# Cucurbit Genetics Cooperative

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The <b>Cucurbit Genetics Cooperative</b> (CGC) was organized in 1977 to develop and advance the genetics of economically important cucurbits. Membership to CGC is voluntary and open to individuals who have an interest in cucurbit genetics and breeding. CGC membership is on a biennial basis. For more information on CGC and its membership rates, visit our website (http://www.umresearch.umd.edu/cgc/) or contact Tim Ng at (301) 405-4345 or tn5@umail.umd.edu.
<b>CGC Reports</b> are issued on an annual basis. The Reports include articles submitted by CGC members for the use of CGC members. None of the information in the annual report may be used in publications without the consent of the respective authors for a period of five years.
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#### 2002 CGC Meeting I (Toronto CA)

Tim Ng, CGC Chair

CGC met on Sunday, 11 August 2002, in Toronto, Canada, in conjunction with the 2002 Annual Conference of the American Society for Horticultural Science. This meeting was not the official annual business meeting, which was scheduled to be held in December in conjunction with Cucurbitaceae 2002; however, many CGC members attend the ASHS Annual Conferences, and this served as a good opportunity for CGC members to get together. Fifteen members and guests were in attendance.

#### **Comments:**

From the CGC Coordinating Committee: CGC Report No. 27 will be published in August 2004. Contributors to the CGC Report should check the website (http://www.umresearch.umd.edu/cgc/) for deadlines, and for instructions on preparing and submitting manuscripts. As always, we are eager to hear from CGC members regarding our current activities and future direction of CGC.

From the CGC Gene List Committee: Lists of known genes for the Cucurbitaceae have been published previously in HortScience and in reports of the Cucurbit Genetics Cooperative. CGC is currently publishing complete lists of known genes for cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), and *Cucurbita* spp. on a rotating basis.

It is hoped that scientists will consult these lists as well as the rules of gene nomenclature for the Cucurbitaceae before selecting a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature (published in each CGC Report) were adopted in order to provide guidelines for the naming and symbolizing of genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

From the CGC Gene Curators: CGC has appointed curators for the four major cultivated crops: cucumber, melon, watermelon and *Cucurbita* spp. Curators are responsible for collecting, maintaining, and distributing upon request stocks of know marker genes. CGC members are requested to forward samples of currently held gene stocks to the respective Curator.

After introductions, an update of CGC Report No. 25 was presented. Tim Ng indicated that the production was behind schedule, but that the 25<sup>th</sup> anniversary issue would be printed and distributed sometime in early 2003. The cover would feature a graphic logo solicited from among CGC members for the 25<sup>th</sup> anniversary. The submitted logos were circulated among the members, and the one designed by Amanda Neill was selected for the CGC cover. In addition, a logo submitted by Tarek Kapiel was selected for the CGC webpage.

Two upcoming meetings of interested to CGC members were announced. Don Maynard announced that Cucurbitaceae 2002, scheduled for December 2002 in Naples, FL, was on schedule and that they were looking forward to an exciting and stimulating meeting. It was also announced that the 8<sup>th</sup> Eucarpia Cucurbitaceae 2004 Meeting on Cucurbit Genetics And Breeding was scheduled to be held in the Czech Republic in 2004, and that it was being organized by Aleš Lebeda and others at Palacký University in Olomouc-Holice. Further conference information would be available at the conference website: http://www.cucurbitaceae.upol.cz/

Tim then talked about the movement of the CGC website to a new server. As old-timers might recall, the website was initially established on Tim's personal account at the University of Maryland, then moved to a server at the National Agricultural Library (NAL) in Beltsville, MD. When the NAL server operations were closed, Cornell agreed to take over the web-hosting for the plant gene databases as well as organizations like CGC. In 2002, Cornell sadly announced that it would no longer be able to host these websites, so Tim moved the CGC website to the server for the Division of Research at the University of Maryland, where it will reside for the time being.

Tim then brought up the issue that www.amazon.com was now listing CGC Reports, as well as reports of The Cucurbit Network (TCN), on their website with no consultation with the publishers. Also, the annual subscription rates were far higher than what these organizations charge their members. Neither the CGC nor the TCN officers are aware of any sales generated by the Amazon listing.

Tim then discussed the expansion of activities which the CGC Chair must undertake, particularly in comparison to the early years of CGC. In addition to maintaining the membership database, billing members and organizations for memberships and subscriptions, keeping track of the financial accounts of CGC, and assembling the final copy of each report for publication (where Coordinating Committee members submitted camera-ready copy of each paper for their sections), the Chair now also must prepare camera-ready copy of each paper formatted for the CGC format, maintain email as well as paper communications, and oversee the updating and development of the website. Unfortunately, this is occurring while Tim's support staff (including secretarial and student help, and print shop personnel) at the University of Maryland was being severely reduced due to budget cuts. Since all CGC officers serve on a voluntary unpaid basis, a discussion ensued as to how to partition out some of the duties (e.g., web maintenance, billing and finance) from the Chair. It was decided that the best solution was to create a few new voluntary positions. and that this would require a revision of the CGC bylaws and a vote by the CGC membership. Tim and a small group of volunteers agreed to pursue this option during the next year, and to report back to the CGC membership in 2003.

There was a discussion about the CGC gene collections, particularly for watermelon where the curation has been in transition for several years. One set of stocks resides with a private seed company which will not return them to CGC, while the other set is badly contaminated with the seed-borne disease watermelon fruit blotch. Todd Wehner indicated that his research is expanding to encompass watermelon, and that he is aggressively trying to recreate the mutant gene stocks at North Carolina State University. Meanwhile, CGC members felt that funding from USDA should be pursued to maintain all of our monogenic collections.

Tim reiterated that the CGC Business Meeting would be held in December in Naples, and the meeting was adjourned.

#### 2002 CGC Business Meeting, Naples FL Tim Ng, CGC Chair

The 2002 CGC Business Meeting was held on Wednesday, 11 December 2002, in Naples FL in conjunction with Cucurbitaceae 2002. Sixty-five members and guests were in attendance. After introductions, CGC Chair Tim Ng described how CGC had provided 25 years of service to the cucurbit

breeding and genetics community. For new members or those unfamiliar with CGC, he went on to describe the many activities CGC provided to ensure that information about cucurbit crops was made available to all interested individuals. These activities also include gene exchanges, gene lists maintenance, and networking. Tim then indicated that CGC 25 was still behind schedule but due for publication within a few months. He said that because of the delay, he would pay for the publishing and mailing out of his own funds, thus giving CGC members a "free year" of membership. Tim also indicated that CGC had recently acquired several boxes of copies of the proceedings from Cucurbitaceae '94 (Padre Island TX), and that these would be made available to CGC members.

Tim then mentioned that the CGC website had officially transitioned to a University of Maryland server following Cornell University's decision not to host any plant genetic databases anymore. transition was somewhat difficult in that Cornell was not particularly helpful in the transition and several pages had to be re-written. Nonetheless the website is now configured and is up and running at the URL http://www.umresarch.umd.edu/cgc/ One of the things Tim wanted to do in 2003 was complete the digitization of the archival issues of the Vegetable Improvement Newsletter (VIN), which was compiled by Henry Munger for many years at Cornell. Despite their age, there is much valuable information in the VIN reports – particularly with regard to dealing with wild progenitors of cucurbit crops - that is not generally available elsewhere.

Two topics were then brought up for discussion, one related to payment of memberships and subscriptions and the other to the proposed restructuring of CGC. Tim indicated that he is now able to accept credit card payments, but only through the University of Maryland; it would be cost-prohibitive to establish CGC as a separate entity to receive credit card payments. It was proposed that perhaps payment could be made through PayPal or some other webbased payment system. Tim indicated he had considered this, but it would also entail separate sets of accounting (e.g., a "shadow" accounting system); however, it would make sense when CGC transitioned over to the next Chair - who would probably not be affiliated with the University of Maryland. With regard to the restructuring of CGC. Tim restated the situation that was described in the CGC meeting in Toronto (see above), and said that

when the proposed changes to the by-laws were finalized, a mail ballot would be conducted among CGC members to finalize the process of restructuring.

#### Cucurbitaceae 2002

Don Maynard, Chair Cucurbitaceae 2002 Organizing Committee

Over 200 cucurbitologists gathered at the Naples (Florida) Beach Hotel and Golf Club in early December 2002 for Cucurbitaceae 2002. Delegates from 25 countries and 27 of the U.S. states participated in the four-day event. The conference was co-sponsored by the University of Florida, American Society for Horticultural Science, Cucurbit Network, and Cucurbit Genetics Cooperative.

Invited presentations were made by D. J. Cantliffe on Current Trends in Florida's Vegetable Production, Amy Goldman spoke on Melons for the Passionate Grower, D. L. Hopkins reported on Bacterial Fruit Blotch of Cucurbits: An Industry-Wide Problem Requiring Industry-Wide Solutions, and William Watson spoke on the National Watermelon Promotion Board.

There were 51 oral and 29 poster presentations delivered in sessions on *Phytophthora capsici*, Breeding and Genetics, Germplasm, Biotechnology, Phytopathology, Entomology, Virology, Crop Physiology, Culture and Management, and Fruit Quality and Postharvest Management. The lecture hall remained full throughout despite the nearby beach and golf course.

One entire day was devoted to professional points of interest in the area. Attendees were asked to chose between two different tours. The highlight of each tour was visits to seed industry research facilities. One tour included Sakata Seed America and Seminis Vegetable Seeds and the other tour visited Syngenta Seeds. Other points of interest were watermelon harvest and packing, cucumber packing, squash production, and transplant production.

Related organizations that met during the conference were Cucurbit Genetics Cooperative, Watermelon Research and Development Working Group, and Cucurbit Crop Germplasm Committee.

It wasn't all work! Breakfasts and lunches and refreshment breaks together provided ample opportunity for informal networking as did a gala welcome reception and a closing beach party dinner

complete with live music. Outside functions in December were a special treat for those from northern areas.

A book, Cucurbitaceae 2002, containing all of the research and educational presentations, is available from the American Society for Horticultural Sciences at: http://www.ashs.org.

The Organizing Committee thanks the many industry sponsors, moderators, tour organizers, and all those attending. The 8<sup>th</sup> EUCARPIA meeting on Cucurbit Genetics and Breeding will be held in Olomoue, Czech Republic in the latter half of July 2004. Cucurbitaceae 2006 will be held in North Carolina at a time and place to be determined.

## Watermelon Research and Development Working Group - 23<sup>rd</sup> Annual Meeting

Benny D. Bruton, Chairman USDA-ARS, Lane Oklahoma

The Annual Meeting of the Watermelon Research & Development Working Group (WRDWG) was held Sunday, 2 February 2003, in Mobile, Alabama in conjunction with the Southern Association of Agricultural Scientists (S.A.A.S.) and the Southern Region American Society for Horticultural Sciences (SR: ASHS).

Following a welcome by Benny Bruton, the following individuals gave reports on seed company releases and updates: Fred McCuistion (Seminis), Don Dobbs (Willhite Seed), Tom Williams (Syngenta Seeds) and Gary Elmstrom (SunSeeds). This was followed by a general discussion on the impact of brokers on the watermelon industry.

The next topic was Statewide Watermelon Trials for 2001, with talks by Warren Roberts of Oklahoma State University ("Watermelon Cultivar Evaluations in Oklahoma"), Don Maynard of the University of Florida ("Review of the Florida Statewide Watermelon Trials"), K. Cushman, R. Snyder, C. Coker, T. Horan and P. Hudson ("Mississippi Triploid Watermelon Cultivar Evaluation: Elongated Genotypes"), Frank Dainello of Texas A&M University ("Review of the Texas Statewide Watermelon Trials"), and George Boyhan of the University of Georgia ("Statewide Watermelon Trials for Georgia, 2001"). Merritt Taylor of Oklahoma State University then talked about "Trends in World Watermelon Production - 1991 to 2001."

Following lunch, Rey Santella (US Department of Agriculture, Foreign Agricultural Service, Horticultural and Tropical Products Division, Washington, DC.) talked about "How to Build Markets Overseas - An Overview of the Foreign Agricultural Service." After this, the following research reports were presented:

- Leskovar, D.I., Bang, H., Bender, D., Crosby, K.
  Texas Agricultural Experiment Station, Dept.
  Horticultural Sciences, Texas A&M University,
  Uvalde, TX 78801; "Environmental Variability
  of Lycopene and Quality"
- Perkins-Veazie, P., Roberts, W., Collins, J., and Perez, K. U.S. Department of Agriculture-Agricultural Research Service, Lane, OK; "Lycopene Variation Among Watermelons: Cultivars, Potassium, and Ripeness"
- Perkins-Veazie, P., Collins, J., Edwards, A. Clevidence, B., and Wiley, E. U.S. Department of Agriculture-Agricultural Research Service, Lane, OK; "Humans and Watermelons: Lycopene Availability from the Human Clinical Trial"
- Shrefler, J. Oklahoma State University, Lane Oklahoma; "Update on New Herbicide Uses in Watermelon"
- Bruton, B.D. USDA-ARS, Lane, Oklahoma. "Squash Bug: Vector of Serratia marcescens, Causal Agent of Cucurbit Yellow Vine Disease"

Following the research reports, there was a general discussion on the impact and future outlook of powdery mildew on the watermelon industry, and on seed sources for fusarium wilt differentials. WRDWG expressed its thanks to Seminis Seeds for providing refreshments for the meeting.

