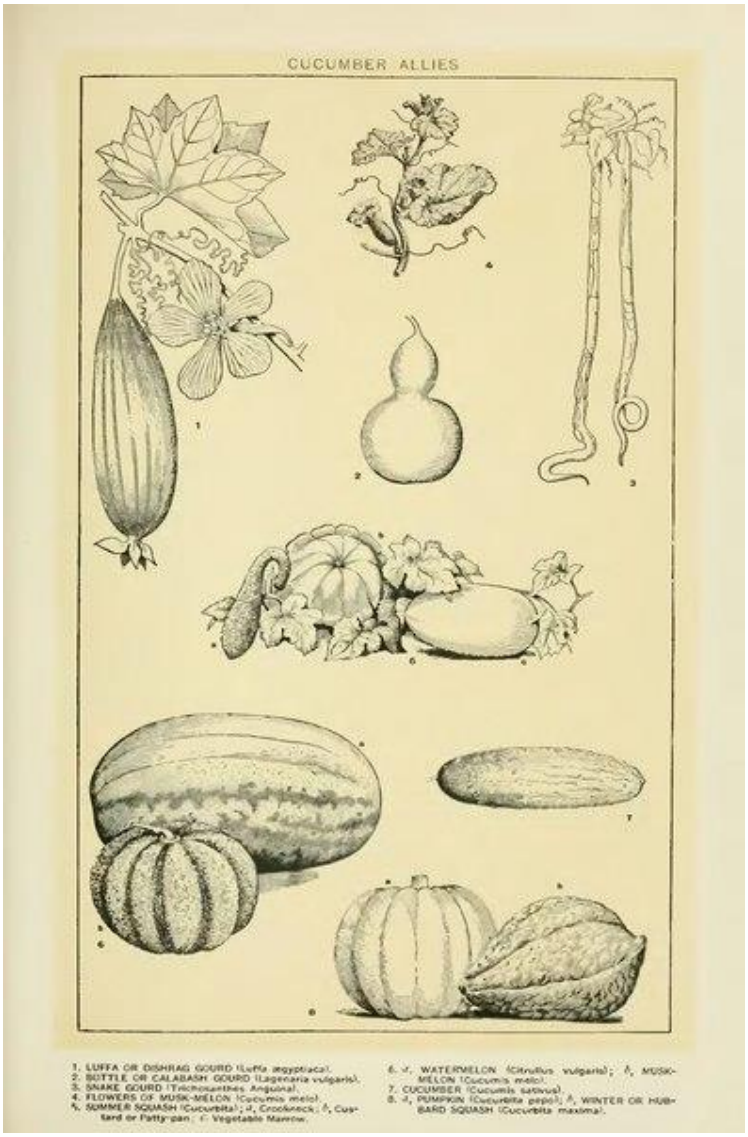
Cucurbit Genetics Cooperative

2021

Report 44



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"Cucumber Allies", *The New International Encyclopædia* (1st ed.), 1905, v. 05, page 759.

The **Cucurbit Genetics Cooperative** (CGC) was organized in 1977 to develop and advance the genetics of economically important cucurbits. Membership to CGC is voluntary and open to all individuals who have an interest in cucurbit genetics and breeding. There are no annual dues. An informal membership list is maintained. If you are interested in becoming a CGC member and receiving email communications concerning CGC, including calls for new contributions to the CGC Report, please contact CGC Chair Amnon Levi at amnon.levi@ars.usda.gov.

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Contents

Citrullus spp. Germplasm Diversity in Tunisia: An Overview

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Introduction

The genus *Citrullus*, a member of the *Cucurbitaceae* family, includes several known diploid species including *C. lanatus* (Thunb.) Matsum. & Nakai, *C. amarus* Schrad., *C. mucosospermus* (Fursa) Fursa, *C. colocynthis* (L.) Schrad., *C. ecirrhosus* Cogn, *C. rehmii* de Winter, and *C. naudinianus* (Sond.) Hook (Chomicki and Renner, 2015; Paris 2015).

The annual *C. lanatus* (2n=22), the dessert watermelon, is the most known among all *Citrullus* species. It is a warm-season annual vegetable fruit with sweet flesh and is one of the most extensively consumed vegetable fruit crops throughout the world. Indeed, it is grown on 3.5 million hectares worldwide and the annual world production exceeded 118 million tons in 2017 (FAOSTAT, 2019). Native to Sudan and Egypt, it includes wild and cultivated forms (Paris, 2015) and is grown for its edible endocarp, rind, and seed oil. The colored flesh, though 93% water, contains significant amounts of carbohydrates, vitamin A, and lycopene (Wehner, 2008). It has been certified as a heart-healthy food by the American Heart Association because it is low in calories, sodium, cholesterol, and fat. In Tunisia, watermelon is largely consumed in summer as a fresh fruit.

Citrullus colocynthis, the colocynth, is considered a putative ancestral or progenitor species of watermelon (Levi et al., 2001a). Also known as bitter apple, it is cultivated for its numerous medicinal properties and the oil of its seeds (Hussain et al., 2014). It is also used as a potential rootstock for watermelon (Bigdelo et al., 2017). *C. colocynthis* is native to the deserts and semi-arid regions of northern Africa and southwestern and central Asia (Paris, 2015). In Tunisia, it grows wild in the arid regions and is used as a medicinal plant. Another wild species, *C. amarus* (previously known as *C. lanatus* var. *citroides*), also known as the citron watermelon or preserving melon, is neither sweet nor bitter. Its rind is used to make pickles and fruits that are fed to livestock (Dane and Liu, 2007) and is also used as a rootstock for watermelon (Thies et al., 2007).

A wide range of phenotypic characteristics, including fruit size, flesh color, rind pattern, and also disease resistance and flesh sweetness, are observed between cultivars. Each growing region has a unique set of cultivars that are widely grown and are suited for cultivation in the local environment (Wehner, 2008; Chikh-Rouhou et al., 2019). Despite considerable geographic and phenotypic diversity, the genetic variation of cultivated watermelon is limited (Levi et al., 2001b).

Watermelon has been cultivated and grown for many centuries in Northern Africa (Jensen et al., 2011). Landraces collected in Northern Africa, including Tunisia, could be a useful source of germplasm for breeding programs. Indeed, the strategic geographic location of Tunisia and the variability of its climate, which varies from humid in the north to arid in the south, have fostered the diversification of several cucurbit species (both landraces and wild genetic resources). In Tunisia, the watermelon collection at the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB, Tunisia) was initiated in 2017 (Figure 1). The accessions collected belong to *C. lanatus*, *C. amarus,* and *C. colocynthis* (Table 1). Several studies were initiated to characterize watermelon landraces using either morphological traits (Chikh-Rouhou et al., 2019), molecular markers (Elbekkay et al., 2021), or phytochemical traits (Tlili et al., 2011). However, watermelon genetic resources in Tunisia are, in general, poorly characterized, and additional studies are needed to properly collect, classify and evaluate them. Unfortunatelly, most landraces have been abandoned and replaced by commercial imported hybrids, except on scattered family farms.

Very few studies have been conducted to characterize the local Tunisian germplasm. Tlili et al. (2011) evaluated

antioxidant components and antioxidant activities of 6 watermelon cultivars and 2 selections (P503 and P403 obtained by the National Institute of Agricultural Research of Tunisia [INRAT]) and found significant differences among accessions for lycopene, phenolics, flavonoids, ascorbic acid (AsA), dehydroascorbic acid (DHA) and total vitamin C (AsA + DHA) contents, as well as in antioxidant activity of their hydrophilic and lipophilic fractions. The results of that study indicated a wide range in the nutritional value of those watermelon accessions and emphasized the need to evaluate watermelon biodiversity for improving nutritional value. Chikh-Rouhou et al. (2019) found wide phenotypic diversity for fruit and seed traits among watermelon landraces collected from Center-East Tunisia. Elbekkay et al. (2021), using RAPD markers, found substantial genetic diversity among watermelon landraces collected from southern Tunisia.

Screening for resistance to *Fusarium oxysporum* f. sp *niveum* (FON), the pathogen causing Fusarium wilt in watermelon, is ongoing to identify germplasm sources useful for breeding programs. Some landraces with a potential source of resistance to FON were identified and are under trial-confirmation (Chikh-Rouhou et al., in preparation). In addition, the phenotyping of the root traits of these landraces is ongoing at CRRHAB. It is essential to phenotype the roots as they are an important component for productive plant performance (Katuuramu et al., 2020). Evaluation of root traits across *Citrullus* spp. is a promising means to identify superior genotypes useful for the improvement and development of elite watermelon cultivars.

We emphasize here the need to collect and evaluate watermelon diversity for more efficient management and utilization of landraces to facilitate sustainable conservation and enrichment of the *Citrullus* spp. germplasm in Tunisia.

Acknowledgments

Research laboratory LR21AGR03-Production and Protection for a Sustainable Horticulture, funded by the Ministry of Higher Education and Scientific Research of Tunisia. PID2020-116055RB-C22 I+D+I project funded by MCIN/AEI/10.13039/501100011003 and the A11-20R project funded by the Aragon Government.

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Figure 1. Diversity of some watermelon genetic resources collected in Center-East Tunisia (Photo H. Chikh-Rouhou)

Table 1. Details of *Citrullus* spp. landraces in the Research Centre on Horticulture and Organic Agriculture (CRRHAB), Tunisia, collection.

