions. Ovary explants and ovules were cultured on two different (MS and CBM) mediums supplemented with 1 mg/l TDZ (thidiazuron), 1 mg/l SPM (spermin) and 1 mg/l TDZ + 1 mg/l SPM. Ovary explants and ovules were incubated at 25-26 °C, having 3000-4000 lux light density, possessing 16/8 h light/dark photoperiod for 8 weeks. After plant regeneration from ovules and ovaries they were transferred to growth regulator free MS and CBM medium.

In ovule culture, the highest plant regeneration rate was 2.5% and obtained from MS + 1 mg/l TDZ and CBM + 1 mg/l SPM medium in ovary explants harvested one day after anthesis. The ovary harvest stage was one day after anthesis. The highest plant regeneration percentage in ovary explants was 10% and achieved from MS +1 mg/l TDZ medium in one day after anthesis period. Totally four plants were obtained from ovary culture. Each plant was coded and cut for propogation. After each line reached 10 plants they were acclimatized and transferred to greenhouse. The pollen formation proved that *in vitro* regenerated watermelon plants from ovaries were diploid.

INTRODUCTION

Watermelon is one of the vegetable crops which displays great development on new cultivar releases. Breeding studies are performed by classical and biotechnological methods in watermelon. Pure lines are obtained via selfing at least 5-6 generations in classical methods. Dihaploidization is a technique which shortens this period and allows developing 100% homozygous lines. Haploid plants can be achieved by *in vitro* methods as ovule-ovarium culture (gynogenesis) and anther culture (androgenesis) (Metwally et al. 1998). Wei at al. (2010) reported that addition of polyamines or plant regulator hormones in culture media can have a

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positive effect on plant regeneration in ovule and ovarium culture.

Haploid plants also can be obtained by using irradiated pollen technique. Favorable results had been reported in Cucurbit species through pollination by irradiated pollens. First haploid plants were developed by Sauton and Dumas de Vaulx (1987) in melon, by Sari et al. (1994) in watermelon, by Kurtar et al. (2002) in squash, by Sauton (1989) and by Niemirowicz-Szczytt and Dumas de Vaulx (1989) in cucumber.

Unfertilizated ovaries are used in ovule and ovary culture for obtaining haploid plants. Ovule and ovary culture provide an affirmative answer to a limited number of Cucurbit species. *In vitro* gynogenesis induction was reported in squash by Chambonnet et al. (1985), Metwally et al. (1998) and in cucumber by Gemes-Juhasz et al. (2002).

The aim of this study was to obtain haploid plants via ovule and ovary culture in watermelon. It is tried to improve the efficiency of this technique by addition of TDZ (thidiazuron) and SPM (spermin) in haploid embryo induction medium (MS and CBM).

MATERIALS AND METHODS

Halep karasi watermelon genotype was used as plant material and grown in glasshouse. Female flowers were picked for ovule and ovary culture at three different growth stages as one day before anthesis, on the day of anthesis and one day after anthesis.

MS and CBM medium were used as basal embryo induction medium. CBM and MS mediums were supplied with 1 mg/l TDZ (thidiazuron), 1 mg/l SPM (spermin) and 1 mg/l TDZ + 1 mg/l SPM to investigate the effect of thidiazuron and spermin on haploid embryo induction. Totally eight different media were used. Study was designed according to split plot with four replications and one petri dish was one replication.

After picking female flowers from the watermelon plants in glasshouse they were brought to laboratory. The flowers were separated from their calix for initial sterilization step. The ovaries were hold for 30 minutes under running tap water then in 70% of ethanol solution for two minutes and rinsed three-four times with sterile distilled water respectively. Subsequently, they were placed in 30% of sodium hypocloride solution for 20 minutes and rinsed three times. Wet ovaries were transferred on a filter paper for removing excessive water.

In ovule culture, ovules were excised from sterilized ovaries under binocular microscope. Ten ovules were placed in each petri dish. Split plot design was used with eight replications and one petri dish was one replication. Petri dishes were incubated in climate room at 25-26 °C, having 3000-4000 lux light density, possessing 16 hours light, eight hours dark photoperiod.