## **Availability of Cucurbit Conference Proceedings**

Since 1980, research conferences on the Cucurbitaceae have been held on an irregular, and more recently regular, basis in the U.S., Europe and internationally. Many of the proceedings from these conferences are available in print form for those seeking archival copies of research sometimes found nowhere else. The following is a listing of current sources for the proceedings from these conferences.

#### **U.S. Cucurbitaceae Conferences**

#### **Cucurbitaceae '80 (Cornell University)**

Available as: *Bates, D.M., R. W. Robinson, and C. Jeffrey, eds. 1990. Biology and Utilization of the Cucurbitaceae. Cornell Univ. Press, Ithaca, NY* 

#### Cucurbitaceae '89 (Charleston, SC)

Out of print (available from CGC via special arrangement – at cost – for photocopying, shipping and handling)

#### **Cucurbitaceae 1994 (Padre Island TX)**

CGC - \$10.00 (including surface mail)

#### Cucurbitaceae 1998 (Monterrey CA)

http://www.ashs.org/ashspress/cucurbit.html ASHS & non-ASHS - \$15.95 + S&H

#### **Cucurbitaceae 2002 (Naples FL)**

http://www.ashs.org/ashspress/cucurbit\_02.html ASHS - \$35.95 + S&H; non-ASHS - \$44.95 + S&H

## Eucarpia Meetings on Cucurbit Genetics and Breeding

## VII Eucarpia Meeting on Cucurbit Genetics and Breeding – 2000

Ma'ale Ha Hamisha, Israel <a href="http://www.actahort.org/books/510/index.htm">http://www.actahort.org/books/510/index.htm</a> € 87 (Available in print or CD format)

#### **ISHS International Symposia on Cucurbits**

### I International Symposium on Cucurbits – 1999

(Adana, Turkey)

ISHS Acta Horticulturae 588

http://www.actahort.org/books/492/index.htm € 71 (Available in CD format only)

#### II International Symposium on Cucurbits – 2001

(Tsukuba, Japan)

ISHS Acta Horticulturae 588

http://www.actahort.org/books/588/index.htm

€ 77 (Available in print or CD format)

## In Memoriam Frank William Zink, Jr.: 1923-2001

James D. McCreight

U.S. Department of Agriculture, Agricultural Research Service, 1636 East Alisal Street, Salinas, CA 93905 U.S.A.

A graduate of the University of California, Davis, Frank began his research career in 1948 as an Associate in the Experiment Station, Division of Truck Crops with the University of California in Imperial Valley where he worked on lettuce and melon disease problems, most notably the "melon virus complex," which later was sorted into watermelon mosaic virus, papaya ringspot virus watermelon strain, and, perhaps, cucumber mosaic virus. He was quickly promoted to Assistant Specialist in 1949. In the mid-1950s, Frank was transferred by the University to work on lettuce disease and nutrition problems in the cool, coastal Salinas Valley, but he yearned to be on campus and was transferred in the early 1960s to Davis where he began to work on the breeding and genetics of cantaloupe and honeydew melons adapted to California melon production areas.

Frank developed five melon bush breeding lines: U.C. SR-91 Bush, U.C. Top Mark Bush, U.C. Perlita Bush (Zink, 1978), U.C. Honeydew Bush (Zink, 1979a), and U.C. Crenshaw Bush (Zink, 1980a). He released 'U.C. Honeyloupe', an orange-fleshed honey dew (Zink, 1979b). He released 'U.C. West Side', which is resistant to crown blight, a disease of uncertain origin, and Verticillium wilt, and is adapted for Spring production in the desert valleys of California and summer production in the San Joaquin Valley (Zink, 1980b). Frank released three melon lines resistant to Fusarium wilt incited by Fusarium oxysporum f. sp. melonis Snyd. and Hans.: U.C. PMR 45 Fom-1 (Zink and Gubler, 1986), U.C. PMR 45 Fom-3 (Zink and Gubler, 1987a), U.C. Top Mark Fom-1 (Zink and Gubler, 1987b) and U.C. Top Mark Fom-3 (Zink and Gubler, 1987a). He was working earnestly on sulfur tolerance in melon when he retired December 1989, and intended to complete the genetic analysis during the ensuing years.

Frank was a great friend and colleague to many of us. His keen intellect brought new insights; his sense of humor was always refreshing. He was always willing to share his knowledge and experience. Many cucurbit researchers owe him a debt of gratitude for the learning experiences he provided to us – always in a humorous vein. He was truly one of those

"unforgettable" characters who enrich our existence and make the world a better place.

The following obituary was published in the "The Davis Enterprise," October 23, 2001, (Yolo County) 315 G Street, Davis, CA 95616.

"Frank William Zink Jr. died peacefully in his home Oct. 13, 2001, after a lengthy illness. He was 78 years old.

He was born on June 17, 1923, in Pullman, Wash. He was a graduate of UC Davis and contributed 42 years of invaluable research as a plant breeder for the UC Davis Vegetable Crops Department, retiring on March 2, 1990.

He received recognition and honors for his melon research by the California Melon Research Board and appreciation as a plant breeder for his productive lettuce research by the Iceberg Lettuce Research Advisory Board. He published many research papers in his field. He had also served in the U.S. Navy in World War II.

After retirement, he enjoyed sailboating, gardening and traveling with his wife.

He is survived by Mary, his wife of 28 years and a resident of Winters; sister Miriam Coleman and husband Sam of Pasadena; son Howard Zink and his wife June and granddaughter Kaili of Fresno; son Ted Zink and his wife Cheryl; and stepgrandchildren Tami and Carri of Palmdale; stepdaughter Claudia Smyth of Winters; and stepgrandson Daniel Smyth of Woodland."

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Zink, F. W. 1979b. 'U.C. Honeyloupe' muskmelon. HortScience 14:549.

Zink, F. W. 1980a. U.C. Crenshaw Bush breeding line. HortScience 15:318-319.

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Zink, F. W. and W. D. Gubler. 1987b. U.C. Top Mark Fusarium wilt-resistant (*Fom-l*) muskmelon breeding line. HortScience 22:1342.

#### In Memoriam Dermot P. Coyne: 1929-2002

Timothy J Ng, University of Maryland, College Park, Maryland 20742-5125

Dermot P. Coyne was born in Dublin, Ireland, in 1929 and came to the United States in 1954 to study plant breeding with H.M. Munger at Cornell University. Coyne earned his doctorate in plant breeding from Cornell in 1958, and in 1961 he accepted a "1-year" position in the Department of Horticulture at the University of Nebraska, where he ultimately spent the rest of his productive and illustrious career. He retired from the University of Nebraska as the George Holmes Distinguished University Professor of agronomy and horticulture in 2001, but continued to work one-quarter time there up until his death.

Coyne was probably best known nationally and internationally for his work with the genetics and breeding of dry beans. In the Spring 2002 issue of "Colorado Bean News" (published by the Colorado Bean Network, Colorado State University, Fort Collins CO), Coyne's accomplishments were described as follows:

"Coyne achieved many professional and technical successes throughout his career, developing several varieties of pinto, pompadour and great northern beans that were resistant to bean common mosaic virus, rust, common bacterial blight and other bacterial diseases, with architectural escape from white mold. Those developments helped feed people in such countries as the Dominican Republic, which has since developed self-sufficiency in bean production thanks in part to Coyne's work. Moreover his disease-resistant germplasm releases and varieties are parents of numerous bean varieties grown in hundreds of thousands of hectares in the Americas, Africa, Asia, and Europe.

His latest recognition by the international bean community occurred at the 2001 Bean Improvement Committee Annual Meeting in Fargo, North Dakota last November where he was the inaugural recipient of the Frazier-Zaumeyer Distinguished Lectureship. Dr. Coyne presented the keynote address entitled, 'Breeding and Genetics of Great Northern and Pinto Dry beans for Multiple Disease Resistance,

Adaptation, Seed Quality, Yield, and Plant Architecture.' The BIC community is very grateful that they had this opportunity to honor their dear friend who has imparted his experiences and wisdom over the past forty years."

Coyne was also deeply devoted to graduate education, and often considered his graduate students as his scientific progeny and his greatest contribution to the field of plant genetics and breeding. He once said "There's a great multiplier effect with graduate students and great pleasure in seeing them develop, mature and accomplish great things in their careers."

Of course, those of us in CGC also remember Coyne for his breeding accomplishments with winter squash, and his passion for cucurbits in general. Among his releases was 'Butter Bowl' – a novel, butternut-type winter squash that was almost completely spherical in shape. Some of us still remember him agonizing about whether to release it, because he felt it was "darn near ugly" despite the fact that it was of high edible quality and that its shape gave it great potential for cooking in microwave ovens.

In honor of Coyne's life-long accomplishments at the University of Nebraska, and aided by contributions from friends and colleagues, the university established the "Dermot P. Coyne Lectureship in Plant Breeding and Genetics" and the "Dermot Coyne Exemplary Service to International Students Award" programs in 2002.

## **Upcoming Meetings of Interest to Cucurbit Researchers**

Organization/Meeting	Dates	Location	Contact
Cucurbit Genetics Cooperative (in conjunction with the ASHS 2003 Centennial Conference)	3 Oct 2003 1:30 to 2:30 p.m.	Rhode Island Convention Center Room 550B Providence, Rhode Island, USA	Timothy J Ng  tn5@umail.umd.edu  http://www.umresearch.umd.edu/cgc/
Pickling Cucumber Improvement Committee (PCIC) (in conjunction with the 2003 Pickle Packers International Annual Meeting & Trade Show)	22 Oct 2003	Sheraton Hotel & Convention Ctr New Orleans, Louisiana, USA	James Adkins  adkins@udel.edu
Watermelon Research & Development Working Group (in conjunction with the 2004 Southern Association of Agricultural Scientists Meeting)	13-18 Feb 2004	Tulsa, Oklahoma, USA	Benny Bruton  bbruton-usda@lane-ag.org  http://www.lane-ag.org/H2omelon/watermelon.htm
8 <sup>th</sup> EUCARPIA Cucurbitaceae 2004	July 2004	Czech Republic	Aleš Lebeda  lebeda@prfholnt.upol.cz  http://www.cucurbitaceae.upol.cz/
2nd International Oil Pumpkin Conference (in conjunction with 8 <sup>th</sup> EUCARPIA Cucurbitaceae 2004)	July 2004	Czech Republic	Penelope Lichtenecker (pslicht@nextra.at) Harry Paris (hsparis@volcani.agri.gov.il) Tamas Lelley (lelley@ifa-tulln.ac.at) Thomas Andres (tom@andres.com)
3 <sup>rd</sup> ISHS International Symposium on Cucurbits	2005	Australia	Gordon Rogers gordon@ahr.com.au
Cucurbitaceae 2006	2006	North Carolina, USA	Gerald Holmes (Gerald_Holmes@ncsu.edu) Jonathan Schultheis (Jonathan_Schultheis@ncsu.edu) Todd Wehner (Todd_Wehner@ncsu.edu) http://cuke.hort.ncsu.edu/cucurbit/meetings/ccrbtceae06mtg.html

#### Dihaploid Female Lines, Mother Component of F<sub>1</sub> Pickling Cucumber Hybrids

#### Vesselina Nokolova, M. Alexandrova, and V. Stoeva

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**Introduction:** Haploid cucumber plants (*Cucumis sativus* L.) (n=x=7) have reduced fertility or often exhibit full sterility (Nikolova and Niemirowicz-Szczytt, 1995) The use of different techniques such as colchicine treatment (Nikolova and Niemirowicz-Szczytt, 1996), (Chen and Staub, 1997) and regeneration from callus (Faris et al., 1996) gives the possibility of creating fertile dihaploid (DH) genotypes. This is a prerequisite for stabilization of homozygous DH lines (Nikolova and Alexandrova, 2001), which are valuable starting materials for a heterosis breeding in cucumber.