Code	*Species*	Flesh color	Weight (kg)	Total soluble solids (°Brix)
P1	*C. lanatus*	Dark red	8.60±1.2	10.10±0.5
P2	*C. lanatus*	Red	4.50±0.5	9.98±0.8
P3	*C. lanatus*	Pinkish-red	5.73±1.2	9.49±0.5
P4	*C. lanatus*	Red	4.63±0.5	9.25±0.3
P5	*C. lanatus*	Dark red	5.80±0.8	9.90±0.5
P6	*C. lanatus*	Dark red	5.70±0.5	8.50±0.4
P7	*C. lanatus*	Dark red	6.60±0.6	8.19±0.4
P8	*C. lanatus*	Red	10.00±1.0	9.50±0.5
P9	*C. lanatus*	Light red	4.70±0.6	9.55±0.4
P10	*C. lanatus*	Dark red	7.75±0.7	9.25±0.5
P11	*C. colocynthis*	White	0.50±0.1	3.00±0.1
P12	*C. lanatus*	Red	3.35±0.2	8.10±0.1
P13	*C. lanatus*	Dark red	4.56±0.3	8.50±0.3
P14	*C. amarus*	Light yellow	6.60±0.5	2.60±0.1
P15	*C. amarus*	Light yellow	5.80±0.5	1.54±0.1

Citrullus naudinianus Genome Assembly

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The genome assembly of the watermelon-related *Citrullus naudinianus,* aka gemsbok cucumber, has been completed. This unusual *Citrullus* (formerly *Acanthosicyos*) species is native to southern Africa including Botswana, Namibia, Mozambique, Zambia, Zimbabwe, and South Africa. Like all *Citrullus* species, *C. naudinianus* possesses an array of unique physiological and morphological characteristics that enable it to survive, and thrive, in an extremely hostile environment. In its native habitat, the fruit of this 'cucumber' are eaten by gemsbok, mole-rats, jackals, honey badgers and likely other fauna as well.

Among the 7 species of *Citrullus* currently recognized, *C. naudinianus* represents the basal branch in the taxonomic tree of *Citrullus* and the species most distantly related to the common watermelon. *Citrullus naudinianus* is, unlike other members of the genus *Citrullus,* dioecious having separate male and female plants. The genetic mechanism accounting for the change from dioecy (*C. naudinianus*) to monoecy (other *Citrullus* species) in this genus currently awaits determination. In addition to its rather small but numerous fruits, this species produces multiple storage roots that are both large and dense.

Although distantly related to *C. lanatus*, successful hybridizations, producing viable progeny, between *C. naudinianus* and several other *Citrullus* species (i.e., *C. rehmii*, *C. eirrhosus* and *C. colocynthis*) have been made. Information on the ability to hybridize *C. naudinianus* with other *Citrullus* spp. does not appear to be available. The crossability of various *Citrullus* species (with *C. lanatus*) exhibits a substantial genotypic effect when *C. lanatus* is used as the maternal parent. However, obtaining flowers of *C. naudinianus* (grown in the greenhouse) has proven to be a challenge, limiting attempts to obtain additional hybridization/crossability data. The full extent of the potential of this taxon to contribute to the improvement of cultivated forms remains undetermined.

Not surprisingly, the fruit of *C. naudinianus* are bitter due to the presence of terpenes common in the fruit of many *Citrullus* species. However, the cooked fruit are apparently edible. The bushmen of the Kalahari have been reported to eat the fruit after the fruit have been roasted in a fire or boiled (the cooking renders the terpenes harmless). The fleshy fruits are also known to serve as a source of water for man and animal and have been used to make pickles. This species is also a locally important source of edible oil and protein. The plant yields a crop quickly, the fruit are easily harvested, the plant is ecologically adapted to a wide range of environments, and it is readily propagated by seed or storage roots. Hence, it has been suggested as a candidate for development and domestication - although studies on the extent of genetic and phenotypic diversity within this species are yet to be conducted.

The genome sequence of the gemsbok cucumber serves to provide an evolutionary anchor point for a pan-genus study on genome evolution in the genus *Citrullus.* It also facilitates an examination of the evolution of the gemsbok cucumber's unique reproductive traits and its many adaptive traits that allow it to survive in a true desert environment. Links to the assembly can be found at:
https://www.dnazoo.org/assemblies/Citrullus_naudinianus.

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Figure 1. Photo of the interior of fruit of mature *C. naudinianus*.

Figure 2. Photo of storage roots of *Citrullus naudinianus*.

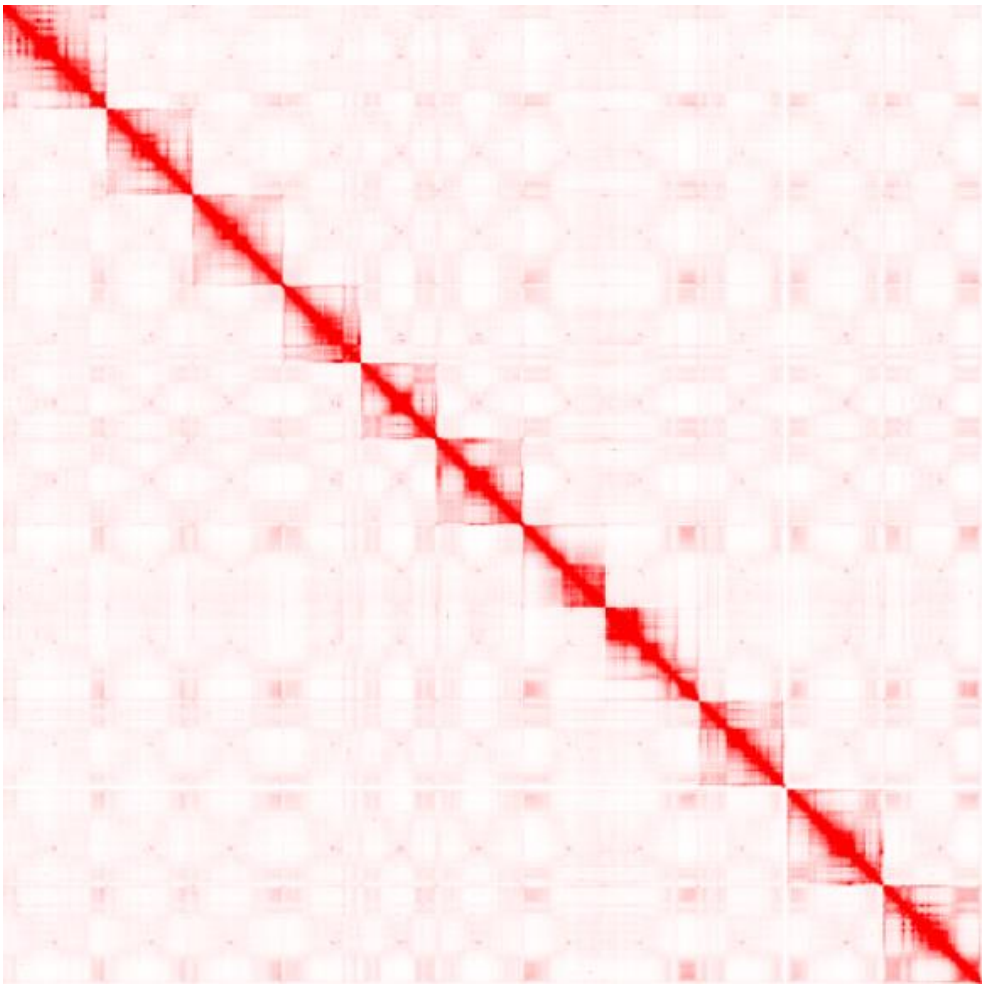

Figure 3. Hi-C contact map of the assembled (n=11) chromosomes of *C. naudinianus* (with permission of DNAZoo.org).

Melon Germplasm from Tunisia with Immense Breeding Value

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Melon (*Cucumis melo* L., 2n = 24) is a morphologically diverse horticultural crop of high nutritional value and economic importance in Tunisia and the Mediterranean regions. The geographical position of Tunisia and range of climatic conditions, from humid in the north to arid in the south, have contributed to melon diversity in terms of landraces and wild genetic resources. Melons are cultivated in various areas in the country and largely consumed in summer, as a fresh fruit, appreciated for its sweetness.

Melon classification is a topic of great interest. It has been historically organized from a botanical viewpoint with the recent designation of three subspecies, ssp. *agrestis*, ssp. *melo*, and ssp. *meloides* (Endl et al., 2018). Botanical groups (*varietas*) have been defined within ssp. a*grestis* and ssp. *melo* (Pitrat et al., 2000). Melon may also be organized in a horticultural framework according to the International Code of Nomenclature for Cultivated Plants (Brickell et al., 2009). Burger et al. (2010) defined 16 Horticultural Groups within the two subspecies *agrestis* and *melo.* Pitrat (2016) later described 19 Horticultural Groups without reference to the subspecies. Horticultural Groups Cantalupensis*,* Flexuosus, Inodorus and Reticulatus, as defined by Burger et al. (2010), are the most important in Tunisia (Chikh-Rouhou et al., 2021c).

The agronomic traits and fruit characteristics of Tunisian landraces are similar to the commercial types demanded by Mediterranean markets, which facilitates breeding programs directed at this market. They also carry adaptation to the range of environments and cultivation methods in Tunisia, which suggests this diverse germplasm could be a great resource for melon breeding in a changing environment. In this paper, the local genetic resources of melon are reported and the breeding programs in which they are involved are summarized to demonstrate the potential of Tunisian melon landraces as a valuable genetic reservoir and the need to plan strategies for its conservation and utilization in breeding programs.

Melon collections (Figure 1) at the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB) Tunisia were initiated in 2014. Accessions were collected and characterized for morphological traits (Chikh-Rouhou et al., 2021c; Trimech et al., 2013) and molecular markers (Trimech et al., 2015). Genetic diversity and population structure of the Tunisian melon collection were characterized by combining phenotypic and molecular data; specific alleles related to agronomic traits of interest were detected in two landraces, which constitute a potentially valuable gene pool for melon breeding (Chikh-Rouhou et al., 2021b).