In ovary culture rinds of the ovaries were peeled slightly with a sterile lancet. Ovaries were cut into eight pieces from one end to the other and five pieces were put in each petri dishes. Split plot design was used with four replications and one petri dish was accepted as one replication.

Cultured ovules and ovariums were observed approximately for two months. Developed putative haploid explants and calluses were placed onto MS and CBM media without growth regulators for regeneration and each were coded. Later growing plants were transferred to MS medium including 1mg/l IAA to induce root formation. Root formed plantlets were propagated. The steps of ovule and ovary culture were presented in Fig. 1. After ten plants were obtained from each line they were acclimatized and transferred to greenhouse. Pollen formation was controlled to determine if the plants were diploid or haploid.

RESULTS AND DISCUSSION

Callus development and plant regeneration results from ovules were presented in Table 1. The callus (total number of calluses/total number of ovules) rate was varied from 0% to %75 among the media used and the stage of ovaries from which ovules were excised. In one day before anthesis, callus formation was 12.5% in MS + 1 mg/l TDZ, 37.5% in MS + (1mg/l TDZ + 1 mg/l SPM) and 27.5% in CBM + 1 mg/l SPM medium. In the day of anthesis, it was 75% in MS+ 1mg/l TDZ, 67.5% in MS+ (1mg/l TDZ + 1 mg/l SPM), 75% in CBM + 1mg/l TDZ and 32.5% in CBM + (1mg/l TDZ + 1 mg/l SPM) media. Callus induction was observed in each medium when the ovules were excised from the ovaries at the stage of one day after anthesis. The callus formation rates were 50% in MS, 75% in MS + 1 mg/l TDZ, 15% in MS + 1 mg/l SPM, 75% in MS + (1mg/l TDZ + 1 mg/l SPM), 20% in CBM, 52.5% in CBM + 1mg/l TDZ, 2.5% in CBM + 1mg/l SPM and 50% in CBM + (1mg/l TDZ + 1 mg/l SPM).

Frequency of plant regeneration (total number of plantlets/total number of ovules) was quite low (0-2.5%) among the media and the stages of ovaries. Plants were only regenerated from unfertilezed ovules which were excised from ovaries harvested one day after anthesis. The rate was 2.5% in both MS + 1 mg/l TDZ and CBM + 1mg/l SPM media.

Callus development and plant regeneration percentages obtained from ovaries were presented in Table 2. Callus developed percentage from ovaries (total number of callus/total number of ovaries) were changed between 0-70% among the media used and stage of ovary. In one day before anthesis, it was 50% in MS + 1 mg/l TDZ, 65 % in MS + (1mg/l TDZ + 1 mg/l SPM) and 10% in CBM + (1mg/l TDZ + 1 mg/l SPM) media. In the period of anthesis it was 65% in MS + 1 mg/l TDZ, 70% in MS + (1mg/l TDZ + 1 mg/l SPM), 20% in CBM + 1mg/l TDZ, 15% in CBM + 1mg/l SPM and 30% CBM + (1mg/l TDZ + 1 mg/l SPM).

Obtention of callus development was rather high in one day after anthesis period. In each medium, callus development was induced between 30-70%. The highest rate was 70% in MS + 1 mg/l TDZ, while the lowest was 30% in CBM + 1mg/l SPM medium.

In case of plant regeneration from ovary explants, only 10% success was achieved from one day after anthesis period when MS + 1 mg/l TDZ medium used while plants were regenerated neither from one day before anthesis nor from the day of anthesis. During the transfer and propagation plants obtained from ovules were died due to fungal contamination. Four different lines derived from ovarium explants were acclimatized and transferred to greenhouse, however after control of pollen formation it was determined that the plants were diploid.