For our cucumber genetic and breeding programs, it was necessary to evaluate the morphological traits of homozygous DH lines and to study the possibilities of including them in heterosis breeding; to evaluate the qualities of new  $F_1$  hybrids; and to estimate the size and structure of their fruit yield, compared to the yield of the standard Bulgarian variety Toni  $F_1$ .

Materials and Methods: Some morphological peculiarities and sex expression of five dihaploid lines of pickling cucumbers, Nos. 43, 44, 45, 50 and 51 were studied during 1999. Their F<sub>1</sub> hybids were obtained by crossing with lines highly-resistant to powdery mildew and tolerant to CMV, i.e., monoecious lines 8/9, 8/19, T/19 and K-2 cultivated under the greenhouse conditions during April-August 2000-2001. The development of the hybrids, sex expression and fruit morphology were evaluated during the growth of 50 F<sub>1</sub> plants. The marketable and total fruit yields were assessed by performing comparative variety experiments, using a block method in four replications. Toni F<sub>1</sub>, the standard pickling cucumber variety in Bulgaria, was used as a control. Harvesting was done every second day. The total yield was determined from the mass of the standard (length 3-12 cm) and non-standard fruits, and the marketable yield from extra, first and second quality fractions with fruit sizes of 3-6 cm, 6-9 cm and 9-12 cm, respectively.

**Results and discussion:** The DH pickling cucumber lines Nos. 43, 44, 45, 50 and 51 had well developed,

vigorous plants of gynoecious type and their flowers and leaves exhibited a morphology typical for diploid *C. sativus* genome. The fruits had light green color with light strips, warts and white spines.

The  $F_1$  hybrids between DH lines and lines 8/9, 8/19, T/19 and K-2 were vigorous, and of gynoecious type. At the start of growth, single male flowers at the bottom of the stem were observed. In some plants, the extremely high temperature during the summer, also provoked formation of male flowers on one or (rarely) more nodes of the central stem and the probably branches. This was due to heterozygosity in the F<sub>1</sub> and to the incomplete expression of the dominant genes determining female sex expression. In homozygous DH lines only female flowers were observed irrespectively, due to the temperature. unfavourable summer The fruit morphology typical for DH lines dominated in the F<sub>1</sub>.

The results from the comparative study of seven F<sub>1</sub> hybrids and the standard Toni F<sub>1</sub> are given in Table 1. The data in Table 1 shows that the  $F_1$  hybrids slightly exceed the standard in total yield (by 0.6-8.9%). This yield was lower (91.7%) only in the hybrid combination DH 51 x 8/9. Similar trends were observed when analyzing marketable yield. Data in the table demonstrates that in five hybrid combinations (DH 44 x T/19, DH 45 x T/19, DH 43 x 8/9, DH 51 x 8/19 and DH 50 x K-2), the yields from the fractions 3-6 cm (extra quality) and from 6-9 cm (first quality) had higher values compared to those in the standard. Two F<sub>1</sub> hybrids, DH 44 x T/19 and DH 45 x T/19, had the highest yield of extra and first quality fruits. They exceeded Toni F<sub>1</sub> by 33.4 and 20.5%, respectively, for fraction 3-6 cm, while for fraction 6-9 cm, it was 10.9 and 13.0%. In these two hybrids the total marketable produce (3-12 cm) was higher by 10.5 and 8.0%, respectively.

The differences between the new hybrids and variety Toni  $F_1$  were more clearly expressed in analyzing the structure of the marketable yield. It was established that in the  $F_1$  combinations DH 44 x T/19 and DH 45 x T/19, the extra and first quality fruits had a greater

Table 1. Results from comparative study of new F<sub>1</sub> hybrids of pickling cucumbers (average for the period 2000-2001)

		Marketal	Marketable yield from fruits with length	m fruits wit	h length		Total marketable	ırketable	Yield from fruits	m fruits	Total vield	vield
Hybrid	3-6  cm	5 cm	6-9  cm	cm	9 - 12  cm	2 cm	yield	eld	longer then 12 cm	n 12 cm	10141	yıcıu
TIYOTA	kg/ha	relative yield %	kg/ha	relative yield %	kg/ha	relative yield %	kg/ha	relative yield %	kg/ha	relative yield %	kg/ha	relative yield %
1. Toni F1 (st)	14649.7	100.0	29888.3	100.0	19472.2	100.0	64010.2	100.0	11160.3	100.0	91937.5	100.0
2. DH 43 x 8/9	16430.2	112.2	33769.4	113.0	20040.7	102.9	70240.3	109.7	10443.1	93.6	98766.6	107.4
3. DH 43 x 8/19	14593.5	99.6	32084.9	107.3	22532.2	115.7	69210.6	108.1	10548.5	94.5	94780.8	103.1
4. DH 44 x T19	19547.1	133.4	33136.3	110.9	18074.8	92.8	70758.2	110.5	11503.8	103.1	100123.5	108.9
5. DH 45 x T19	17652.3	120.5	33784.7	113.0	17679.9	90.8	69116.9	108.0	9010.3	80.7	96811.5	105.3
6. DH 51 x 8/9	14145.7	96.6	27419.7	91.7	16195.0	83.2	57760.4	90.2	9649.7	86.5	84327.2	91.7
7. DH 51 x 8/19	17063.0	116,5	31458.2	105.3	17534.1	90.0	66055.3	103.2	7968.6	71.4	92557.5	100.6
8. DH 50 x K2	15505.0	105.8	30743.5	102.9	19948.8	102.4	66197.3	103.4	13078.9	117.2	97726.6	106.3

proportion (74.4%) of marketable yield, and in compliance with this the hybrids exceed the variety Toni  $F_1$  (69.6%) by about 5%. The proportion of the second quality fruits (9-12 cm) in the formation of this yield was 25.6%.

The marketable yield structure was similar in other  $F_1$  hybrids, DH 51 x 8/19, DH 51 x 8/9, and DH 43 x 8/9, in which the yields from the 3-6 cm and 6-9 cm fractions were 73.5, 72.0 and 71.5% higher, respectively, from the standard produce. The better structure of marketable yield in the new  $F_1$  hybrids probably was due to the dihapliod mother components.

On the basis of the obtained results we consider that the use of homozygous DH lines in the heterosis breeding of pickling cucumbers raises the possibility of obtaining of higher fruit yields from extra and first quality fractions (3-6 and 6-9 cm). These fractions are the most desired ones for processing industry.

**Conclusions:** The dihaploid lines Nos 43, 44, 45, 50 and 51 are of gynoecious type, with good morphological characters of the fruits and could be used as mother components in the heterosis breeding of pickling cucumbers. The  $F_1$  hybrids of the dihaploid lines with monoecious lines – 8/9, 8/19, T/19 and K-2 – give higher total and marketable yield compared to the control Toni  $F_1$ . The marketable yield structure of the new hybrids is better. The hybrid combinations DH 44 x T/19 and DH 45 x T/19 are the most promising ones. Their yield from the extra fruit quality fraction (3-6 cm) exceeds the standard variety Toni  $F_1$  by 33.4 and 20.5%, respectively and from 6-9 cm fraction (first quality) - by 10.9 and 13.0%.

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# Enterobacter cloacae Bacterium as a Growth Regulator in Greenhouse Cucumbers (Cucumis sativus L.)

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**Introduction:** There is a great diversity of microorganisms inhabiting the plant rhizosphere and exerting an influence on their development. Some of them - the fungi Trichoderma sp. and Gliocladium sp., the bacteria Streptomyces griseoviridis and Pseudomonas putida are used for disease control in vegetable crops (3, 5, 8). It is known that the phytopathogene antagonists stimulate plant growth and increase plant yield (1). The bacteria from genus Enterobacteriaceae play an important role in the biocenose links in cereals by promoting nitrogenfixation (6). Enterobacter cloacae is described as an antagonist of Pythium sp., which causes cucumber root rot, and increases soil suppression in soils infected with Fusarium oxysporum. It can also be used as a seed treatment (4, 7). Possibilities for the use of *Enterobacter cloacae* for control of powdery mildew and downy mildew in greenhouse cucumbers have been studied (2). In this article we shall discuss the results from the investigation of the *Enterobacter* cloacae properties to stimulate cucumber growth.

Material and Methods: We multiplied the Enterobacter cloacae bacterium (isolate 1B) on an agar-meat infusion (AMI) at t +32°C within 72 hours. The bacterial suspension with a titre of 1.10<sup>10</sup> cells/ml was used for the pre-sowing soaking of seeds for 8 hours and for watering the soil after sowing by 5 l/m<sup>2</sup>. The germinated plant percentage, plant height two weeks after germination, stem height, leaf number and the phenophase one month after planting were recorded. In order to be determine the germination percentage, we used 100 seeds. We planted 20 plants on constant place for each treatment. In the control treatments we sowed seeds without preliminary soaking in the bacterial suspension and water for 8 hours. An analysis of variance of the data at P = 5% was made.

**Results and Discussion**: The results from the experiments showed that the soaking of cucumber seeds in the bacterial liquid for 8 hours before sowing stimulates germination (Table 1). It was established

that in the control treatment without preliminary seed soaking there were 73.0-80.0% germinated plants twenty days after the sowing. With soaking in water, there were 80.0-90.0% germinated plants, and in the treatment with soaking in bacterial suspension there were 86.0-95.0% germinated plants. Watering of the sown seeds with the bacterial suspension stimulated the germination as in the treatment with soaking of seeds in water. The effect of this treatment is manifested two weeks after germination (Figure 1). The analysis of the seedlings conditions two weeks after germination showed that in the treatment where the soil is watered with bacterial suspension, 55% of the plants had a stem height of 10-15 cm, 39% had a stem height over 15 cm, and only 6% had a stem height below 10 cm. Seedlings from the group with treated seeds also are near to these values. For comparison, in the control treatment 37% of the plants were below 10 cm in height, 13% were over 15 cm in height, and 50% were between 10 and 15 cm. Therefore the seedlings were bigger when the soil and seeds were treated with bacterial suspension. The measuring of some biometric plant indices one month after their planting in a constant place showed that in the treatments with processing of the seeds and soil, the plants are more vigorous and had larger vegetative mass (Table 2). The fruit formation in these treatments started earlier and the yield per plant was considerable higher compared to the treatments where the bacterial suspension had not been applied. The stimulation effect of the bacterium *Enterobacter* cloacae on growth and yield in greenhouse cucumbers probably is due to the ability of bacteria from this genus to produce extracellular polysaccharides, serving as growth regulators.

The results from these investigations expand the possibilities for the use of *Enterobacter cloacae* in greenhouse cucumbers. Preliminary soaking of cucumber seeds in bacterial suspension from *Enterobacter cloacae* ( $10^{10}$  c/ml) stimulates seed germination with 13 - 15% compared to the control. The bacterial suspension from *Enterobacter cloacae* 

Table 1. Effect of bacterial suspension on cucumber seed emergence.

<u>No</u>	Treatment	Percentage of eme	erged plants (%)
	Heatment	1 year	2 year
1	Seed soaking in bacterial suspension	86.0	95.0
2	Soil watering with bacterial suspension	80.0	90.0
3	Seed soaking in water	80.0	90.0
4	Seeds without preliminary soaking	73.0	80.0

Table 2. Effect of bacterial suspension on some biometric traits, phenophase and yield in cucumbers

No	Treatment	Stem height	Leaf number	Phenophase	Yield kg/plant
1	Seed treatment	70.5	12	Sets	6.20
2	Soil treatment	78.0	15	Sets	5.30
3	Free of treatment	55.8	8	Full anthesis	4.05
4	Soaking in water	60.0	10	Full anthesis	4.95

$$F_{table.} = 2.68$$
  $F_{empiric} = 30.0$   $P = 5\%$ 

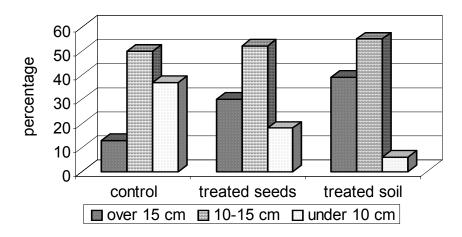


Figure 1. Percentage according to stem height two weeks after emergence.

(10<sup>10</sup> c/ml) used for preliminary soaking of the seeds for 8 hours or soil watering with 5 l/m<sup>2</sup> after sowing exert an influence on cucumber seedlings size. The treatment of seeds and soil with bacterial suspension from *Enterobacter cloacae* accelerates the elapse of phenophases and increases the yield from cucumbers.

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## Evaluation of Several Accessions and Wild Relatives of *Cucumis melo* against Cucumber Vein Yellowing Virus (CVYV)

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Cucumber vein yellowing virus (CVYV) was described in Israel in 1960 by Cohen and Nitzany. Later it was also observed in Jordan (1), Turkey (6) and recently, has been described affecting protected melon crops in Almería, Spain (3). Since the fall of 2000, the virus has spread drastically, causing serious economic losses in protected cucurbits crops in the Southeast of Spain.