Tunisian melon landraces have been identified as highly resistant to many biotic stresses such as powdery mildew (Chikh-Rouhou et al., 2020), Fusarium wilt incited by *Fusarium oxysporum* f.sp. *melonis* (FOM) (Chikh-Rouhou et al., 2018, 2021a), and melon aphid (*Aphis gossypii*) (Chikh-Rouhou et al., 2019). These studies combined phenotypic and molecular strategies to identify resistant accessions (Table 1). Molecular markers tightly linked to FOM and melon aphid resistance genes, *Fom*-1 and *Fom*-2, and *Vat*, respectively (Oumouloud et al., 2012, 2015; Dogimont et al., 2009), and controlled inoculations were used to determine melon landraces resistant to FOM and melon aphid (Chikh-Rouhou et al., 2021a, 2019, 2018). Thirteen of the 27 landraces carried *Fom*-1, confirming their resistance to FOM races 0 and 2; two of them were also resistant to FOM race 1 (Table 1). Two accessions of Inodorus and Flexuosus groups with a high level of resistance to the most virulent race 1.2 of FOM

have been reported; the number of accessions resistant to race 1.2 is very low, and almost all of them belong to Groups Makuwa and Conomon (Chikh-Rouhou et al., 2010, 2011), which are common to parts of India and East Asia. Thus, the two FOM 1.2-resistant Tunisian landraces are very promising resistance sources to stem losses from this race, but further characterization of resistance to FOM 1.2 in these two landraces is needed before it can be incorporated into melon breeding programs. Resistance to race 1.2 is complex, controlled by multiple recessive genes with epistatic effects, which make selection difficult (Chikh-Rouhou et al., 2011; Perchepied and Pitrat, 2004).

Regarding resistance to melon aphid, Chikh-Rouhou et al. (2019) reported one of 15 landraces evaluated with the *Vat* gene, which confers resistance to melon aphid colonization and the viruses transmitted by this aphid (Table 1). This accession is also promising because several genomics studies focused on the region containing *Vat* showed that the density of host plant resistance genes in melon is highest in this region (Garcia-Mas et al., 2012). Thus, 28 genes of the NLR family have been identified in a 1-Mb region containing *Vat* (González et al. 2014), as well as the resistance genes to powdery mildew incited by *Podosphaera xanthii* (Yuste-Lisbona et al., 2001) and cucumber vein yellowing virus (CVYV) (Ibn Oaf, 2012), the *Fn* gene (Pitrat and Lecoq, 1984), which triggers plant necrosis in response to some isolates of zucchini yellow mosaic virus (ZYMV), and the quantitative trait loci (QTL) *FomV-2,* which confer partial resistance to FOM race 1,2 (Perchepied et al., 2005).

Particular attention should be given to landrace Chamem, an Ananas type, that carries *Vat* and *Fom*-1 (Chikh-Rouhou et al., 2019; Chikh-Rouhou et al., 2021a) and was also found resistant to *P. xanthii* race 2 (Chikh-Rouhou et al., in preparation). It is a potential landrace with immense value as a donor of multiple pest resistances for melon breeding programs to develop commercial melons of Ananas type, which are highly appreciated not only in Tunisia but in other Mediterranean countries. Most reported sources of resistance to *P. xanthii* come from India (e.g., PI 124112, PI 414723, PI 134198, PI 313970) and a few have been reported in Groups Momordica and Acidulus (Nunés et al., 2017), but their agronomic and fruit characteristics are usually unsuitable for the Mediterranean market, which makes them problematic as sources of powdery mildew resistance.

Comparative studies of disease resistant and tolerant melon genotypes for differences in their microbiomes are ongoing at CRRHAB in order to identify key microorganisms potentially involved in modulating the defensive/resistance responses that may be taken into account in future breeding programs. A recent study of Aydi-Ben-Abdallah et al. (2021) on fungal and bacterial rhizosphere microbiomes associated with selected Tunisian melon landraces demonstrated the following: 1) genotypic differences for quantum and diversity of their microbiomes and 2) soil microbial structure–melon genotype interactions that may be exploitable for development of melon lines with high-level, stable yield potential by inclusion of holobiont selection in breeding programs. All these investigations have led to the identification of local (Tunisian) germplasm of high interest for their resistance to biotic stress, fruit quality, and agronomic behavior. Hybrids from the promising parents identified could improve heterosis for quality traits and yield in melon. Breeding of local hybrid varieties was initiated in 2019 at CRRHAB and newly created Fı hybrids resistant to Fusarium wilt are currently under evaluation in different sites in order to select the best ones for quality and yield. These lines are also the subject of diallel, heritability, GCA (General Combining Ability), and SCA (Specific Combining Ability) analyses in order to optimize selection of elite materials for Tunisia.

Acknowledgements

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Table 1. Horticultural characteristics–Horticultural Group, total soluble solids (°Brix), external aroma–of 27 Tunisian melon landraces and their resistances to Fusarium wilt incited by *Fusarium oxysporum* f.sp. *melonis* and melon aphid (*Aphis gossypii*), as determined by molecular markers.

Accession					Resistance marker	
Code	Name	Group[z]	°Brix	Aroma[z]	Fusarium wilt[x]	Melon aphid
TUN-1	Maazoun Chott-Mariem	Inodorus	9.8 ± 0.6	no	*Fom*-1/*fom*-1	*vat*
TUN-2	Maazoun Menzel Chaker	Inodorus	10.2 ± 1.1	no	*Fom*-1/*Fom*-1	*vat*
TUN-3	Maazoun Mehdia (MM2009)	Inodorus	11.0 ± 1.9	no	*Fom*-1/*Fom*-1	*vat*
TUN-4	Maazoun Fethi	Inodorus	10.9 ± 0.5	no	S[y]	*vat*
TUN-5	Fakous (FL)	Flexuosus	4.7 ± 0.5	no	*Fom*-1/*fom*-1 *Fom*-2/*fom*-2	–
TUN-6	Fakous Salem 2014	Flexuosus	–	–	*Fom*-1/*fom*-1	–
TUN-7	Trabelsi	Inodorus	9.7 ± 0.3	no	S	*vat*
TUN-8	Galaoui	Reticulatus	10.1 ± 0.4	yes/no	S	*vat*
TUN-9	Dziri (DZ P5 2011)	Inodorus	9.6 ± 0.9	no	*Fom*-1/*Fom*-1	*vat*
TUN-10	Lobneni	Reticulatus	9.5 ± 0.2	yes/no	*Fom*-1/*Fom*-1	–
TUN-11	Arbi	Inodorus	–	–	S	–
TUN-12	Horchay	Chate	7.4 ± 0.4	no	*Fom*-1/*fom*-1 *Fom*-2/*fom*-2	–
TUN-13	Arbi 1	Inodorus	9.8 ± 0.1	no	S	*vat*
TUN-14	Arbi 2	Inodorus	8.4 ± 0.2	no	*Fom*-1/*Fom*-1	–
TUN-15	Arbi 3	Inodorus	10.2 ± 1.1	no	S	–
TUN-16	Sarachika	Inodorus	9.8 ± 0.5	yes/no	*Fom*-1/*fom*-1	*vat*
TUN-17	RD	Cantalupensis	11.8 ± 1.1	yes	S	*vat*
TUN-18	Rupa	Cantalupensis	9.7 ± 0.7	yes	*Fom*-1/*fom*-1	*vat*
TUN-19	Chamem (Ananas type)	Reticulatus	10.0 ± 0.2	yes	*Fom*-1/*Fom*-1	***Vat***
TUN-20	HTM Kairouan	Reticulatus	–	–	S	–
TUN-21	Acc Jendouba	Inodorus	–	–	S	–
TUN-22	Dziri (Menzel Kamel)	Inodorus	–	–	S	–
TUN-23	Ecotype arbi Dz	Inodorus	–	–	S	–
TUN-24	Maazoun (Kairouan)	Inodorus	9.5 ± 0.6	no	S	*vat*
TUN-25	Asli	Inodorus	11.43± 0.5	no	S	*vat*
TUN-26	Stambouli	Inodorus	8.93± 1.1	no	*Fom*-1/*Fom*-1	*vat*
TUN-27	V4 autoféc	Inodorus	11.5 ± 1.3	no	S	–

[z]Burger et al. 2010

[x] Chikh-Rouhou et al. 2021a

[y]S: susceptible

Figure 1. Diversity of Tunisian melon landraces. (Photo H. Chikh-Rouhou)

Setting up a Selection Method for Drought Tolerance in Melon Seedlings

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Introduction

Climate change causes serious concerns to growers and breeders. Drought affects a significant proportion of the global population, particularly those living in semi-arid and arid regions.

Melon is one of the most important fruit crops. Approximately 42 million tons of melons were produced in 2020 worldwide, with more than 1.4 million ha harvested. Development of melon cultivars adapted to abiotic stress and with high quality standards is required by global markets. This includes tolerance or resistance to drought, particularly in the Mediterranean region where there is predicted an important increase of arid areas (Turral et al., 2011).

Although plants can be adversely affected by drought at any time of their life, some of the most critical stages are during seedling growth. Previous observations (Sarria-Villada, personal communication) pointed to the existence of certain drought tolerance in the Zimbabwean accession TGR1551 during that stage.

With the aim of setting up a method that allows the confirmation of drought tolerance in TGR1551 and the reliable subsequent selection of genotypes with tolerance to drought in a RIL population derived from this melon accession, we carried out a pilot experiment, based on the work done by Zhang et al. (2016) in watermelon.

Material and Methods

The Spanish cultivar Bola de Oro and the accessions TGR1551 and C278 were included in the present study to determine their drought tolerance responses during the seedling stage under extreme water stress conditions in a temperature-controlled greenhouse.

To ensure a minimum number of seedlings (14) per entry, two to three seeds were sown in each nursery tray cell; two types of trays were used, standard trays (5 cm diameter x 5 cm tall cells) routinely used in melon nurseries, and forest trays (6x6 cm square x12 cm tall cells). Trays were placed in an insect-free greenhouse where the temperature ranged from 17-27 ºC and the natural lighting period was 14/10h day/night. When the first true leaf appeared, seedlings were thinned to one per cell. After sowing, two water regimes were applied: half of the trays were watered daily and the other half every other day (alternating), until the seedlings reached the stage of two or three true leaves. Seedlings were then subjected to two consecutive water-stress periods as follows: trays were placed in a water container for 2 min and no watering was applied for 4-days, when they were again placed in a water container for 2 min and left without watering until the end of the experiment.