As a result the most effective medium was determined to be MS + 1 mg/l TDZ for plant regeneration in ovule and ovary culture among the eight media tested. The haploid plant regeneration was also low in similar studies. Chambonnet and Dumas De Vaulx (1985) used unfertilized ovules of squash (*Cucurbita pepo* L.) for the obtention of haploid plants. They obtained the highest plant induction from the ovules which were excised one or two days before anthesis. They observed 4-7 regenerated plants from 100 ovules however most of the plants were determined to be diploid, haploid-diploid cimeric, aneuploid or polyploid. Ergin Yılmaz (2005) reported that any plants were achieved when 0.01mg/l TDZ, 0.1mg/l TDZ and 1mg/l TDZ included CBM mediums were used for haploid plant induction from unfertilized ovaries of squash (*Cucurbita pepo* L.). Gémes-Juhasz et al. (2002) were investigated the effect of optimal stage of female gametophyte and heat treatment on *in vitro* gynogenesis induction in cucumber. They reported that the highest frequency of gynogenesis was 18.4% and the highest plant regeneration was 7.1%. They demonstrated that using high temperature like 35°C and growth regulators like TDZ increased the efficiency of gynogenesis.

In this study it can be concluded that the most effective medium was MS + 1 mg/l TDZ for plant regeneration in ovule and ovary culture and the effect of different stages of female gametophyte should be investigated for higher plant regeneration in watermelon.

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Table 1. The effect of different media and stage of ovaries on callus development and plant regeneration in ovule culture of watermelon.

Mediums	One day before anthesis		On the day of anthesis		One day after anthesis	
	Callus development rate (%)	Plant regeneration rate (%)	Callus development rate (%)	Plant regeneration rate (%)	Callus development rate (%)	Plant Regeneration rate (%)
MS	0	0	0	0	50	0
MS1	12.5	0	75	0	75	2.5
MS2	0	0	0	0	15	0
MS3	37.5	0	67.5	0	75	0
CBM	0	0	0	0	20	0
CBM1	0	0	75	0	52.5	0
CBM2	0	0	0	0	2.5	2.5
CBM3	27.5	0	32.5	0	50	0

MS: MS control; MS1: MS + 1mg/l TDZ; MS2: MS + 1 mg/l SPM; MS3: MS + (1 mg/l TDZ + 1 mg/l SPM) CBM: CBM control; CBM1: CBM + 1 mg/l TDZ; CBM2: CBM + 1 mg/l SPM; CBM3: CBM + (1 mg/l TDZ + 1 mg/l SPM)

Table 2. The effect of different media and stage of ovaries on callus development and plant regeneration in ovary culture of watermelon

Mediums	One day before anthesis		On the day of anthesis		One day after anthesis	
	Callus development rate %	Plant regeneration rate %	Callus development rate %	Plant regeneration rate %	Callus development rate %	Plant regeneration rate %
MS	0	0	0	0	60	0
MS1	50	0	65	0	70	10
MS2	0	0	0	0	50	0
MS3	65	0	70	0	65	0
CBM	0	0	0	0	50	0
CBM1	0	0	20	0	40	0
CBM2	0	0	15	0	30	0
CBM3	10	0	30	0	65	0

MS: MS control; MS1: MS + 1mg/l TDZ; MS2: MS + 1 mg/l SPM; MS3: MS + (1 mg/l TDZ + 1 mg/l SPM); CBM: CBM control; CBM1: CBM + 1 mg/l TDZ; CBM2: CBM + 1 mg/l SPM; CBM3: CBM + (1 mg/l TDZ + 1 mg/l SPM)