The virus is transmitted by the sweet potato whitefly, *Bemisia tabaci* (Gennadius) in a semipersistant manner. Its host range seems to be restricted to cucurbits (5), although the virus has been also found affecting other species (4).

The symptomology is characterised by a yellowing of the leaf vein area, becoming systemic, with chlorosis of the youngest leaves. In extreme infections, plant stunting and fruit damage have been described. Fruits affected show chlorotic spots on their skin and/or internal necrosis.

The high virus transmission efficiency and the difficulty of *B. tabaci* control make necessary the search for genetic resistance/tolerance to the virus. Presently, there is not any total or efficient resistance to CVYV described in melon, and the behaviour of any wild *Cucumis* species against the virus is unknown. Once the mechanical inoculation technics were optimized and an accurate molecular diagnostic method established, we started an evaluation of the melon and wild *Cucumis* species collection maintained at the Experimental Station La Mayora, CSIC, Spain for CVYV resistance/tolerance.

During last year, 152 melon genotypes coming from different geographic areas have been evaluated as well as several accessions of the following species: *C. myriocarpus* (one), *C. metuliferus* (one), *C. africanus* (two), *C. zeyheri* (one), *C. dipsaceus* (two), *C. prophetarum* (one), *C. meeusii* (one), *C. ficifolius* (one), and *C. anguria* var *anguria* (one). The commercial cultivar Rochet has been used as a susceptible control in the experiments. Ten

plants/accession has been mechanically inoculated with the virus and two plants/accession were used as control.

The virus isolate, CVYV-A1LM, was obtained by the authors from a commercial cucurbit crop in Almería (Spain). The virus isolate was maintained in melon plants of 'Rochet'. Ten plants of each accession were inoculated mechanically by rubbing active carbon and carborundum-dusted cotyledons with from these infected foliar homogenized in 0.01 M K<sub>2</sub>HPO<sub>4</sub> buffer (pH 7). Plants were inoculated at the fully expanded cotyledon stage. Mock-inoculated and non-inoculated controls were included routinely. To ensure efficent virus inoculation, five days after the first inoculation, plants were reinoculated also in the cotyledons. Presence or absence of virus symptoms was recorded for each plant ten days after the second inoculation. The asymptomatic accessions and the plants with no clear symptoms were tested for the virus inoculated by molecular hybridization using a RNA digoxigenin probe containing a cDNA insert of 1.5 Kb.

All plants of the susceptible control, 'Rochet', showed systemic infection 12 days after artificial inoculation. Plant showed strong interveinal chlorosis in the second true leaf, more serious in the growing tips.

All plants of the 152 melon accessions artificially inoculated showed systemic response 12-15 days after inoculation. Two melon accessions, C-29 (Casaba Golden Beauty) and C-867 (Ambrus-Fele) showed a heterogeneous response to the virus, since only 3 plants showed symptoms of infection. If this response is confirmed, those melon accessions could be of interest in resistance/tolerance breeding.

Plants of *C. prophetarum* showed only local lesions in the cotyledons and no virus was found in the plant. The plants of the species *C. metuliferus*, *C. africanus*, *C. dipsaceus*, and *C. zeyheri* do not show any symptoms. But, after molecular hybridization, CVYV

was found in plants of *C. metuliferus*, *C. zeyheri* and one of the accessions of *C. africanus*. The rest of the plants were totally resistant. Strong sexual barriers between the wild species and *C. melo* make the use of these resistance sources very difficult.

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#### Correlation among Growth, Yield and Quality Characters in Cucumis melo L.

#### Taha, M.<sup>1</sup>, K. Omara<sup>2</sup>, and A. El Jack<sup>3</sup>

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Abstract: Correlation among growth, yield and quality attributes in Cucumis melo L. was estimated using the randomized complete block design Thirteen variable lines representing (RCBD). different melon types were used in this study. Positive and significant associations were found between the number of fruits/vine with the number of primary branches (+0.82), netting development with number of primary branches (+0.69), netting development with total soluble solids (+0.67), number of primary branches with number of secondary branches (+0.63), fruit weight with plant length (+0.59), earliness with flavor (+0.42), and netting development with flesh thickness (+0.39). Earliness with netting development (-0.82), total soluble solids with earliness (-0.71), and the number of primary branches with stem length (-0.55) were found to be negatively associated.

**Introduction**: Information on the correlation and linkage among different horticultural characteristics is of primary importance in the field of crop improvement. Linkage relationships can be used to increase breeding efficiency by allowing earlier selection and reducing plant population size during selection (5). Linkage has been reported between yellow fruit color and absence of sutures on the rind (3). Linkage studies were also undertaken with genes that control disease resistance, flower biology, or vegetative characters of *Cucumis melo* L. and eight linkage groups were identified (4). Much research in melon genetics is now focusing in gene mapping and development of marker-assisted selection (MAS).

In correlation studies with melon, yield per plant has been reported to be positively correlated with the number of fruits, average fruit weight, number of nodes on the main stem, stem length, internode length, and fruit shape index (8). Fruits per vine and fruit weight were positively correlated with yield and they were recommended as selection criteria for yield. High fruit density is correlated with thick flesh and small seed cavity in melon (7). The seed size of

melon cultivars differed significantly with fruit size (2). The main objective of this research was to study the correlation among different agronomic and horticultural characteristics of melon.

Materials and Methods: Thirteen breeding lines representing different melon types were grown in replicated trials to estimate correlation coefficients among different agronomical and horticultural characteristics in melon, using a RCBD design with three replicates. Experiments were conducted twice in winter seasons of 1999 and 2000. The land was disc plowed, harrowed, and then divided into growing units (10 x 2.5 cm<sup>2</sup>). Three seeds were planted per hole with 40 cm spacing between holes. Plants were thinned to two plants per hole 20 days after sowing. One dose of superphosphate was added pre-sowing and two doses of urea were added 15 and 45 days after sowing. The crop was irrigated at an interval of five to seven days in the first two months, after which the irrigation interval was increased gradually up to ten days at the maturity stage. Hand weeding and chemical spraying against insects and fungal diseases was done whenever necessary.

Plant Material: To diversify the plant material, thirteen lines representing different melon types were selected and used in this study. The plant material included: 'PMR 5', 'PMR 45', and 'Hale's Best Jumbo' (American types); 'Nantais Oblong', 'Cantalon', 'Vedrantais', 'Virgos', and 'Charentais' (Charentais or French types); 'Amarillo' and 'Rocket' (Spanish types); 'Ananas' (Ananas type); 'Ogen' (Ogen type); and 'U.G. 00171' (local Sudanese type).

Characters studied were: (a) number of fruits per plant; (b) earliness – ET. 40 (referring to the time elapsed until 40% of the fruits of each line in each replication were harvested (1)); (c) netting development of different fruits of a given line; (d) fruit weight; (e) total soluble solids; (f) flavor; (g) cavity/diameter ratio of the fruit; (h) average plant

Table 1. Simple correlation coefficients on pairs of different characteristics affecting yield and quality of melon.<sup>z</sup>

Character	Yield/plant	<b>Earliness</b>	Netting	SST	Flavor	Plant	Primary	Y.	Cav./Dia.	F. wt
			Development			Length	branches	branches		
Yield/plant		+0.07	50.0-	-0.37	+0.12	+0.05	** 57.0+	+0.1	-0.14	50.0+
Earliness	+0.09		-0.82**	-0.62**	+0.42*	+0.28	+0.03	+0.03	+0.38	+0.08
<b>Netting Development</b>	-0.01	-0.75**		+0.60**	-0.08	-0.22	+0.69**	+0.37	+0.39*	*85.0
TSS	-0.42	-0.71**	**76.0+		-0.36	-0.01	+0.08	+0.32	-0.04	-0.14
Flavor	+0.13	+0.37*	-0.13	-0.17		-0.15	+0.17	+0.45*	-0.01	40.0+
Plant Length	+0.14	+0.32	-0.15	-0.03	-0.03		-0.55**	-0.32	-0.20	+84.0+
Primary branches	+0.82**	+0.06	**85.0+	+0.13	+0.10	-0.43**		+0.47*	-0.03	+0.27
Secondary branches	+0.15	+0.04	+0.21	+0.20	+0.23	-0.12	+0.63*		-0.56**	-0.15
Cav./Dia.	-0.09	+0.34	+0.32*	-0.10	-0.16	-0.03	-0.01	-0.26		-0.02
F. wt	+0.01	+0.15	-0.19	-0.07	+0.03	+0.59**	+0.35	-0.27	-0.23	

<sup>&</sup>lt;sup>z</sup> Upper figures in the diagonal represent results of the first season, whereas the lower figures represent results of the second season. TSS = total soluble solids; Cav. = cavity; Dia. = diameter; F. wt = fruit weight.

Table 2. Correlations among pairs of the major characteristics in melon.

Character	Netting development	TSS (%)	Flavor	Earliness	Fruit weight
Netting development TSS (%) Flavor Earliness Fruit weight		+ H <sup>z</sup>	- L - M	- V.H - H + M	- H + L + L + L

<sup>&</sup>lt;sup>z</sup> Where V.H = very high coefficient of correlation (ranging from 1.0 to 0.8); H = high (ranging from 0.8 to 0.6); M = moderate (ranging from 0.6 to 0.3); L = lw (ranging from 0.3 to 0.1); and V.L = very low (< 0.1).

length; (i) number of primary branches; and (j) number of secondary branches. Simple correlation coefficients (6) were estimated among pairs of ten parameters.

Results and Discussion: The correlation coefficients among the major characteristics are given in Table 1. Results were almost the same for the two seasons with slight differences. A character by character examination showed that different characters were differentially associated with each other. Number of fruits/vine and earliness (ET. 40) were found positively correlated with plant length, number of primary branches, number of secondary branches, fruit weight, and flavor. They were found negatively correlated with netting development and TSS. Most of the coefficients of correlation showed nonsignificance except in the number of fruits/ vine with the number of primary branches (+0.82), earliness with flavor (+0.42), earliness with TSS (-0.71), and earliness with netting development (-0.82). Results indicated the importance of the number of primary branches as one of the main yield components. It was obvious that selection for earliness might negatively affect TSS and netting development of fruits with a moderate improvement on flavor.

TSS, number of primary branches, number of secondary branches, and cavity/diameter ratio were found positively correlated with netting development; however, flavor, plant length and fruits weights were found negatively correlated with netting development. The correlation of netting development was significant with the TSS (+0.67) and number of primary branches (+0.69).

Correlation between fruit weight and plant length was found significant (+0.59). This might be due to the

increase in the rate of photosynthesis as the plant length increased. Correlation among the major quality attributes in melon were demonstrated and symbolized in Table 2. In general, non-significant coefficients of correlation indicate that selection for the different characteristics could be done simultaneously and independently. Results indicate the possibility to produce high yielding and quality melon with moderate earliness and fruit size.

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# **Determination of Fruit Sampling Location for Quality Measurements in Melon** (*Cucumis melo* L.)

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Introduction. Fruit quality assessment and characterization is an important objective in many melon improvement programs. There are several simply inherited genes that control melon fruit ripening, shape and flesh color. Examples of such fruit quality genes are flesh color (gf), fruit abscission (Al-3, Al-4), mottled ring pattern (Mt-2), pentamerous (p; five carpels), presence of vein tracts on the rind (s), mealy flesh texture-2 (Me-2), sour taste (So), empty cavity (Ec), and white testa (Wt) (Pitrat., 2002). Possible linkages were found between wt-2 and s-3, and between Me-2 and Ec (Perin et al., 1999).

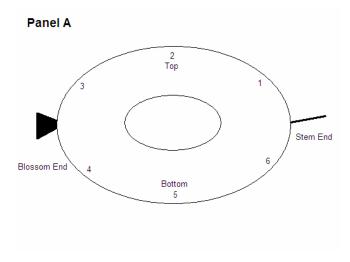
Our laboratories have been interested in collaborative mapping of yield components in a cross between a line designated as USDA 846 and 'Top Mark'. USDA 846 was derived from mating between an exotic accession obtained from Costa Rica and 'Top Subsequent backcrossing (BC<sub>2</sub> to 'Top Mark') and selfing (S<sub>4</sub>) of progeny from this initial mating were selected for fruit size and number, multiple lateral branching, and early crown-setting ability and self-pollinated to produce USDA 846. The fruit of line USDA 846 does not fit into a defined market class, having unique epidermal and mesocarp fruit characteristics. We are interested in improving the fruit quality of lines derived from USDA 846, and thus are developing strategies to evaluate specific fruit characteristics for selection and genetic mapping. We report herein the assessment of different fruit sampling locations for the determination of fruit firmness and total soluble solids in commercial hybrids, experimental lines, and a hybrid between USDA 846 and 'Top Mark' in two growing locations.