Drought tolerance evaluations were done on the fourth and the seventh day after the second water stress period by careful examination of each individual seedling for their drought-induced injury symptoms on each accession. Following Zhang et al. (2016) the following rating scale was used: 0 = cotyledons and first true leaf remained in a normal horizontal position; 1 = cotyledons pointed upward while the first true leaf remained horizontal; 3 = cotyledons curled downward and the first true leaf pointed upward; 5 = cotyledons curled downward and the first true leaf curled upward; 7 = whole plant showed wilting. Mean drought responses for each combination of genotypes, trays and watering regimes were compared by using Tukey-b post-hoc test after one-way ANOVA.

Results and discussion

Four days after last watering, several plants of C278 growing in standard trays showed mild symptoms of sensitivity to drought regardless of water regime (Fig.1). In these trays, plants of 'Bola de Oro' showed low or mild symptoms of sensitivity to drought, when the water applied was daily or alternate, respectively. All plants of TGR1551 growing in standard trays showed tolerance regardless of the water regime. Plants of any of the genotypes growing in forest trays showed no drought symptoms under daily water regime but they showed some level of sensitivity when the water regime was alternate.

Seven days after last watering, plants of C278 showed drought sensitivity when growing in standard trays and mild symptoms when growing in forest trays. 'Bola de Oro' only showed high drought sensitivity when growing in standard

trays with alternate watering. Plants of TGR1551 showed drought tolerance response in all cases.

Accession C278 was the most susceptible to drought. It was the accession with the highest mean score and plants showing serious injuries or wilting regardless of treatment. 'Bola de Oro' also showed a susceptible response, though the mean score was significantly lower than in C278.

Although four days after last watering significant differences between TGR1551 and 'Bola de Oro' and C278 were observed, the drought responses of the two latter genotypes were mild. Seven days after last watering, significant differences among the three genotypes were observed but the highest mean score was observed for standard trays and alternate watering regime. Under these severe conditions, significant and clear differences among susceptible and tolerant genotypes were observed (Fig 1).

The combination of sowing in standard trays, watering every two days, and evaluating plant responses seven days after the last watering appears to be the most suitable technique for selection of melon genotypes to study tolerance to drought. We expect this technique to be useful in establishing the genetic basis of drought tolerance in the TGR1551 x Bola de Oro RIL population we are evaluating.

Acknowledgement

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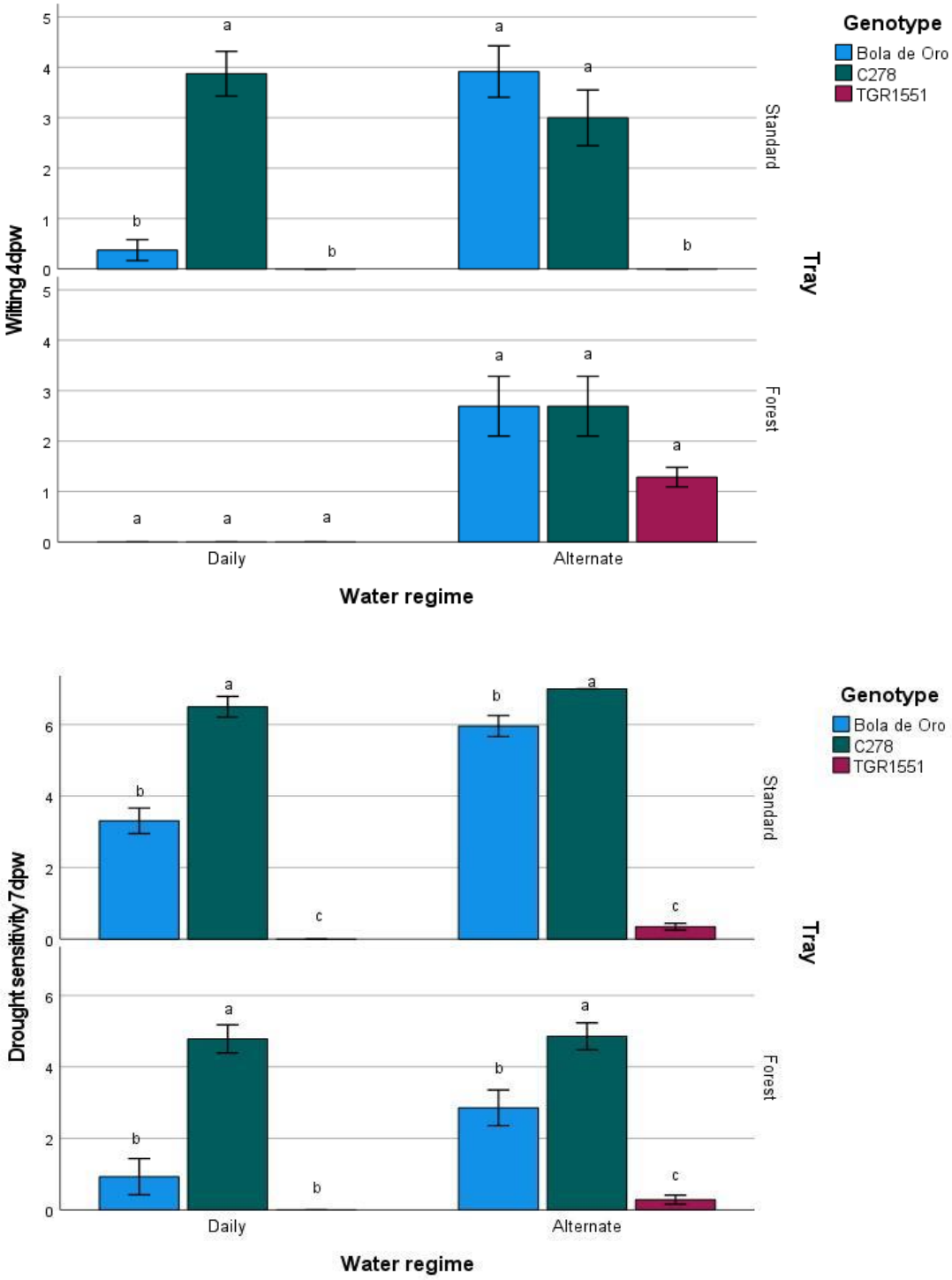

Figure 1. Drought sensitivity means (± SE) of plants from the Spanish cultivar Bola de Oro, and accessions C278 and TGR1551 at the 4th day (top) and 7th day (bottom) evaluations after last watering in standard trays (5 x 5 cm/cell) or forest trays (6 x 12 cm/cell) and watered daily or on alternate days from sowing to two-three leaves stage. Means with the same letter are not significantly different (Tukey-b test, *P*<0.01)

Development and Availability of a Melon Differential Set for Determination of Virulence Variation of Cucurbit Powdery Mildews (*Podosphaera xanthii* and *Golovinomyces orontii*)

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Introduction

Cucurbit powdery mildew (CPM), a disease of field and greenhouse cucurbit crops, is caused most frequently by the two obligate erysiphaceous ectoparasites *Golovinomyces orontii* (Go) and *Podosphaera xanthii* (Px) that greatly vary in their ecology, specificity of host-pathogen interactions and virulence (Lebeda *et al*., 2021). Go and Px are distributed worldwide (Braun and Cook, 2012), and differ in their spatio-temporal and geographic distribution (Křístková *et al*., 2009). Changes in species spectrum are substantially influenced by ecological factors (Trecate *et al*., 2019) and climate change (Lebeda *et al*., 2009, 2021). Economically important cucurbit crops (*Cucumis sativus*, *C. melo*, *Cucurbita* spp., *Citrullus lanatus*, *Momordica charantia* and many others) host Go and Px (Lebeda *et al*., 2007, 2021).

The first attempts to breed melon (*Cucumis melo*) for resistance to CPM (likely Px) took place in California starting in the 1920s (for a review see Pryor *et al*., 1946). Similar attempts were made later for other cucurbits (Jahn *et al*., 2002; Sitterly, 1972). Success of these efforts was complicated by the presence of different pathogen biotypes (races) (Lebeda *et al*., 2008; McCreight, 2006), later confirmed by the enormous variation in virulence at the population level of both CPM species (Lebeda *et al*., 2018a, 2021). Characterization of virulence variation is basic to understanding host-pathogen interactions and developing strategies for CPM resistance breeding (Lebeda *et al*., 2021).

Melon CPM (MCPM) races 1 and 2 have been commonly observed since the 1920s. Race 3 was observed in 1976 (Thomas, 1978). New MCPM-melon interaction patterns were observed in pathogen populations from the beginning and in the mid-1980s and continuing through the early 2000s (Lebeda *et al*., 2011; McCreight, 2006; Pitrat et al., 1998). Investigators used various host differentials (mostly *C. melo*) and different race denomination systems, which confounded understanding and communication within the CPM research, and melon and cucurbit production communities (Lebeda *et al*., 2011). Lack of a standard set of differentials and clear and uniform descriptions of the genetic variation in the virulence of the CPM pathogens on melon and other cucurbits limit study of the genetics of resistance, resistance breeding, plant protection and production (Lebeda *et al*., 2018b, 2021). Recognition of these obstacles thus led to the development of an international differential set and a methodology of race determination, description, and denomination (Lebeda *et al*., 2008, 2011, 2016a), supported by study of virulence variation at the population level (Lebeda *et al*., 2021).