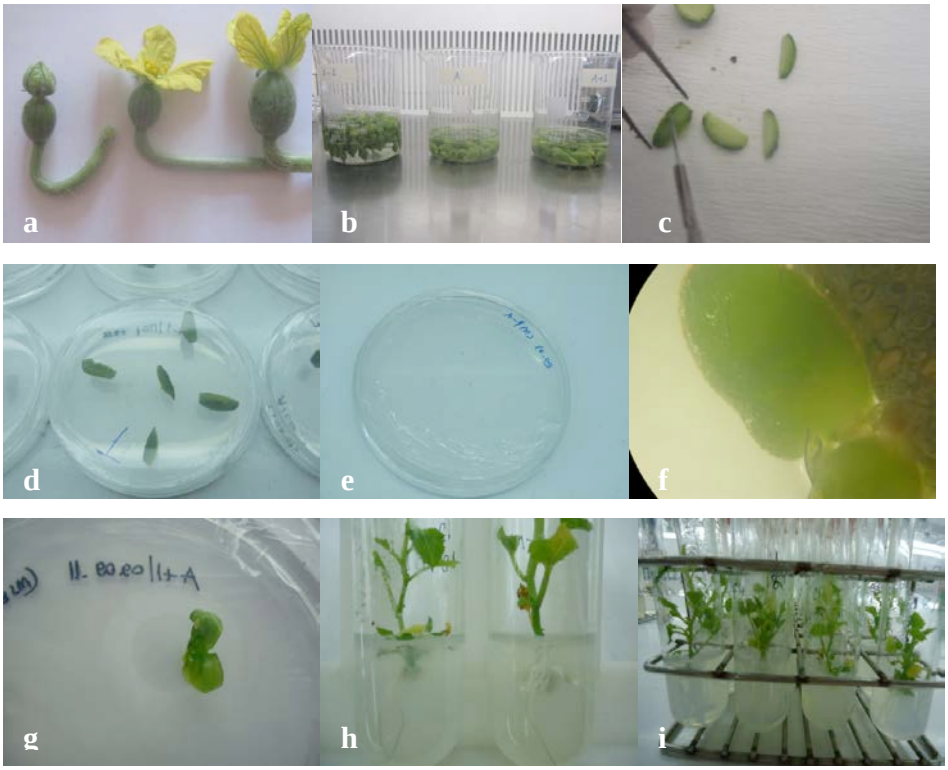

Fig. 1. Steps of ovule and ovary culture in watermelon; a: different stages of watermelon flowers; b: disenfection of ovaries; c: ovary slices; d: placement of watermelon ovary slices in petri dish; e: placement of watermelon ovules in petri dish; f: plant regeneration from callus tissue; g: transferred plantlest to new medium; h: *in vitro* regenerated and rooted plants; i: propogated watermelon plants.

Effect of sowing date and genotypes on the quality of different watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) cultivars grown in Tunisia

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Keywords: Growing season, lycopene, yield, agronomic characteristics, soluble solids

Abstract

To evaluate growing period variations in carpometric characteristics and lycopene content of watermelon cultivars (*Citrullus lanatus* (Thunb.) Mansfeld). Fruits from plants grown under low plastic tunnel and on open-field conditions were collected. Five watermelon cultivars (four commercial cvs Crimson Sweet, Dumara, Giza, Aramis, and new selection P503 produced by the National Agricultural Research Institute of Tunisia) were compared. The variations in the solar radiations received and temperature changes in the fruit load over the study period significantly affected the yield, soluble solids content (°Brix) and lycopene content of watermelon. The lycopene content in the watermelon cultivars ranged from 50,17 (check the guidelines and see if US or French punctuation is supposed to be used, then make the punctuation consistent. ('.' Or ',') to 110,36 mg / Kg FW. The mean lycopene content of the five cultivars was 23% higher in full season (March- July) than in early season (January - May).

This study indicates that the antioxidant components of watermelon can vary considerably with the changes in environmental conditions within the early and full seasons.

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) is a popular horticultural crop in the Mediterranean area, because of its economic importance and nutritional properties. Watermelon provides a wide variety of dietary antioxidants such as carotenoids (lycopene and beta-carotene), phenols, vitamins (A, B, C and E) and specific amino acids (citrulline and arginine) (Perkins-Veazie 2002; Perkins-Veazie et al. 2007, Tlili et al. 2011), which are thought to exert a protective role in reducing the risk of certain types of cancers, cardiovascular diseases and age-related

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degenerative pathologies (Rice-Evans et al. 1996; Giovannucci 1999; Rao 2006). In order to maximize the content of the important phytonutrient, the influences of genetics, agricultural practices and environment have been investigated. To develop information on seasonal variations in watermelon, we compared some quality characteristics and lycopene content of watermelon fruit, grown in two different seasons.