Materials and Methods. Five fruits of experimental melon lines (USDA 3022, USDA 3157) and cultivars ('Top Mark', 'Sol Real') were selected from a replicated melon trial (plants 30 cm apart in rows and rows placed on 2.1 m centers) in El Centro, Calif. (June 2003) for examination of fruit firmness and

total soluble solids concentration at no-slip, and halfand full-slip fruit maturity. In Hancock, Wisc. (September 2003), plants were grown under the same spacing, and three to five fruits from 'Esteem', 'Top Mark', 'Sol Dorado', and the USDA experimental hybrid 846 x Top Mark were sampled from a replicated yield trial for soluble solid concentration and firmness analysis at half- and full-slip maturity.

In California, 10 fruits from each entry were analyzed for total soluble solids evaluation using a digital BRIX refractometer (Model DR103L, QA Supplies, Norfolk, Va., USA) and firmness in using a fruit pressure tester, i.e., penetrometer, Model# FT 011, Effigi, Alfonsine, Italy. Five fruit were cut in transverse section and five were cut longitudinally, samples (~ 3 cm³) were taken from each fruit at specific locations (Figure 1). Based on results from the California location, sampling of three to five fruits at half- and full-slip maturity were performed using transverse sections only at Hancock (Figure 1, Panel B).

Results and Discussion. El Centro. Means and standard deviations for firmness and soluble solids concentration were variable and depended on relative maturity and position of sampling (Table 1). The mesocarps of fruits that did not detach from the stem. i.e., no slip (NS), were firmer than those at half-slip maturity, which were firmer than those at full maturity, i.e., full-slip. Standard deviations from the mean were generally lower in fruits sampled in transverse section when compared to those sampled in longitudinal section. Thus, sampling of fruit at Hancock was restricted to transverse sections at halfor full-slip maturity. After initial sampling of fruits for soluble solid estimations it became clear that sampling at positions 1 and 3 in transverse section or 2 and 5 in longitudinal section provided the most consistent results, i.e., mean and SD. Thus, only these measurements are reported herein.



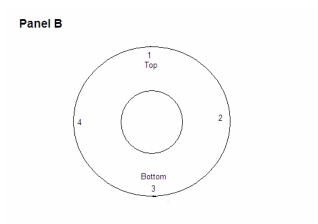


Figure 1: Diagrammatic representation of longitudinal (Panel A) and transverse (Panel B) sampling locations of melon (*Cucumis melo* L.) for mesocarp sugar content and pressure analysis (without epidermis).

Table 1. Firmness and soluble solids concentration of melon fruit grown at El Centro, Calif. cut in horizontal and transverse section and sampled at different mesocarp locations according to Figure 1 (data for 3 or 5 fruit per entry).

		SD		1.3	8.0	6.0	0.1	0.3			0.0	9.0	0.3	0.5	0.4			0.5	0.2	0.4	8.0	1.6			0.4	1.6	2.2		(continued)
ı (Brix)		Mean		10.8	7.7	10.2	5.4	5.1			6.9	7.8	4.9	6.7	8.2			15.1	12.9	14.1	14.3	0.6			13.7	13.7	13.1		(cont
centration		5																											
Soluble solids concentration (Brix)	Sampling position	3		6.6	8.3	9.5	5.4	4.9	9.7	2.3	6.9	7.3	5.1	6.3	7.9	6.7	1.1	14.7	13.0	14.4	14.9	10.1	13.4	2.0	13.9	12.5	14.6	13.7	T: I
Soluble	Samplin	2																											
		-		11.7	7.1	10.8	5.3	5.3	8.0	3.0	6.9	8.2	4.7	7.0	8.5	7.1	1.5	15.4	12.7	13.8	13.7	7.9	12.7	2.9	13.4	14.8	11.5	13.2	1.7
		$SD^3$		0.7	1.2	0.5	1.8	1.4			1.4	0.4	0.4	0.3	0.3			0.4	1.0	1.0	9.0	8.0	7.5		0.4	0.4	0.4		
bs.) <sup>2</sup>		Mean	section	5.8	6.4	5.3	6.9	7.9			8.1	6.1	6.7	9.8	7.7			8.0	8.0	8.9	6.7	7.1	8.0		5.7	5.2	4.7		
Fruit firmness (pressure to compress in lbs.) <sup>2</sup>		9	Transverse section																										
ire to con	uo	5	T																										
ss (pressu	Sampling position	4		5.0	5.3	5.4	7.8	7.3	6.2	1.3	6.4	5.8	10.0	8.2	8.0	7.7	1.7	8.5	8.7	6.2	10.0	9.9	8.7	1.6	5.5	4.6	4.5	4.9	9.0
uit firmne	Sampl	3		5.5	8.0	5.9	5.3	6.9	6.3	1.1	8.0	5.8	10.0	8.7	7.5	8.0	1.5	7.5	8.2	7.9	10.0	8.1	6.7	1.0	6.2	5.3	4.9	5.5	0.7
F		2		9.9	6.7	5.0	9.6	7.5	6.3	1.0	8.6	6.7	9.6	9.8	7.9	8.5	1.3	8.2	9.9	7.2	8.8	7.2	9.7	6.0	5.7	5.4	4.2	5.1	8.0
		_		0.9	5.7	4.8	0.6	10.0	7.1	2.3	8.0	5.9	9.1	0.6	7.5	7.9	1.3	7.8	8.5	5.7	10.0	6.4	8.3	1.7	5.2	5.5	5.0	5.2	0.3
	Relative <sup>1</sup>	maturity		$\mathrm{HS}^2$	$NS^3$	NS	SN	SN	Mean	SD	SN	SN	NS	SN	SN	Mean	SD	$FS^4$	FS	FS	HS	HS	Mean	SD	FS	$^{\mathrm{LS}}$	$\Sigma$	Mean	SD
		Entry		3022							3157							Top Mark							Sol Real				

Table 1. continued.

			I	Fruit firmness (pressure to compress in lbs.)	ess (pressu	rre to con	npress in	lbs.)			Soluble	solids co	Soluble solids concentration (Brix)	ı (Brix)	
	Relative			Sampl	Sampling position	u(					Sampling	Sampling position			
Entry	maturity		2	3	4	5	9	Mean	SD	-	2	3	5	Mean	SD
						Loi	Longitudinal section	ıl section							
3022	HS	6.4	5.4	9.9	9.9	4.5	0.9	5.9	8.0		9.9		6.1	6.4	0.4
	SZ	0.9	4.4	5.7	5.3	5.4	6.2	5.5	9.0		7.7		7.9	7.8	0.1
	NS	6.4	6.5	6.5	7.0	5.0	5.9	6.2	0.7		5.5		0.9	5.8	0.4
	SN	5.3	4.5	6.3	7.0	0.9	7.8	6.2	1.2		8.9		6.7	9.3	9.0
	$S_{N}$	8.5	4.5	5.8	5.8	5.8	6.4	6.1	1.3		11.6		10.0	10.8	1.1
	Mean	6.5	5.1	6.2	6.3	5.3	6.5				8.1		7.9		
	SD	1.2	6.0	0.4	8.0	9.0	8.0				2.3		1.9		
3157	NS	9.7	8.4	8.6	6.9	8.7	7.6	8.9	3.1		6.7		7.1	6.9	0.3
	NS	10.2	5.5	5.0	7.0	8.5	8.0	6.5	2.7		7.8		7.0	7.4	9.0
	SZ	9.8	5.4	6.2	8.5	9.4	10.0	6.9	2.3		8.3		7.4	7.9	9.0
	SZ	8.0	4.7	5.6	8.9	9.4	7.6	6.2	2.3		7.7		7.4	7.6	0.2
	NS	0.6	7.0	10.0	10.0	8.0	9.6	7.7	2.9		7.1		7.5	7.3	0.3
	Mean	8.7	6.2	7.1	7.8	8.8	0.6				7.5		7.3		
	SD	1.0	1.5	2.1	1.4	9.0	1.1				9.0		0.2		
Top Mark	HS	0.9	7.7	5.4	7.6	7.3	7.5	5.8	2.6		11.9		12.4	12.2	0.4
	FS	9.6	8.5	8.9	9.7	8.0	7.0	6.9	3.0		13.4		11.0	12.2	1.7
	FS	7.5	7.0	6.2	9.5	6.7	7.4	6.3	2.9		13.8		12.3	13.1	1.1
	FS	10.0	6.1	6.9	10.0	8.6	9.8	7.2	3.7		7.4		8.8	8.1	1.0
	HS	8.5	8.5	8.2	0.6	8.0	7.0	7.1	3.4		14.0		13.3	13.7	0.5
	Mean	8.3	7.6	6.7	8.7	8.0	7.5				12.1		11.6		
	SD	1.6	1.0	1.0	1.1	1.2	0.7				2.8		1.7		
Sol Real	FS	6.4	5.0	5.0	5.2	4.4	5.5	4.4	2.1		13.2		11.8	12.5	1.0
	FS	8.9	5.2	5.8	5.7	4.8	5.9	4.8	2.3		15.2		13.9	14.6	6.0
	FS	5.9	5.0	5.8	5.5	4.9	6.5	4.5	2.2		13.5		15.1	14.3	1.1
	Mean	6.4	5.1	5.5	5.5	4.7	0.9				14.0		13.6		
	SD	0.5	0.1	0.5	0.3	0.3	0.5				1.1		1.7		
1 10 1 10 1.	Gen satingation		J. 2000	tors and sate days	7. 0	, L	C.11 -1:				1	4	1-1F	£1	2

<sup>1</sup>HS = half-slip maturity reflects relative ease of detachment from fruit compared to full-slip maturity, NS = no-slip maturity indicates no tendency to detach from fruit, and FS = full-slip maturity reflects tendency to completely detach from fruit.  ${}^{2}Measurements$  taken with a penetrometer.  ${}^{3}SD=standard$  deviation.

Table 2. Firmness and soluble solids concentration of melon fruit grow at Hancock, Wisc. cut in horizontal and transverse section and sampled at different mesocarp locations according to Figure 1 (data for 3 or 5 fruit per entry).

Fruit firmness

			Pressu	ire to con	npress (ll	os.) <sup>2</sup>		Soluble so	olids conc	entration	(Brix)
	Relative <sup>1</sup>	S	ampling	position				Sampling	position		
Entry	maturity	1	2	3	4	Mean	$SD^3$	1	3	Mean	SD
Esteem	FS	3.5	4.2	4.2	3.6	3.9	0.4	6.5	8.2	7.4	1.2
	FS	4.0	4.2	4.7	4.2	4.3	0.3	6.9	6.5	6.7	0.3
	FS	3.8	3.5	3.6	3.8	3.7	0.2	6.9	6.7	6.8	0.1
	Mean SD	3.8	4.0	4.2	3.9			6.8	7.1		
Top Mark	FS	6.0	5.9	6.1	6.1	6.0	0.1	8.4	9.2	8.8	0.6
-	FS	6.2	7.2	6.8	6.9	6.8	0.4	9.4	8.9	9.2	0.4
	HS	6.4	6.2	7.7	6.6	6.7	0.7	8.8	9.5	9.2	0.5
	HS	7.5	7.6	6.8	7.7	7.4	0.4	6.5	6.9	6.7	0.3
	HS	5.5	5.4	5.0	6.6	5.6	0.7	6.5	6.1	6.3	0.3
	Mean SD	6.3	6.5	6.5	6.8			7.9	8.1		
Sol Dorado	FS	1.5	1.2	1.2	1.6	1.4	0.2	8.2	8.4	0.3	0.3

2.0

1.9

1.9

2.9

2.1

1.9

3.7

2.5

6.9

7.2

4.4

2.0

1.8

1.9

2.5

2.2

3.5

2.9

6.8

8.2

0.1

0.3

0.4

0.3

0.2

0.3

0.6

0.8

1.5

8.0

6.9

7.5

9.0

8.0

7.6

8.3

10.0

5.3

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7.0

6.0

7.8

5.7

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6.5

9.0

8.9

6.8

6.4

7.5

1.8

1.3

1.3

2.4

1.6

2.2

3.6

2.5

6.9

7.5

4.5

FS

FS

FS

FS

Mean

SD

FS

FS

FS

HS

HS

Mean

SD

2.0

1.9

2.2

2.4

2.0

2.4

3.1

3.8

7.7

4.3

2.0

2.0

2.0

2.3

1.9

2.2

3.6

2.9

5.7

10.0

4.9

846 x Top Mark

7.5

6.5

7.7

7.4

7.1

8.7

9.5

6.1

7.2

0.7

0.6

0.2

2.3

0.8

0.5

0.8

1.1

1.1

<sup>&</sup>lt;sup>1</sup> HS = half-slip maturity reflects relative ease of detachment from fruit compared to full-slip maturity, and FS = full-slip maturity reflects tendency to completely detach from fruit.