Research and Initiative Leading to Differential Set

The process of developing a set of differentials was initiated at the end of the 1970s by carrying out some basic studies related to CPM (Lebeda, 1983, 1984, 1986; Lebeda *et*

al., 2008; Lebeda and Sedláková, 2010). Discussions at the IXth Eucarpia Meeting on Cucurbitaceae in Avignon, France in 2008, and the collaboration between the Czech Republic (A. Lebeda *et al*.) and the U.S. (J. D. McCreight) led to the International Cucurbit Powdery Mildew Initiative (ICPMI) (Lebeda *et al*., 2011, 2016a,b, 2018a,b). Discussion at Cucurbitaceae 2016, XIth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae (Warsaw, Poland) by the authors and representatives of nine international melon breeding companies led to the unanimous agreement to implement the proposed system for uniform virulence variation and race determination, and denomination of MCPM, as well as CPM on other cucurbits, as summarized in recent publications (Lebeda *et al*., 2016a,b, 2018a,b, 2021).

The proposed triple septet of 21 MCPM differentials (Lebeda *et al*., 2016a,b, 2018a,b) was the next logical step in facilitating communication within and among researchers, commercial breeders, plant pathologists, extension specialists and crop consultant communities (Table 1). During this process, a fourth septet was established with the addition of melon accession SVI105, designed as 4.1 (Table 1). Additional melon accessions deemed crucial for characterization of CPM virulence variation in the future may be added to the fourth septet.

Establishment of a uniform system for MCPM virulence characterization, and race determination and denomination is based upon four components: (1) a standard set of race differentials, (2) a uniform screening methodology, (3) a uniform code for the host-CPM interactions/scores (Lebeda *et al*., 2016a,b; 2018a,b), and (4) a system that is open to the addition of new differential accessions. The proposed set of 22 melon differentials was acceptable to commercial melon breeders and pathologists attending Eucarpia Cucurbitaceae 2016 (Warsaw, Poland) (Lebeda *et al*., 2016a). Rijk Zwaan Breeding B.V. (De Lier, The Netherlands) took on the task of increasing the entire differential set for distribution to the international melon CPM community. The seeds have recently been deposited in the cucurbit collection of the United States Department of Agriculture (USDA), National Plant Germplasm System (NPGS), North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, U.S.A., which will be responsible for distribution of the seed material to requestors. Ales Lebeda coordinated the process of purification, multiplication, and deposition from 2016 through 2021. Details about this activity and results are summarized below.

Genetic Homogenization and Multiplication of Differential Set

Seed multiplication of differentials was done by Rijk Zwaan at its Breeding Support Location, Arusha, Tanzania. The materials were grown in strict quarantine conditions from the seedling stage through fruit harvest; seedlings and mature plants were checked for presence of seed-transmissible diseases. Seventy-five to 150 plants per accession per project cycle were placed in a greenhouse compartment. Leaf disk samples of all plants in the greenhouse were taken and sent to De Lier and stored in a freezer for DNA extraction and SNP-marker analysis. Well-trained personnel performed the required controlled, hand-pollinations. Plants were pruned, prepared and female flowers emasculated the day before pollination. The different origins (Table 1) and backgrounds of the accessions ensured highly variable flowering and fruit setting patterns between and within the differentials and required extended pollination periods to obtain fruit from all differentials. Fruits were harvested when maturity signs were showing. Seeds were threshed and washed the same day, followed by drying in dedicated cabinets.

Clear phenotypic variation for leaf, plant and fruit characters was observed in many of the differentials in the initial planting; this was expected based on their origins. Self-pollinations were, therefore, used in order to achieve phenotypic homogeneity within each of the accessions.

DNA samples were analyzed using a dedicated set of markers spread evenly over the chromosomes in order to assess the genetic homozygosity and used to select the seeds of each differential for the next cycle of seed multiplication. It was also used to check on similarity of all individuals within the same accession. The set of markers used, specifically designed for this project, were evenly spread over the chromosomes.

Once the individuals in each accession were similar in phenotype and exhibited the same genetic marker profile, the decision was taken to finalize purification and proceed with the multiplication to produce 10,000 to 15,000 seeds. The plants and seed in the final multiplication were checked in order to certify freedom from important seed-transmissible pathogens, e.g., melon necrotic spot virus (MNSV).

Seed Deposition, Maintenance, Availability and Distribution

USDA, NPGS, NRCPIS received seeds of 21 of the 22 differentials (Table 1), designated by ICPMI in November

2021. NCRPIS which maintains the NPGS *Cucumis* collection will distribute, but not maintain, the differential set. The missing differential, PI 414723, will be increased in 2022 and deposited in the near future as pollination and quarantine procedures are completed.

The Rijk Zwaan-generated seed lots were shipped to the USDA-APHIS (Animal and Plant Health Inspection Service) for quarantine inspection prior to being shipped to NCRPIS. Accession passport data including source, donor, identifiers, inventory, etc., have been uploaded to the GRIN-Global database. The 100-seed and total seed weights were determined for each differential inventory lot and the total quantity of seeds calculated (total seed weight divided by the 100-seed weight × 100). Prepacks of the ICPMI differential set, which consists of individually packaged, 25-seed lots of each differential, have been placed in -20 °C storage to facilitate order processing. A 100-seed backup sample of each accession will be sent to the National Laboratory for Germplasm Resources Preservation in Fort Collins, Colorado, USA.

Accessions in the ICPMI differential set will be distributed only as a set, not individually, and can be ordered via the Public GRIN-Global website at https://npgsweb.ars-grin.gov/gringlobal/ search. Requestors must first create a log-in account in order to submit a request *via* the website. To query the ICPMI set, first select the "Advanced Search" tab. Under "Additional search criteria", select "Accession Group" from the drop-down list, then select "Melon Differential Powdery Mildew (International)" from the list of group names, and select the search button. Requestors can select the individual links for each accession to see additional information and they can add the accessions to a shopping cart and submit an order.

In accordance with the NPGS plant germplasm distribution guidelines, germplasm will be supplied to scientists, educators, producers and other *bona fide* research and education entities. There is no charge for the germplasm, though requestors may be asked to provide shipping costs, especially when expedited domestic or international services are requested. All germplasm provided to cooperators outside the U.S. must follow phytosanitary regulations specific to the samples transferred between the U.S. and the importing country. APHIS is contacted before such orders are filled for information regarding the importing country's phytosanitary regulations. APHIS provides, as required, a phytosanitary certificate to accompany seed samples attesting to freedom from specified pests and pathogens. These seed lots were prepared in The Netherlands by Rijk Zwaan which provided seed analysis certificates indicating a representative sample of seeds was tested and found to be negative for: cucumber green mottle mosaic virus (CGMMV), squash mosaic virus (SqMV), MNSV, *Didymella bryoniae*, and *Acidovorax citrulli*. Though we can include electronic copies of these documents for an order, the requestor may need to seek a waiver if the importing country does not accept them or if additional declarations are indicated on the import permit.

Utilization of Differential Set

This differential set was composed with the main idea that it could be used internationally and by everyone, i.e., researchers, academics, plant breeders, seed producers, growers, agricultural testing institutions, etc., who need valid, understandable and internationally comparable information on pathogenic variation of CPM species Go and Px occurring on melon as well as other cucurbits (Lebeda *et al*., 2016a,b). This strategy and approach enable comparisons and understanding of CPM variation in pathogenicity and virulence among CPM isolates and populations across countries and continents. This is the main difference between our system and the system developed by the International Seed Federation Disease Resistance Terminology Working Group (ISF DRT WG), and which had the objectives of 1) defining a more manageable subset of differentiating melon hosts, 2) assembling commercially relevant Px races, and 3) developing a uniform testing protocol for routine disease resistance testing in order to support commercial claims of CPM resistance (Grimault *et al*., 2020).

The ICPMI approach was developed because race denominations of Go and Px (Lebeda *et al*., 2011; McCreight, 2006) over the past century often hampered direct comparisons of results obtained by different research groups (Lebeda *et al*., 2016a,b, 2021). This system enables objective and uniform description of Go and Px virulence variation at the individual (virulence-factors /v-factors/, v-phenotypes and races) and population level (frequencies of v-factors and v-phenotypes) (Lebeda *et al*., 2021), thus allowing a thorough understanding of the prevalence and dynamics of race-specific v-factors, which play a crucial role in deployment of resistance sources and/or specific R-genes (resistance genes) in plant breeding, as demonstrated in long-term population studies (Lebeda *et al*., 2018a, 2021).

Conclusions

After nearly a century of research of CPM species virulence variation, the contributions of generations of scientists and plant breeders have been critically analyzed, organized, and tested in long-term virulence studies. This

research yielded the first comprehensive and internationally (globally) applicable differential set and system for CPM virulence description and denomination as a background for better communication and breeding of melon and other cucurbits for resistance to CPM. The differential set is publicly available and open for future enlargement and development.

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Table 1. GRIN accession numbers and septet group identifications of melon cucurbit powdery mildew (CPM) race differentials designated by the International Cucurbit Powdery Mildew Initiative (ICPMI) (Lebeda *et al.*, 2016a,b, 2018a,b, unpubl.).[z]

GRIN no.	Septet no.	Differential	Other designation(s)	Source[y]	Country
ICPMI 1 01	1.1	Iran H	–	INRA	Iran
ICPMI 1 02	1.2	Védrantais	M 319[y]	INRA	France
ICPMI 1 03	1.3	PI 179901	Teti	USDA	India
ICPMI 1 04	1.4	PI 234607	Sweet Melon	USDA	South Africa
ICPMI 1 05	1.5	AR HBJ	AR Hale's Best Jumbo	USDA	USA
ICPMI 1 06	1.6	PMR 45	M 321[x]	USDA	USA
ICPMI 1 07	1.7	PMR 6	Ames 26810	USDA	USA
ICPMI 1 08	2.1	WMR 29	M 322[x]	USDA	USA
ICPMI 1 09	2.2	Edisto 47	NSL 34600	Clemson Univ.	USA
ICPMI 1 10	2.3	PI 414723	LJ 90234	USDA	India
ICPMI 1 11	2.4	PMR 5	Ames 26809	USDA	USA
ICPMI 1 12	2.5	PI 124112	Koelz 2564	USDA	India
ICPMI 1 13	2.6	MR-1	Ames 8578	USDA	USA
ICPMI 1 14	2.7	PI 124111	Koelz 2563	USDA	India
ICPMI 1 15	3.1	PI 313970	PI 315410; VIR 5682	USDA	India
ICPMI 1 16	3.2	Noy Yizre´el	–	Bar Ilan Univ.	Israel
ICPMI 1 17	3.3	PI 236355	–	USDA	England
ICPMI 1 18	3.4	Negro	–	Univ. Zaragoza	Spain
ICPMI 1 19	3.5	Amarillo	–	Univ. Zaragoza	Spain
ICPMI 1 20	3.6	Nantais Oblong	M 320[x]	INRA	France
ICPMI 1 21	3.7	Ames 31282	–	USDA	China
ICPMI 1 22	4.1	SVI105	–	INRA	France

[z]The complete set of differentials is available by request: https://npgsweb.ars-grin.gov/gringlobal/search

[y]INRA = L'Institut National de la Recherche Agronomique, Montfavet (France); USDA = United States Department of Agriculture, Agricultural Research Service.