MATERIALS AND METHODS

Experiments were carried out at the Research and Experimental Station of Teboulba, Monastir, Tunisia. Plants were grown during two different growing periods (early season under low plastic tunnel and full season under field conditions). Sowing was performed on January 19th and March 2008 4th, respectively. Ripe watermelons were harvested late May under low plastic tunnel production and middle of July under field condition. Watermelon fruits were quickly delivered to the laboratory and immediately cut longitudinally from stem-end to blossom-end through the ground spot. Flesh samples were taken from the heart area (between locular and the fruit centre) of each watermelon. Soluble solids content was measured immediately using a digital refractometer. Approximately 1 kg of the obtained samples was homogenized in a mixer for 5 min. The homogenates were frozen at -80°C and used to determine lycopene content.

Lycopene extraction and determination were conducted as described by Fish et al. (2002). The method uses a mixture of hexane/ethanol/acetone (2:1:1 by vol.) containing 0.05% butylated hydroxytoluene (BHT). The absorbance of the hexane extract was measured at 503 nm with a Cecil BioQuest CE 2501 spectrophotometer and results were expressed as mg/kg fw.

RESULTS AND DISCUSSION

Marketable yields, soluble solids (°Brix) and lycopene content of the watermelon cultivars grown under the early (January - May) and full seasons (March - July) are presented in Table 1. The results showed that marketable yields, soluble solids and lycopene content were significantly different between cultivars ($P < 0.001$). Regarding the marketable yield, Aramis ranked among the best cultivar in both trials. Watermelon marketable yields were significantly affected by the season grown, whatever the cultivar ($P < 0.001$), and were higher in full season in all the studied cultivars. The average value was 6.12 and 9.78 kg per plant in early and full seasons, respectively. The fruit yield differences was likely mostly due to the climatic conditions over the growing period.

Concerning soluble solids, Aramis ranked among the best cultivar in both trials. The results showed that the watermelon soluble solids were also significantly affected by the growing seasons whatever the cultivar ($P < 0.001$). Watermelon

soluble solids were higher in full season in all cultivars. The average value was 9.36 and 11.48 in early and full seasons, respectively. This is likely due to what Winsor and Adams (1976) reported; that an increase in the amount of solar radiation received by the plant leads to an increase in the rate of photosynthesis by the plants, which leads to an accumulation of carbohydrates (mainly in the form of sugars) in the tomato fruit.

Regarding lycopene content, P503 and Giza ranked among the best cultivars for lycopene content in both trials. The lycopene content was also characterized by marked variations between growing period whatever the cultivar, ranging from 67.78 to 87.49 mg / Kg fw in early and full seasons, respectively. The mean lycopene content of the five cultivars was 23% higher in full season (March - July) than in early season (January - May). This fact correlate to an increase in the amount of solar radiation associated with an increase in temperature received by the plants. Heinonen et al. (1989) observed that lycopene concentration was relatively high in summer (June to August) (3800-6600 µg/100 g fw) and low in winter (October to March) (2600-3100 µg/100 g fw) in tomatoes purchased from retail food stores in Finland. This last sentence really has no relevance to your study. Winter tomatoes are usually picked green in the fall and stored for months before they are gassed to force ripens, and/or they are picked green in the southern hemisphere and shipped to the northern hemisphere.

Table 1. Physicochemical properties of the different studied watermelon varieties within different sampling areas.

Cultivars	**Yield fruit weight per plant (kg)**		**Soluble solids (°Brix)**		**Lycopene (mg kg^{-1} FW)**	
	Early season	Full season	Early season	Full season	Early season	Full season
Crimson Sweet	6.24a	9.24ab	9.8a	12.1a	46.87c	69.58c
Giza	6.75a	8.41b	8.3 a	9.9a	91.95 a	119.75a
Dumara	6.13a	10.48ab	9.7 a	12a	45.62 c	54.71b
P503	5.11a	7.68b	9.2 a	10.5a	97.00 a	123.72a
Aramis	6.40a	13.12a	9.7 a	12.7a	57.42b	89.65b
Significance	ns	**	ns	ns	**	**

Signification: ** Probability level of 1%; ns: not significant. Values in the same column followed by the same letters do not differ significantly (LSD test, $P<0.05$).

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