<sup>&</sup>lt;sup>2</sup> Measurements taken with penetrometer.

<sup>&</sup>lt;sup>3</sup> SD = standard deviation.

solids concentration varied with maturity (half- and full-slip having relatively high values compared to NS) and the hybrids examined. Standard deviations were highest in half- and full-slip fruits.

Hancock. Mean fruit firmness values varied (1.4 to 7.4) among the hybrids examined and standard deviations were relatively low ranging from 0.1 to 1.5 (Table 2). In contrast, soluble solid concentration values were less variable (6.1 to 9.5) and standard deviations were remarkably high ranging from 0.1 to 2.3 (Table 2).

These results indicate that fruit firmness may be a trait that, when measured under replication, could provide information for inheritance and genetic mapping studies. The precise estimation of total fruit soluble solids concentration is difficult, i.e., highly

variable, and placement of this trait on a genetic map will likely require the measurement of fruit having similar maturity (half- or full-slip) and the examination of several fruit, perhaps as many as 10, from a replication. Studies of inheritance will likely require relatively high replication (perhaps 6) and multiple measurements of plants within a plot (perhaps 5-10).

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#### Resistance to Fusarium Wilt and Root-knot Nematode in Watermelon Germplasm

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Watermelon is an important vegetable crop in the United States with close to 81,000 ha in production, which is concentrated in Texas, Georgia, and Florida (22). Root knot nematodes (*Meloidogyne* spp.) and Fusarium wilt (*Fusarium oxysporum* Schlechtend.:Fr. f.sp. *niveum* (E. F. Sm.) Snyd. & Hans.) race 2 can be serious problems in many areas, particularly in soils with a history of the diseases, which are persistent. Soil fumigants have proven to be effective in controlling these pathogens, but there is growing concern about their environmental and health effects as well as their costs. In addition, one of the most widely used fumigants, methyl bromide, is scheduled to be removed from the market in 2005 (23).

There is widespread interest in nematode and Fusarium wilt resistance in vegetable crops. Resistance to root-knot nematode has been reported in several different vegetable crops, however, there have not been widely accepted sources of root-knot nematode resistance with the exception of tomatoes (17). As an example, Khelu et al. (10) found tomato cv. Karla, cucumber cv. Capris, and pepper cv. Clovis to be resistant to root-knot nematodes (*M. arenaria* (Neal (Chitwood) and *M. javanica* (Treub) Chitwood)) compared to the susceptible entries.

Watermelons are attacked by several species of root-knot nematodes, and are susceptible to all 4 races of *M. incognita* (Kofoid & White) Chitwood), *M. javanica*, and both races of *M. arenaria* (16). Zhang et al. (24) found several watermelon lines to have resistance to root-knot nematodes including 'Crimson Sweet'. This finding is particularly interesting since 'Crimson Sweet' is generally considered susceptible. They used *M. incognita* race 2, *M. arenaria* race 2, and *M. javanica* in their screening. For resistance to be effective and widely adopted, it must apply to all species and races of root-knot nematode.

Boyhan et al. (3) found differences in susceptibility to *M. incognita* races 3 and 4 in watermelon, with subsequent testing showing some of this material

retaining a high level of resistance at 7000 eggs/plant. These promising results suggest that additional sources of resistance may be found in the USDA germplasm collection.

There are at least 3 races (0, 1, 2) of Fusarium wilt that attack watermelon (5, 11). Race 2 has recently received the most intense scrutiny because there were no known sources of resistance until Netzer and Martyn (13) reported race 2 resistance in PI 296341. In addition, Dane et al. (6) reported resistance to Fusarium wilt race 2 in PI 271769. These results suggest that other sources of Fusarium wilt race 2 resistance may be present in the USDA germplasm collection.

Root-knot nematode infection of watermelon has been shown to enhance the susceptibility of watermelon to Fusarium wilt even in those lines showing Fusarium tolerance or resistance (8, 19, 20).

The objective of this study was to evaluate the USDA watermelon germplasm collection for resistance to Fusarium wilt race 2 and root-knot nematode race 3 with an emphasis on finding resistance to both diseases in a single accession.

Materials and Methods: All screening was conducted in the greenhouses at the Bamboo Farm and Coastal Gardens Extension-Research Center in Savannah, GA. Greenhouse temperatures during the screening ranged from 20-35 deg. C. Accessions from 58 countries were evaluated in two different groups for Fusarium wilt resistance with 1,034 in the first screening and 377 in the second screening. Flats (28 x 56 cm) were filled with soil mix (Metromix 300, Scotts-Serria Products Co., Marysville, OH) and nine seed were planted per replication with three replications in a randomized complete block design (RCBD). Fusarium wilt race 2 inoculum (culture 62939, Amer. Type Culture Collection, Manassas, VA) was obtained from Dr. Fenny Dane (Auburn University, Auburn, AL) and sufficient quantity was grown for 2 weeks in an agitated potato dextrose

broth at 20 deg. C. The inoculum was adjusted to 1.5x10<sup>6</sup> microspores per ml with a hemacytometer. Seed were planted for the first screening on 7 October 1998 and each plant was inoculated during 26 to 28 October 1998 with 50 ul of inoculum injected into the plant's stem just above the soil line with a 50 unit insulin syringe (Becton Dickinson Co., Frankin Lakes, NJ). Plants were evaluated on 17 to 21 November 1998 on a 0-9 scale with 0, no sign of disease. The scale represents increasing levels of symptoms including discoloration and lesions on the stem and wilting of the plant culminating with death of the plant having a rating of 9. This scale was used comport with the Germplasm Resource Information Network maintained by the USDA.

The second screening was conducted in the same fashion as the first. Seed were planted on 27 January 1999, inoculated 26 February 1999, and evaluated on 1-2 April 1999. Individual plants, which showed no sign of disease were self-pollinated to produce seed for later studies.

Root-knot nematode race 3 was obtained from Dr. Richard Davis (University of Georgia, Athens, GA) and were increased on okra [Abelmoschus esculentus (L.) Moench] planted on Ocilla-Pelham-Albany association (loamy fine sand) soil. Excess soil was rinsed from the okra roots, which were then agitated in a 10% bleach solution for 4 minutes. The resulting solution was passed through 180 um, and 75 um screens, and the nematode eggs were collected on a 25 um screen. The nematode inoculum was adjusted to 10,000 eggs per ml after counting them on a hemacytometer.

Seed of 1,235 accessions were sown in 28 x 56 cm flats with #809 inserts (8 packs of 9 cells, 3.8x3.8x6.4 cm) filled with field soil. The design was a RCBD with three plants per replication and three replications. One ml of inoculum was applied to each seed at the time of planting.

Seed were sown and inoculated on 30 August to 9 September 2000 and plants were evaluated 25 September to 11 October 2000 in the greenhouse. The soil was washed from the roots of each plant and the roots were visually evaluated on a 0-3 scale with 0, no sign of galling, 1, up to 25% of roots galled, 2, >25% to 50% roots galled, and 3, >50% galling. Individual plants with no signs of disease were self-pollinated for further testing.

Results and Discussion: There were 1,411 watermelon accessions evaluated for Fusarium wilt and 1,235 evaluated for root-knot nematode resistance. PIs 534536, 386522, 270524, 543212, 482273, 385964, 512383, 299378, 482308, 169233, and 482299 had mean ratings for Fusarium wilt resistance of 3.5 or less (Table 1). In addition, individuals without symptoms were saved from 63 accessions for self-pollination (Table 2). Overall, the majority of the tested accessions for Fusarium wilt had ratings between 5 and 8 (Figure 1).

There were 10 PIs with root-knot nematode resistance ratings of 1.5 or less (Table 3). The majority of accessions tested for root-knot nematodes had ratings of 2-3 (Figure 2).

None of the PIs tested exhibited resistance to both Fusarium wilt and root-knot nematodes. Many PIs have been reported as sources of resistance to a variety of diseases including root-knot nematodes, gummy stem blight, anthracnose, watermelon mosaic virus, and zucchini yellows mosaic virus (1, 4, 3, 6, 7, 12, 14, 15, 18, 21) Testing of PIs, with previously reported resistance to various pathogens, did not result in those materials having favorable ratings for resistance to Fusarium wilt or root-knot nematode (Table 5). PI 482299, which had previously had reported resistance to ZYMV (15) had a rating of 3.6 for Fusarium wilt. This was the only PI in this study with the promise of multiple disease resistance.

Breeding for resistance to Fusarium wilt has been problematic because of the complex interaction of the host, pathogen, and soil environment. Hopkins et al. (9) found in monoculture that the level of Fusarium wilt changed dramatically from one year to the next and the specific watermelon cultivar appeared to have a suppressive effect on disease incidence in subsequent watermelon plantings. In addition, testing conditions also appear to have an effect. In previous tests of PI 296341-FR, a selection with resistance to Fusarium wilt, it did not perform any better than other cultigens tested (2). Similarly in this screening, PI 296341 with a mean rating of 6.3 appeared guite susceptible to Fusarium wilt. The testing method might play an important role in these contradictory results. Although we used a peat based artificial media in our test, a testing method that excludes potential effects of the media might have been a better choice. To confirm their work with PI 296341-

Table 1. Fusarium wilt evaluation of accessions with mean evaluations of 3.5 or lower.

Accession Number	Seed Source	Evaluation Mean <sup>a</sup>	Number of Plants Evaluated
PI 534536	Syria	0.9	9
PI 368522	Yugoslavia	2.7	26
PI 270524	Israel	2.9	25
PI 543212	Bolivia	3.0	35
PI 482273	Zimbabwe	3.0	18
PI 385964	Kenya	3.1	21
PI 512383	Spain	3.2	29
PI 299378	South Africa, Transvaal	3.3	23
PI 482308	Zimbabwe	3.4	27
PI 169233	Turkey	3.5	35
PI 482299	Zimbabwe	3.5	24

<sup>&</sup>lt;sup>a</sup>Scale: 0-9 with 0-no symptoms, 9-plant death.

Table 2. Plant introductions (Pls) from which plants without Fusarium wilt symptoms were saved.

PI 255137	PI 482350	PI 278010
PI 500327	PI 482273	Grif 1732
PI 385964	PI 438671	PI 278031
PI 167126	PI 438671	PI 368493
PI 482299	PI 521383	PI 357720
PI 190050	PI 482329	PI 182934
PI 181937	PI 482350	PI 368493
PI 482265	PI 249008	PI 183300
PI 487458	PI 482318	PI 344298
PI 534588	PI 482286	PI 370434
PI 482265	PI 249008	PI 306365
PI 357695	PI 378613	PI 357661
PI 181937	PI 378613	PI 278050
PI 181937	PI 299379	PI 278004
PI 532809	PI 378611	PI 368498
PI 490378	PI 482324	PI 532809
PI 378617	PI 457916	PI 270144
PI 490378	PI 482252	PI 482265
PI 487459	PI 378616	PI 482265
PI 482273	PI 295850	PI 482265
PI 326515	PI 512384	PI 378617

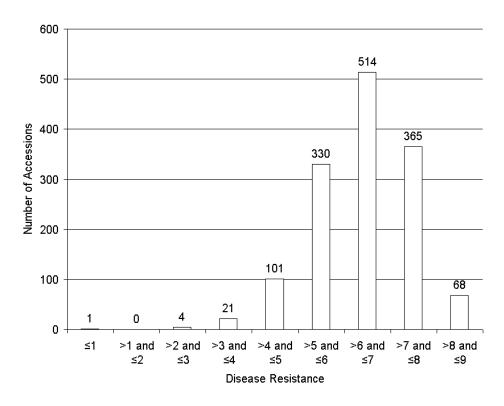


Figure 1. Frequency distribution of Fusarium wilt ratings of watermelon germplasm.

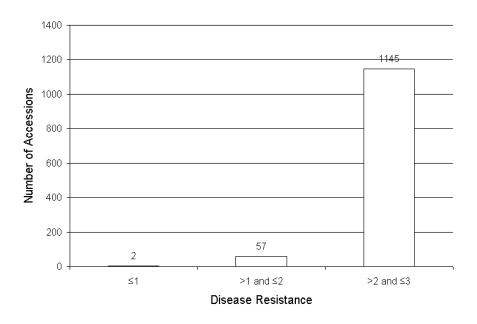


Figure 2. Frequency distribution of root-knot nematode ratings of watermelon germplasm.