[x]designation by M. Pitrat, INRA, Montfavet (France) of seed provided to A. Lebeda in 1997.

Heterosis and Inbreeding Depression Seldom Occur in Tropical Pumpkin

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Introduction

Like most cucurbits, tropical pumpkin (*Cucurbita moschata* Duchesne) is a cross-pollinated, monoecious species. Many monoecious species exhibit heterosis or hybrid vigor with crossbreeding, and inbreeding depression with inbreeding. Heterosis is the improved performance of F_1 hybrid progeny compared to their parents calculated on a midparent (MP) or better parent (BP) basis. Inbreeding depression is the tendency of the phenotypic mean to decline because of inbreeding. Bushnell (1922) demonstrated that inbreeding can take place in *C. maxima* without being accompanied by inbreeding depression; Scott (1934) made the same observation in *C. pepo*. Wehner (1999) estimated that F_1 cultivars of summer squash (*C. pepo*) and winter squash (*C. maxima* and *C. moschata*) yielded up to 44% greater than open pollinated cultivars but did not present any direct data. López Anido et al. (2004) observed strong heterosis for yield when crossing crooknecks or straightnecks [belonging to subspecies *texana* (= *ovifera*)] to cocozelles, vegetable marrows or zucchinis (subspecies *pepo*), but not when crossing cultivars within the same cultivar (subspecies) group. They concluded that this response could be used to increase yields while maintaining the desirable fruit attributes of a particular cultivar group. Many authors have indicated that hybrid vigor is a result of genetic diversity between parents although Flint-Garcia et al. (2009), working with maize, found this to be true for some, but not all traits. Jansi et al (2018) studied heterosis and inbreeding depression for flowering, yield number and weight, and average fruit weight in three crosses of tropical pumpkin (*C. moschata*) and concluded that midparent and high parent heterosis and inbreeding depression were common. However, our review of their data and statistical approach makes us question their conclusions. For most traits in most crosses, the F_1 was not significantly better than the BP nor the MP value. The F_2 performed more poorly than the F_1 in only about a third of the cases (little inbreeding). El-Shoura and Abed (2018) also reported high levels of heterosis in *C. pepo* but again, when we did a further analysis of means of F_1 hybrids, we concluded that the F_1 was seldom significantly better than either the MP or the BP. Both Jansi et al. (2018) and El-Shoura and Abed (2018) appeared to have converted individual plot data into percentage heterosis, and used analysis of variance to analyze these percentages. This same approach was used in several other studies we reviewed that claimed high amounts of heterosis in *C. moschata* (Jahan et al., 2012; Tamilselvi et al., 2015). This approach can lead to a conclusion of "significant" heterosis even when there is no significant difference between trait means *per se*. For example, a BP mean of 10 versus a F_1 mean of 12 corresponds to 17% BP heterosis. Likewise, a BP mean of 30 versus a F_1 mean of 36 also gives 17% BP heterosis. But if the LSD is, say, 5, then the 17% heterosis in the first example is not "significant" while the 17% heterosis in the second example is. Lamkey and Edwards (1999) point out that there is no good direct statistical test of percentage heterosis. The same challenge applies to testing percentage inbreeding depression.

Our objective was to determine how common heterosis and inbreeding depression are in diverse crosses within tropical pumpkin, and between tropical pumpkin and temperate *C. moschata* germplasm.

Materials and Methods.

In Expt. 1, eight lines and eight F_1 hybrids from those lines were planted in Lajas, Puerto Rico. The lines have a semi-bush growth habit and were derived from crosses between temperate x tropical germplasm (Table 1). A randomized complete block design with three replications was used. Plots consisted of a single 7.6 m-long row, with seven plants/row. Rows were 366 cm apart. In Expt. 2, five parents and their F_1 and F_2 populations (10 populations each, no reciprocals; Table 2) were planted in Juana Díaz, Puerto Rico. A randomized complete block design with two replications was used. Plots consisted of three 18.3-m-long rows with a distance of 183 cm between rows. Long-vine tropical genotypes ('La Segunda' and 'Soler') and their F_1 and F_2 progenies were planted at 183 cm between plants. The remaining genotypes [TP312 and BN111 (lines derived from bush-butternut temperate x tropical germplasm), and

Waltham Butternut (a temperate cultivar; Mountain Valley Seed Company, Salt Lake City, UT, USA)] were planted at 91 cm between plants (including all F_1 and F_2 populations with these genotypes as parents). Flowering (male and female) was recorded as number of days after planting when 50% of the plants in a plot had at least one open flower. Number of fruit and fruit yield was measured on a per hectare basis in Expt. 1 and on a per plant basis in Expt. 2. Percentage heterosis and inbreeding depression was calculated as follows using MP, BP [or low parent (LP) in the case of flowering], F_1 and F_2 means:

%MP heterosis = $[(F_1 - MP)/MP] * 100$
%High parent heterosis = $[(F_1 - BP)/BP] * 100$
%Inbreeding depression = $[(F_2 - F_1)/F_1] * 100$

For flowering, where lower phenotypic values are preferred (earlier flowering)
%Midparent heterosis = $[(MP - F_1)/MP] * 100$
%Low parent heterosis = $[(LP - F1)/LP] * 100$
%Inbreeding depression = $[(F_1 - F_2)/F_1] * 100$

We considered the percentage heterosis to be significant when the mean of the F_1 hybrid was significantly better (earlier in the case of flowering, greater in the case of fruit number, yield, and average weight) than the BP according to a test with a single-degree-of-freedom linear contrast at the 0.05 probability level. Similarly, the percentage inbreeding depression was considered significant when the mean of the F_1 hybrid was significantly better than that of the F_2 according to the linear contrast. It should be noted that this is an indirect test of the significance of heterosis and inbreeding depression.

Results and Discussion

Heterosis: In Expt. 1, both staminate and pistillate flowering in the F_1 was earlier than the early parent, but this difference was significant in only half of the crosses (Table 3). When significant, BP (early parent) heterosis ranged from 7.6% to 7.9% for days to male flowering and 10.6% to 17.2% for days to female flowering. The same general trend was observed in Expt. 2: flowering in the F_1 tended to be earlier than that of the early parent (Table 4). However, this trend was never significant. In the only case where there was a significant difference between the F_1 and the early parent, there was negative heterosis (the F_1 was later than the early parent). Only two replications were used in Expt. 2. A greater number of replications might have resulted in better ability to detect significant amounts of heterosis for early flowering.

In Expt. 1, the F_1 tended to produce more fruit/ha than the BP, but this difference was significant in only two cases (Table 5). In Expt. 2, the F_1 tended to produce more fruit/plant than the BP in about half of the cases, but this difference was never significant. For yield/ha (Expt. 1) or yield/plant (Expt. 2), the (non-significant) tendency was for the F_1 to perform more poorly than the BP (Tables 7 and 8). This was also true for average fruit weight (Tables 9 and 10).

Inbreeding depression: Across all traits, there were no significant differences between F_1 and F_2 populations, nor were any non-significant trends observed (Tables 4, 6, 8 and 10). Wehner (2022) suggests that inbreeding depression is not observed in watermelon, cucumber, and melon because small populations were used by farmers during domestication and therefore a high degree of natural inbreeding occurred. This is also likely the case for squash and pumpkins.

The breeding of hybrid cultivars of tropical pumpkin may be of benefit for the purposes of protection of intellectual property (inbred lines) or for easily combining dominant traits. Our study suggests that inbreeding depressing is not an impediment for the development of inbred lines. However, our study also indicates that for earliness of flowering and yield there is little heterosis to exploit in tropical pumpkin.

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Table 1. Pedigrees of semi-bush lines used in Expt. 1. 'Seminole' is a subtropical landrace. 'La Segunda' and 'La Primera' were derived from crosses between tropical germplasm and 'Seminole'. 'Bush Butternut' and NY441 are temperate butternut types.

TP111	Bush Butternut x La Segunda
TP121	Bush Butternut x La Segunda
TP211	(Bush Butternut x La Segunda) x Seminole
TP241	(Bush Butternut x La Segunda) x Seminole
TP331	((Bush Butternut x La Segunda) x La Primera) x Seminole
TP341	((Bush Butternut x La Segunda) x La Primera) x Seminole
TP411	NY441 x La Primera
TP423	NY441 x La Primera

Table 2. Pedigrees/origin of lines used in Expt. 2. 'La Segunda' and 'Soler' are long-vine tropical genotypes. TP312 and BN111 are lines derived from bush-butternut temperate x tropical germplasm. Waltham Butternut is a temperate cultivar.

TP312	((Bush Butternut x La Segunda) x La Primera) x Seminole
BN111	Bush Butternut x La Segunda
Waltham Butternut	Temperate butternut cultivar
Soler	OP cultivar from Puerto Rico
La Segunda	OP cultivar derived from crosses between tropical germplasm and Seminole

Table 3. Mean days to flowering of parents and their F_1 populations, significance of linear contrasts of means of the F_1 vs. midparent and F_1 vs. early flowering parent (= best parent, BP), and percentage heterosis in tropical pumpkin (*Cucurbita moschata*) planted in Lajas, Puerto Rico (Expt. 1).

		Days to 50% flowering[1]				Significance of linear contrast		Heterosis (%)	
Late parent	Early parent	Late parent	BP	Midparent value	F_1	F1 vs midparent	F1 vs BP	Below midparent	Below BP
					Staminate flowers				
TP111	TP411	51.4	50.7	51.05	46.7	**	*	8.5	7.9
TP111	TP341	51.4	47.1	49.25	46.9	NS	NS	4.8	0.4
TP121	TP241	54.2	51.3	52.75	47.3	**	*	10.3	7.8
TP121	TP331	54.2	50.1	52.15	46.3	**	*	11.2	7.6
TP211	TP341	52.5	47.1	49.80	46.1	**	NS	7.4	2.1
TP241	TP331	51.3	50.1	50.70	49.1	NS	NS	3.2	2.0
TP241	TP423	51.3	50.8	51.05	47.7	*	NS	6.6	6.1
TP423	TP331	50.8	50.1	50.45	48.5	NS	NS	3.9	3.2
					Pistillate flowers				
TP411	TP111	55.3	46.2	50.75	39.6	**	**	22.0	14.3
TP341	TP111	58.2	46.2	52.20	41.3	**	*	20.9	10.6
TP241	TP121	60.1	48.7	54.40	41.3	**	**	24.1	15.2
TP331	TP121	58.2	48.7	53.45	40.3	**	**	24.6	17.2
TP341	TP211	58.2	43.3	50.75	42.4	**	NS	16.5	2.1
TP241	TP331	60.1	58.2	59.15	54.1	*	NS	8.5	7.0
TP241	TP423	60.1	56.7	58.40	53.9	*	NS	7.7	4.9
TP331	TP423	58.2	56.7	57.45	53.9	NS	NS	6.2	4.9

[1]Days from planting to 50% of plants in flower.

Table 4. Mean days to flowering of parents and their F_1 and F_2 populations, significance of linear contrasts of means of the F_1 vs. midparent, F_1 vs. early flowering parent (=best parent, BP), and F_1 vs. F_2, and percentage heterosis and inbreeding in tropical pumpkin (*Cucurbita moschata*) planted in Juana Díaz, Puerto Rico (Expt. 2).

		Days to 50% flowering[1]					Significance of linear contrast			Heterosis (%)		Inbreeding depression (F_1 vs F_2) (%)
Late parent	Early parent	Late parent	BP	Mid-parent	F_1	F_2	F_1 vs. mid-parent	F_1 vs. BP	F_1 vs. F_2	F_1 vs. mid-parent	F1 vs. BP	
						Staminate flowers						
TP312	BN111	48.5	44.8	46.65	43.4	44.8	NS	NS	NS	7.0	3.1	-3.2
TP312	Waltham	48.5	44.9	46.70	43.2	48.5	NS	NS	NS	7.5	3.8	-12.3
Soler	TP312	49.4	48.5	48.95	47.6	51.8	NS	NS	NS	2.8	1.9	-8.8
La Segunda	TP312	51.1	48.5	49.80	51.9	44.8	NS	NS	NS	-4.2	-7.0	13.7
Waltham	BN111	44.9	44.8	44.85	43.3	43.2	NS	NS	NS	3.5	3.3	0.2
Soler	BN111	49.4	44.8	47.10	40.5	44.3	*	NS	NS	14.0	9.6	-9.4
La Segunda	BN111	51.1	44.8	47.95	42.8	43.1	NS	NS	NS	10.7	4.5	-0.7
Soler	Waltham	49.4	44.9	47.15	48.3	49.4	NS	NS	NS	-2.4	-7.6	-2.3
La Segunda	Soler	51.1	49.4	50.25	52.5	41.7	NS	NS	NS	-4.5	-6.3	20.6
						Pistillate flowers						
TP312	BN111	50.7	35.4	43.05	33.4	37.7	*	NS	NS	22.4	5.6	-12.9
TP312	Waltham	50.7	34.3	42.50	32.5	36.1	*	NS	NS	23.5	5.2	-11.1
Soler	TP312	58.5	50.7	54.60	47.6	50.8	NS	NS	NS	12.8	6.1	-6.7
TP312	La Segunda	50.7	50.2	50.45	54.3	53.3	NS	NS	NS	-7.6	-8.2	1.8
BN111	Waltham	35.4	34.3	34.85	33.4	32.7	NS	NS	NS	4.2	2.6	2.1
Soler	BN111	58.5	35.4	46.95	31.3	36.5	*	NS	NS	33.3	11.6	-16.6
La Segunda	BN111	50.2	35.4	42.80	35.3	37.5	NS	NS	NS	17.5	0.3	-6.2
Soler	Waltham	58.5	34.3	46.40	46.8	39.5	NS	**	NS	-0.9	-36.4	15.6
Soler	La Segunda	58.5	50.2	54.35	52.6	49.2	NS	NS	NS	3.2	-4.8	6.5

[1]Days from planting to 50% of plants in flower.

Table 5. Mean number of fruit/ha of parents and their F_1 populations, significance of linear contrasts of means of the F_1 vs. midparent and F_1 vs. high parent (=best parent, BP), and percentage heterosis in tropical pumpkin (*Cucurbita moschata*) planted in Lajas, Puerto Rico (Expt. 1).

		Number of fruit/ha				Significance of linear contrast[2]		Heterosis (%)	
Low parent	High parent	Low parent	BP	Midparent value	F_1	F1 vs midparent	F1 vs BP	Above midparent	Above BP
TP111	TP411	7,774	8,970	8,372	11,661	**	*	39.3	30.0
TP341	TP111	6,877	7,774	7,326	7,774	NS	NS	6.1	0.0
TP241	TP121	5,382	7,475	6,429	7,475	NS	NS	16.3	0.0
TP121	TP331	6,877	7,475	7,176	9,568	*	NS	33.3	28.0
TP211	TP341	6,877	6,877	6,877	10,465	**	*	52.2	52.2
TP241	TP331	5,382	6,877	6,130	9,269	**	NS	51.2	34.8
TP241	TP423	5,382	5,682	5,532	5,980	NS	NS	8.1	5.2
TP423	TP331	5,681	6,877	6,279	8,970	*	NS	42.9	30.4

Table 6. Mean number of fruit/plant of parents and their F_1 and F_2 populations, significance of linear contrasts of means of the F_1 vs. midparent, F_1 vs. high parent (=best parent, BP), and F_1 vs. F_2, and percentage heterosis and inbreeding in tropical pumpkin (*Cucurbita moschata*) planted in Juana Díaz, Puerto Rico (Expt. 2).

		Number of fruit/plant					Significance of linear contrast			Heterosis (%)		
Low parent	High parent	Low parent	BP	Midparent	F_1	F_2	F_1 vs midparent	F_1 vs BP	F_1 vs F_2	Above midparent	Above BP	Inbreeding depression (F_1 vs F_2) (%)
TP312	BN111	2.1	5.3	3.7	5.6	3.9	NS	NS	NS	50.8	5.6	30.5
TP312	Waltham	2.1	4.4	3.3	4.4	4.1	NS	NS	NS	34.2	-0.5	6.4
TP312	Soler	2.1	4.3	3.2	4.9	4.2	NS	NS	NS	52.0	14.1	13.4
TP312	La Segunda	2.1	3.2	2.7	2.9	2.1	NS	NS	NS	10.6	-7.6	28.3
Waltham	BN111	4.4	5.3	4.9	4.2	5.0	NS	NS	NS	-13.7	-21.1	-19.3
Soler	BN111	4.3	5.3	4.8	6.3	7.1	NS	NS	NS	31.2	18.1	-13.2
La Segunda	BN111	3.2	5.3	4.2	5.0	6.7	NS	NS	NS	17.0	-6.6	-35.1
Soler	Waltham	4.3	4.4	4.3	3.2	3.5	NS	NS	NS	-26.2	-27.5	-9.7
La Segunda	Waltham	3.2	4.4	3.8	5.0	6.9	NS	NS	NS	32.1	13.6	-38.0
La Segunda	Soler	3.2	4.3	3.7	4.2	3.8	NS	NS	NS	13.7	-0.7	10.0

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Table 7. Mean yield/ha of parents and their F_1 populations, significance of linear contrasts of means of the F_1 vs. midparent and F_1 vs. high parent (best parent, BP), and percentage heterosis in tropical pumpkin (*Cucurbita moschata*) planted in Lajas, Puerto Rico (Expt. 1).

		Yield (kg/ha)				Significance of linear contrast[2]		Heterosis (%)	
Low parent	High parent	Low parent	BP	Midparent value	F_1	F1 vs midparent	F1 vs BP	Above midparent	Above BP
TP111	TP411	6,957	18,526	12,742	12,979	NS	NS	1.9	-29.9
TP111	TP341	6,957	14,837	10,897	9,873	NS	NS	-9.4	-33.5
TP121	TP241	6,374	14,335	10,355	10,904	NS	NS	5.3	-23.9
TP121	TP331	6,374	12,125	9,250	11,433	NS	NS	23.6	-5.7
TP211	TP341	7,269	14,837	11,053	12,545	NS	NS	13.5	-15.4
TP331	TP241	12,125	14,335	13,230	22,201	**	**	67.8	54.9
TP241	TP423	14,335	17,021	15,678	13,047	NS	NS	-16.8	-23.3
TP331	TP423	12,125	17,021	14,573	26,867	**	**	84.4	57.8

Table 8. Mean yield/plant of parents and their F_1 and F_2 populations, significance of linear contrasts of means of the F_1 vs. midparent, F_1 vs. high parent (best parent, BP), and F_1 vs. F_2, and percentage heterosis and inbreeding in tropical pumpkin (*Cucurbita moschata*) planted in Juana Díaz, Puerto Rico (Expt. 2).