Table 3. Root-knot nematode evaluation of accessions with mean evaluations of 1.5 or lower.

		Evaluation	Number of Plants
Accession Number	Seed Source	Mean <sup>a</sup>	Evaluated
PI 482271	Zimbabwe	1.0	4
PI 512833	Spain	1.0	3
PI 169276	Turkey	1.4	5
PI 169248	Turkey	1.5	4
PI 214316	India	1.5	3
PI 271770	South Africa, Transvaal	1.5	3
PI 278000	Turkey	1.5	4
PI 295845	South Africa, Transvaal	1.5	3
PI 357738	Yugoslavia	1.5	4
PI 482309	Zimbabwe	1.5	6

<sup>&</sup>lt;sup>a</sup>Scale: 0-3 with 0-no root galling, 3-severe root galling.

Table 4. Pls from which plants without root-knot nematode symptoms were saved.

		J 1	
PI 278057	PI 392291	PI 254743	PI 500347
PI 379246	PI 179878	PI 270140	PI 192937
PI 278057	PI 368511	PI 512342	PI 525096
PI 500307	PI 169299	PI 534598	PI 426625
PI 278057	PI 357705	PI 177322	PI 357697
PI 177329	PI 490382	PI 254743	PI 164247
PI 379233	PI 500336	PI 270140	PI 180426
PI 559993	PI 212094	PI 512342	PI 525096
PI 559993	PI 481871	PI 172791	PI 368527
PI 482269	PI 482331	PI 357738	PI 357697
PI 487458	PI 177325	PI 254743	PI 500335
PI 269680	PI 181936	PI 270140	PI 678615
PI 165448	PI 175659	PI 482248	PI 500336
PI 542114	PI 512393	PI 381708	PI 181937
PI 482300	PI 169250	PI 172801	PI 181937
PI 559993	PI 171582	PI 370423	PI 357667
PI 169262	PI 179234	PI 164685	PI 512342
PI 500319	PI 482282	PI 482281	PI 559992
PI 476329	PI 212209	PI 500312	PI 512833
PI 174106	PI 482291	PI 357709	PI 379255
PI 172786	PI 595203	PI 254623	PI 207473

Table 5. Reaction of watermelon germplasm to Fusarium wilt and root-knot nematodes with previous reports of disease resistance.

	Number of plants	evaluated	5	7	~	6	5	4	7	6	2	8	2	5		7	5	9	8	&	7		9	2	
Root-knot Nematode	Race $3^{b}$	Mean	2.2	3.0	2.9	2.9	2.6	3.0	3.0	2.8	2.0	2.9	3.0	1.8		2.3	3.0	2.5	2.9	2.9	2.6		3.0	3.0	
I	Number of plants	evaluated	23	26	34	16	22	19	35	24	23	26	12	12	15	25	24	25	26	27	26	25	44	25	
Fusarium Wilt	Race 2 <sup>a</sup>	Mean	7.5	5.9	6.4	7.2	7.2	9.9	5.0	6.4	6.3	5.2	4.3	5.8	4.9	5.7	3.6	7.6	4.9	4.5	0.9	5.9	6.4	4.9	
	Previous Published	Resistance	nematodes	GSB/Anthracnose	WMV-2	WMV-2	WMV-2	Fusarium race 2	Anthracnose	Anthracnose	Fusarium race 2	Anthracnose	Anthracnose	ZYMV Resistance	ZYMV Resistance	ZYMV Resistance	ZYMV Resistance	nematodes	nematodes	nematodes	nematodes	nematodes	Anthracnose	nematodes	
		Accession Number	PI 164247	PI 189225	PI 189316	PI 189317	PI 248178	PI 271769	PI 271775	PI 271778	PI 296341	PI 299379	PI 326515	PI 386025	PI 386026	PI 482261	PI 482299	PI 494815	PI 500327	PI 500329	PI 500335	PI 506439	PI 512385	PI 532811	

<sup>&</sup>lt;sup>a</sup>Fusarium Reaction: 0-9; 0-no symptoms, 9-dead plant <sup>b</sup>Nematode Reaction: 0-3; 0-no symptoms, 3-severe galling

FR (12) used three different methods of testing, root dip, tray dip, and infested microplots. Considering the number of accessions tested, it was not feasible for us to use multiple testing methods. In addition, we did not have the facilities to fumigate a large volume of media, which may have helped prevent possible interactions. However, even with fumigation, organic media have large numbers of high molecular weight organic compounds whose interaction with the plant and the pathogen are not clearly understood.

We did recognize some limitations with the root-knot nematode screening as well. Because our inoculum consisted of primarily M. incognita race 3, any PI exhibiting resistance would require further testing against other races and species to be truly useful. Because of the size of the experiment we had to increase inoculum under field conditions, which can result in the very real possibility of other races and/or species of root-knot nematodes being present. This we felt was not a problem because watermelon in general is known to be susceptible to all races and species of root-knot nematode. This study was to be an overall characterization of the collection for rootknot nematode resistance rather than specifically addressing a particular root-knot nematode species or race reaction. In addition, we did not have the facilities to sterilize a large quantity of field soil at the particular site for this evaluation, which resulted in the possibility of contamination by other pathogens. We did not see any specific additional disease problem in the root-knot nematode evaluation and we did see well developed galling across most of the material indicating the inoculation was successful.

PIs with previously reported root-knot nematode resistance did not exhibit resistance in this screening. Overall, we were very conservative in our assessment of the accessions, not wishing to erroneously identify a PI with resistance or tolerance that did not exist, which may in part explain these results. In addition, environmental conditions could have played a role as well as the material itself. As these accessions are grown out for increasing seed stocks, crosspollination, inadvertent selection, and the small populations involved, all can contribute to changes in the collection over time.

The results of these screenings may indicate that genetic drift or a high level of heterozygosity is present in the PI collection. Our results were with relatively few individuals, which makes it difficult to draw any conclusive statements about genetic drift or heterozygosity but in light of our results compared to other studies suggest these may be factors. Researchers have been concerned about this for years. How stable is the collection, how much seed should be used to increase and maintain the collection, are we loosing diversity over time? Studies such as these in the aggregate may indicate genetic drift or a greater level of heterozygosity than previously thought. Modern tools for studying the genetic complexity and diversity will have to be employed to answer these questions.

In conclusion, our testing showed a great deal of diversity in the collection to Fusarium wilt and root-knot nematodes. Several PIs showed resistance to either Fusarium wilt or root-knot nematode; none, however, exhibited dual resistance.

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### Mass Production of Gummy Stem Blight Spores for Resistance Screening

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In recent years, much work has been done in breeding cucurbits for resistance to gummy stem blight caused by Didymella bryoniae. Researchers have screened for useful sources of resistance in watermelon (Citrullus lanatus) squash (Cucurbita spp.) (9), melon (Cucumis melo) (10), and cucumber (Cucumis sativus) (7). Germplasm screening often involves a combination of field and greenhouse inoculations, requiring many spores to ensure proper disease intensity. However, researchers have found different spore concentrations to be useful in screening cucurbits for resistance to gummy stem blight among experiments and species: 10<sup>5</sup> spores/ml in watermelon (1), squash (9), and melon (10);  $10^6$  spores/ml (3, 4, 6, 8) or  $10^7$ spores/ml (5) in cucumber. The production of a high number of spores of gummy stem blight often results in the preparation, infection, and harvest of hundreds of Petri plates per hectare of field to be inoculated.

In our study, we tested a new container to be used as an alternative to Petri plates for mass production of spores of gummy stem blight. We also tested two different methods of infection of the culture medium:

1) transfer of a plug of agar from a fungal culture, and 2) preparation of an inoculum solution from a previous culture and using that to flood the medium.

**Methods:** The experiments were conducted in the Horticultural Science pathology laboratory at North Carolina State University. We used a strain of *D. bryoniae* isolated in 1998 from diseased cucumber tissues harvested from naturally infected plants in Charleston, SC. The experiment was a split-plot treatment arrangement in a randomized complete block design, with two types of containers (20 Petri plates vs. 1 Nalgene autoclavable pan), two infection methods (potato dextrose agar plugs vs. inoculum solution), and four replications.

For all treatment combinations, *D. bryoniae* was grown on 50% potato dextrose agar (PDA) and incubated for 4 weeks at  $24 \pm 2^{\circ}$ C under alternating periods of 12 hours of fluorescent light (40 to 90  $\mu$ mol·m<sup>-2</sup>·sec<sup>-1</sup> PPFD) and 12 hours of darkness.

Two different types of containers were used: plastic Petri plates (90 mm internal diameter) and autoclavable Nalgene autoclavable pans (420x340x120 mm). We poured the PDA into the Petri plates after sterilizing it in autoclave, while we autoclaved it directly into the final container for the Nalgene autoclavable pans. The Nalgene autoclavable pans were covered with aluminum foil for sterilization in autoclave and inserted in a sterile plastic, sealable, and transparent bag (Tower Self-Seal TSM, 24x30 inch or 61 x 76 cm, Allegiance Healthcare Corporation, McGaw Park, IL) after infection.

In our experiment each plot included 20 Petri plates or 1 Nalgene autoclavable pan, each containing 1 liter of PDA. We infected the Petri plates and Nalgene boxes following two different methods: 1) we transferred 5mm square plugs of PDA infected by gummy stem blight from previous cultures, and 2) we harvested spores from one Petri plate from previous cultures using a sterile glass rod and deionized water and then flooded the fresh medium with the inoculum solution (the inoculum solution was adjusted to a volume of 200 ml with sterile deionized water). The initial inoculum for each plot was prepared from a single Petri plate (20 plugs of PDA or 200 ml of inoculum solution). When Petri plates were infected using the solution rather than a PDA plug, we poured the solution into one Petri plate, recollected it in a sterile beaker, and then poured onto the next Petri plate until all 20 Petri plates of the plot were infected. With the Nalgene autoclavable pan, we poured the solution into the box and then recollected it in the beaker and disposed of it, since one pan was one plot.

At the end of the 4 week subculture, we harvested the spores from all plots separately: each Petri plate was flooded with 5 ml of sterile deionized water using a sterile repipette, while each Nalgene autoclavable pan received 100 ml of sterile deionized water (corresponding to 5 ml x 20 Petri plates) using a sterile graduated cylinder. The PDA surface was scraped with a sterile L-shaped glass-rod and the solution was then filtered through 4 layers of sterile

Table 1. Mean squares for spores production of gummy stem blight (total time, stock concentration, and total inoculum produced).<sup>z</sup>

Sources	D	Total time	Stock conc.	Total inoculum
of variation	F	(seconds)	(1,000 sores/ml)	(liters)
Replication	3	148.6 ns	1580600039.0 ns	38.5 ns
Type of container	1	525262.6 **	2979112852.0 ns	250.7 *
Inoculation method	1	2047.6 *	10566555039.0 **	200.9 *
Container x method	1	40100.1 **	108550352.0 ns	9.3 ns
Error	9	373.9	955730456.0	26.3

ns, \*, \*\* indicates F value non-significant or significant at a p-value <0.05 or p-value <0.01, respectively.

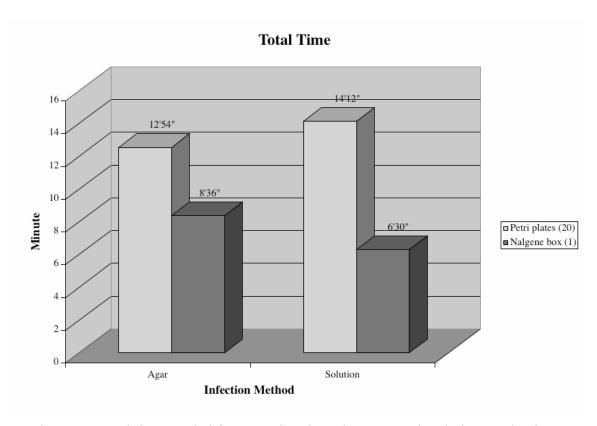


Figure 1. Total time needed from starting the culture to stock solution production.

<sup>&</sup>lt;sup>2</sup> Data are means of 4 replications, 2 types of container (Nalgene box vs. Petri plates), and 2 inoculation methods (infected PDA plugs vs. inoculum solution).