		Yield /plant (kg)					Significance of linear contrast[1]			Heterosis (%)		
Low parent	High parent	Low parent	BP	Mid-parent	F_1	F_2	F_1 vs midparent	F_1 vs BP	F_1 vs F_2	Above midparent	Above BP	Inbreeding depression (F_1 vs F_2) (%)
BN111	TP312	2.57	5.40	5.94	4.03	3.99	NS	NS	NS	49.1	10.0	32.1
Waltham	TP312	4.15	5.40	5.71	6.52	4.78	NS	NS	NS	19.6	5.8	-14.2
TP312	Soler	5.40	23.65	28.40	19.47	14.53	**	NS	NS	95.6	20.1	31.5
TP312	La Segunda	5.40	16.60	14.63	12.08	11.00	NS	NS	NS	33.0	-11.9	17.4
BN111	Waltham	2.57	4.15	3.05	3.14	3.36	NS	NS	NS	-9.1	-26.4	-2.8
BN111	Soler	2.57	23.65	12.18	10.12	13.11	NS	*	NS	-7.1	-48.5	16.9
BN111	La Segunda	2.57	16.60	8.19	10.98	9.59	NS	NS	NS	-14.6	-50.7	-34.1
Waltham	Soler	4.15	23.65	16.96	7.87	13.90	NS	NS	NS	22.0	-28.3	53.6
Waltham	La Segunda	4.15	16.60	12.23	15.39	10.38	NS	NS	NS	17.9	-26.3	-25.8
La Segunda	Soler	16.60	23.65	22.46	24.86	20.13	NS	NS	NS	11.6	-5.0	-10.7

Table 9. Mean individual fruit weight of parents and their F_1 populations, significance of linear contrasts of means of the F_1 vs. midparent and F_1 vs. high parent, and percentage heterosis in tropical pumpkin (*Cucurbita moschata*) planted in Lajas, Puerto Rico (Expt. 1).

		Average fruit weight (kg)				Significance of linear contrast[2]		Heterosis (%)	
Low parent	High parent	Low parent	High parent	Midparent value	F_1	F1 vs midparent	F1 vs high parent	Above midparent	Above high parent
TP111	TP411	0.91	2.10	1.51	1.12	NS	NS	-25.6	-46.7
TP111	TP341	0.91	2.14	1.53	1.29	NS	NS	-15.4	-39.7
TP121	TP241	0.84	2.85	1.85	1.46	NS	NS	-20.9	-48.8
TP121	TP331	0.84	1.80	1.32	1.19	NS	NS	-9.8	-33.9
TP211	TP341	1.06	2.14	1.60	1.20	NS	NS	-25.0	-43.9
TP331	TP241	1.80	2.85	2.33	2.47	NS	NS	6.2	-13.3
TP241	TP423	2.85	3.00	2.93	2.17	NS	NS	-25.8	-27.7
TP331	TP423	1.80	3.00	2.40	3.00	**	NS	25.0	0.0

Table 10. Mean individual fruit weight of parents and their F_1 and F_2 populations, significance of linear contrasts of means of the F_1 vs. midparent, F_1 vs. high parent, and F_1 vs. F_2, and percentage heterosis and inbreeding in tropical pumpkin (*Cucurbita moschata*) planted in Juana Díaz, Puerto Rico (Expt. 2).

		Average fruit size (kg)					Significance of linear contrast[1]			Heterosis (%)		
Low parent	High parent	Low parent	High parent	Midparent	F_1	F_2	F_1 vs midparent	F_1 vs high parent	F_1 vs F_2	Above midparent	Above high parent	Inbreeding depression (F_1 vs F_2) (%)
BN111	TP312	0.48	2.52	1.05	1.06	1.50	NS	NS	NS	-29.8	-58.2	-0.9
Waltham	TP312	0.95	2.52	1.29	1.56	1.74	NS	NS	NS	-25.8	-48.9	-20.8
TP312	Soler	2.52	5.52	5.74	4.70	4.02	*	NS	NS	42.8	4.0	18.1
TP312	La Segunda	2.52	5.18	5.02	5.70	3.85	NS	NS	NS	30.4	-3.1	-13.5
BN111	Waltham	0.48	0.95	0.73	0.63	0.72	NS	NS	NS	1.5	-23.6	13.8
BN111	Soler	0.48	5.52	1.94	1.42	3.00	NS	**	NS	-35.3	-64.8	27.1
BN111	La Segunda	0.48	5.18	1.65	1.68	2.83	NS	**	NS	-41.7	-68.1	-2.0
Waltham	Soler	0.95	5.52	5.30	3.62	3.24	*	NS	NS	63.9	-3.9	31.7
Waltham	La Segunda	0.95	5.18	2.59	2.22	3.07	NS	**	NS	-15.6	-50.1	14.0
La Segunda	Soler	5.18	5.52	5.39	6.48	5.35	NS	NS	NS	0.7	-2.4	-20.3

[1]Significance tested using single-degree-of-freedom linear contrasts comparing days to flower of F_1 vs. midparent, F_1 vs. early parent, and F_1 vs F_2.

In memoriam

Claude Earle Thomas, Plant Pathologist (1940-2021)

Claude E. Thomas, a retired research plant pathologist with the Agricultural Research Service, U.S. Department of Agriculture, died on December 15, 2021, near his home in Charleston, SC. He was 81.

Thomas was born in Spartanburg, SC, on December 4, 1940. Following graduation from Spartanburg High School, Thomas enrolled at nearby Wofford College, where he received his B.S. degree in 1962. From 1962 to 1966, he was a Graduate Fellow in a newly established plant pathology Ph.D. program at Clemson University. Thomas received his M.S. degree in 1964 and his Ph.D. degree in 1966. He was Clemson University's very first recipient of a Ph.D. degree in plant pathology.

Thomas started his professional career in 1966 by accepting a job as a Research Plant Pathologist with the Agricultural Research Service, U.S. Department of Agriculture in Weslaco, Texas. He spent the first 16 years of his career at a USDA research facility co-located with the Texas A&M University agricultural research station at Weslaco. After about 15 years of living in Texas, Thomas and wife June had a budding family and they developed a desire to move back to their home state of South Carolina. In 1982, the Agency approved a request to transfer Thomas and his research program to the USDA/ARS vegetable crops research laboratory in Charleston, SC. In 1990, the Laboratory Director position at the Charleston location became vacant, and Thomas applied for and was appointed Supervisory Research Plant Pathologist and Laboratory Director, U.S. Vegetable Laboratory, Charleston, SC. Claude's tenure in this position lasted 14 years. During this period, the laboratory thrived under his direction and was widely recognized as one of the Agency's most productive and well managed locations. Thomas spent his entire 38-year career working as a USDA scientist.

Thomas developed an international reputation as the leading authority on fungal pathogens of cucurbit crops, especially the melon. He was instrumental in identifying sources of genetic resistances, in determining the inheritances of the resistances, in the identification of pathogen strains, in the development of methodologies to identify and differentiate pathogen strains, in cooperating with efforts to develop molecular markers and map the location of resistance genes on genetic maps, and in developing new breeding lines and varieties exhibiting high levels of resistances. One of Thomas' most recognized achievements was the development and release of the melon breeding line MR-1 (MR meaning "multiple resistance"). MR-1 is resistant to the major melon diseases powdery mildew, downy mildew, Fusarium wilt, and Alternaria leaf blight. MR-1 has been used by numerous melon breeders worldwide to breed disease resistant varieties.

Several other accomplishments need to be mentioned that document Thomas' recognition as a leader in his chosen field of endeavor: 1) he was the organizer and chairperson of the 1989 North American Cucurbitaceae Conference held in Charleston, SC; 2) he was a former president of the Southern Division, American Phytopathology Society; and 3) he was an author of American Phytopathology Society's publication "Compendium of Cucurbit Diseases." This is a widely cited reference work with multiple editions as well as multiple printings.

Thomas was both a renowned research scientist and a consummate educator. He served two terms on the Weslaco, TX, School Board; served as a guest lecturer, Xinjiang Agricultural University, China (1988); completed research and advisory assignments in Peru, Israel, France, Poland, and China; and served on the adjunct graduate faculties of both Texas A&M University and Clemson University, advising and guiding research of M.S. and Ph.D. students. He authored/co-authored over 200 scientific research publications and was an elected member of the honor societies Phi Kappa Phi, Sigma Xi, and Gamma Sigma Delta.

The final act of Claude Thomas' professional career was the management of the design and construction of a 50,000 square foot office and laboratory facility in Charleston, SC, to house 14 USDA research scientists and 6 faculty members of the Clemson University Coastal Research and Education Center. USDA and Clemson University personnel moved into this new state-of-the-art research facility in March 2003. Claude had to delay his planned "early" retirement by 1 year to see the construction project to conclusion. Claude retired from the USDA in early 2004.

Claude Thomas was a devoted husband, loving father and grandfather, and a devout Christian who served as a deacon and church council member at Fort Johnson Baptist Church, Charleston, SC. He is survived by his wife of 61 years, June Oakman Thomas; three sons, Christopher, Andrew, and Matthew; one sister, Dorothy Calvert; three grandchildren; and one great-grandchild. Thomas was a lifelong sportsman who loved nothing more than to be hunting and fishing with his three sons and later his grandchildren. Upon retirement, he became President of Charleston Lowcountry Rose Society and enjoyed growing and exhibiting his championship roses.

(Submitted by Dr. Thomas' former colleague Richard L. Fery.
Dr. Fery is a retired vegetable breeder in Charleston, SC.)

Figure 1. Plant pathologist Claude E. Thomas, 1940-2021.