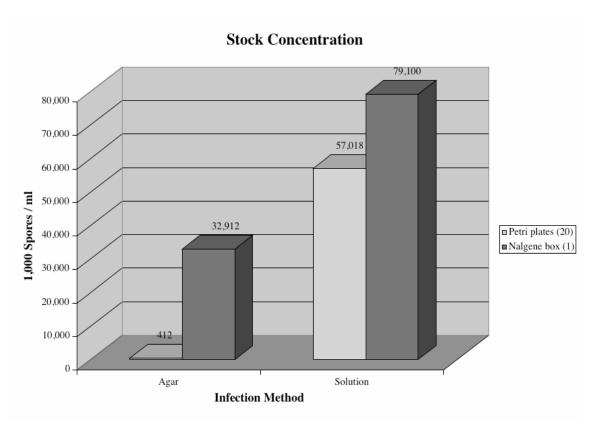


Figure 2. Concentration of the stock solution (1,000 spores/ml).

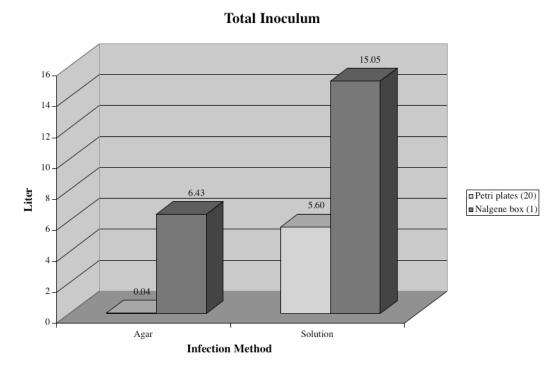


Figure 3. Total inoculum produced at a final concentration of  $5x10^5$  spores/ml.

cheese-cloth into a 100 ml sterile beaker to remove dislodged agar and part of the mycelium. For each stock solution we measured the volume using sterile graduated cylinders (one per solution), and the spore concentration using a hemacytometer. For each stock solution, we calculated the volume of final inoculum that could be produced, assuming a final concentration of  $5 \times 10^5$  spores/ml (2).

We timed each of the following stages of spores production: a) preparation of 1 liter of PDA (including pouring into a flask for the Petri plates plots or into the Nalgene autoclavable pans directly). b) pouring of the PDA into the final container after sterilization in autoclave (pouring time = 0 for the Nalgene autoclavable pans), c) infecting each plot (including spore harvest, preparation of the inoculum solution, or preparation of the PDA plugs, and collocation of the Petri plates on the trays constituting each plot), d) harvesting of the spores, e) cleaning of the container after spore harvest (cleaning time = 0 for the Petri plates, being disposable). We did not consider as factors contributing to the total amount of time needed to produce a final inoculum solution from each plot the following: 1) sterilization in autoclave (it does not require labor and varies depending on the size of the autoclave, the temperature at the beginning of the cycle, and the temperature of the inlet water), 2) counting of the spores at the microscope with the hemacytometer (it is fairly consistent from one to another, and depends mostly on the ability of the counter).

Results: The largest source of variation in the analysis of variance was the type of container. Infection method had a smaller effect, and the interaction with type of container was variable. The infection methods had the only large and significant effect on stock concentration and almost the same effect of the type of container on the total volume of final inoculum produced. No effect was due to interaction between type of container and infection method for the two latter traits (stock concentration and total inoculum produced). Replication was not significant for any of the traits (Table 1).

Considering the time requirement, the stock concentration, and the final inoculum volume produced, the best method for spore production of gummy stem blight was the Nalgene autoclavable pan infected with the inoculum solution of spores (Fig. 1, 2, 3). The Nalgene autoclavable pan required

less time than Petri plates and produced more spores and more inoculum, regardless of infection method.

During the 4 weeks of subculture, we observed that the Nalgene autoclavable pans and Petri plates infected with the inoculum solution were ready for spore harvest at 10 days after infection, while the containers infected with the PDA plugs needed 4 weeks or more to colonize the PDA surface with mycelia.

The fastest growth of mycelia on PDA infected with a spore suspension rather than infected PDA plugs might be explained by the higher number of spores delivered with the first method in addition to the more uniform distribution of the spores on the PDA More difficult to explain is the higher efficiency of the Nalgene autoclavable pans vs. the Petri plates: we hypothesize that the higher volume of air present in the box helps to disperse volatile autoinhibitory compounds eventually produced by the fungus. It may also be that the temperature and humidity in the box are more stable than in the Petri plates, due to the larger air volume and to the larger surface area for exchange between the medium and the air. Further studies might be directed to test the same technique on the cultivation of other fungi and on the understanding of the reasons for the higher efficiency in spore production.

Conclusions: Our experiment indicates that the fastest, cheapest, and easiest method for the production of large quantities of gummy stem blight spores and final inoculum should require: 1) the use of Nalgene autoclavable pans, sealed with a sterile transparent bag, instead of Petri plates, and 2) the infection of the PDA with a suspension of spores in sterile deionized water from previous cultures.

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### Naming the Gene Conferring Resistance to Cool Temperatures in Watermelon

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Many cultivars and landraces of watermelon (Citrullus lanatus L.) are susceptible to seedling injury when grown at cool temperatures (<20°C). Symptoms are chlorotic cotyledons and a foliar mosaic consisting of scattered, irregular, whitish flecks and patches, caused by a partial chlorophyll deficiency (2). The intensity of the symptoms depends on cultivar, with some more affected than others. Warmer temperatures are conducive to a full recovery of affected plants, but their initial retarded growth usually delays fruit maturity. However, there are resistant landraces of C. lanatus able to continue normal growth even when exposed to cool temperatures.

The widespread use of cool-resistant watermelon cultivars can secure significant economic advantages, since they can be planted several weeks earlier than the cool-susceptible cultivars. Although cool-resistant plants will not survive persistent frosts, they usually recover from damages caused by early frosts that are light.

Resistant and susceptible lines were used to determine the inheritance of cool temperature resistance (3). Populations were developed from

crosses and reciprocal backcrosses between the coolsusceptible cultivar New Hampshire Midget and the cool-resistant line PP261-1 (a single plant selection of PI 482261 from Zimbabwe). It was determined that cool sensitivity is related to the single recessive gene *slv* (seedling leaf variegation), and that resistance is conferred by a dominant gene. I propose the symbol *Ctr* (cool temperature resistance) for that gene. Plants of PP261-1 also have a single recessive gene (*zym*) conferring resistance to the Florida strain of zucchini yellow mosaic virus (1).

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## Polygenic Inheritance of Some Vine Traits in Two Segregating Watermelon Families

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Cultivated watermelon is *Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai. The genetics of watermelon have been widely studied and many genes have been identified (1-4, 6). Although the fruit of citron (*C. l.* var. *citroides*) accessions are hard and bitter, the close relative of watermelon is a valuable source of new traits for plant breeders. For example, the vines of citron often are more vigorous and disease resistant than those of cultivated watermelon. We were interested in vine traits of citron such as straight stems, large leaves, rounded leaf blades, and large plant size.

The objective of this study was to measure the inheritance of the vine traits mentioned above. The research was done using two segregating families of watermelon crossed with citron.

**Methods:** The families used in the experiment were developed from the parental crosses 'Calhoun Gray' x PI 490383 (*C. l.* var. *lanatus* x *C. l.* var. *lanatus*) and 'Allsweet' x PI 482283 (*C. l.* var. *lanatus* x *C. l.* var. *citroides*). Six generations (P<sub>a</sub>, P<sub>b</sub>, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>P<sub>a</sub>, BC<sub>1</sub>P<sub>b</sub>) were developed in 2000 to 2002 at the Horticultural Science greenhouses, North Carolina State University.

'Calhoun Gray' and 'Allsweet' were used as parents because their genetic background is common to most of the commercial cultivars available on the market. The citron accessions PI 490383 and PI 482283 were different from the watermelon cultivars for stem pattern (straight vs. zigzag), leaf size (large vs. small), leaf margin (rounded vs. pointed), and plant size (large vs. small) (Figures 1, 2, 3, 4).

The field test was run at the Horticultural Crops Research Station in Clinton, NC in Summer, 2002. The experiment was run with two sets each of two families tested in one field. The data were analyzed for segregation analysis and goodness of fit using the SAS Statistical Package (SAS Institute, Cary, NC) and the SASGENE 1.2 program (5).

**Results:** The  $F_1$  of both families segregated for the following phenotypic traits that were rated and studied: 1) stem pattern (straight vs. zigzag), 2) leaf size (large vs. small), 3) leaf margin (rounded vs. pointed), and 4) plant size (large vs. small) (Tables 1-4). In two cases (leaf size in 'Calhoun Gray' x PI 490383 and leaf margin in 'Allsweet' x PI 482283), the  $F_1$  fit the 1:0 ratio expected in the hypothesis of single gene inheritance. However, all the  $F_2$  and BC segregation data showed  $X^2_{F2} > X^2_{(0.05;1)}$  (Tables 2, 3). Thus, there were no obvious single qualitative genes controlling phenotypic expression. The four traits had mostly polygenic inheritance.

Further experiments specifically designed for a quantitative analysis would be useful to gain a better understanding of the variance components and heritability for these traits in watermelon.

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Figures 1 and 2. Zig-zag and straight stems.



Figure 3. Small and large leaves.

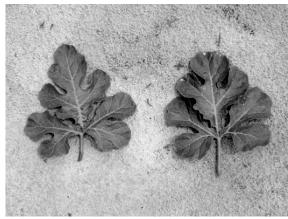


Figure 4. Pointed and rounded leaf margins.

Table 1. Single locus goodness-of-fit-test for stem pattern in watermelon.<sup>z</sup>

Generation	Total	Zig-zag	Straight	Expected	$X^2$	df	P-value
'Calhoun Gray	y' x PI 4903	83					
$P_a$	3	3	0	-	-	-	-
$P_b$	5	0	5	-	-	-	-
$F_1$	13	9	4	-	-	-	-
$F_2$	142	81	61	N/A	-	-	-
$BC_1P_a$	43	23	20	N/A	-	-	-
$BC_1P_b$	29	14	15	N/A	-	-	-
'Allsweet' x PI	482283						
$P_a$	5	5	0	-	-	-	-
$P_b$	5	0	5	-	-	-	-
$F_1$	9	3	6	-	-	-	-
$F_2$	138	73	65	N/A	-	-	-
$BC_1P_a$	41	22	19	N/A	-	-	-
$BC_1P_b$	34	14	20	N/A	-	-	-

<sup>&</sup>lt;sup>2</sup> Data are ratings from two families of *Citrullus lanatus*: 'Calhoun Gray' x PI 490383 and 'Allsweet' x PI 482283

Table 2. Single locus goodness-of-fit-test for leaf size in watermelon.<sup>z</sup>

Generation	Total	Large	Small	Expected	$X^2$	df	P-value
'Calhoun Gray	y' x PI 49038	3					
$P_a$	3	0	3	-	-	-	-
$P_b$	5	5	0	-	-	-	-
$F_1$	13	13	0	-	-	-	-
$F_2$	142	95	47	3:1	4.97	1	0.02
$BC_1P_a$	43	36	7	1:0	0.00	1	1.00
$BC_1P_b$	29	22	7	1:1	0.00	1	1.00
'Allsweet' x PI	482283						
$P_a$	5	0	5	-	-	-	-
$P_b$	5	4	1	-	-	-	-
$F_1$	9	8	1	-	-	-	-
$F_2$	138	65	73	N/A	-	-	-
$BC_1P_a$	41	20	21	N/A	-	-	-
$BC_1P_b$	34	8	26	N/A	-	-	-

<sup>&</sup>lt;sup>z</sup> Data are ratings from two families of *Citrullus lanatus*: 'Calhoun Gray' x PI 490383 and 'Allsweet' x PI 482283

Table 3. Single locus goodness-of-fit-test for leaf margin in watermelon.<sup>z</sup>

Generation	Total	Rounded	Pointed	Expected	$X^2$	df	P-value
'Calhoun Gray	y' x PI 4903	83					
$P_a$	3	0	3	-	-	-	-
$P_b$	5	5	0	_	-	-	-
$F_1$	13	8	5	-	-	-	-
$F_2$	142	93	49	N/A	-	-	-
$BC_1P_a$	43	34	9	N/A	-	-	-
$BC_1P_b$	29	20	9	N/A	-	-	-
'Allsweet' x PI	482283						
$P_a$	5	0	5	_	-	-	-
$P_b$	5	5	0	-	-	-	-
$F_1$	9	9	0	_	-	-	-
$F_2$	138	86	52	3:1	11.84	1	0.00
$BC_1P_a$	41	33	8	1:0	0.00	1	1.00
$BC_1P_b$	34	31	3	1:1	0.00	1	1.00

<sup>&</sup>lt;sup>2</sup> Data are ratings from two families of *Citrullus lanatus*: 'Calhoun Gray' x PI 490383 and 'Allsweet' x PI 482283

Table 4. Single locus goodness-of-fit-test for plant size in watermelon.<sup>z</sup>

Generation	Total	Large	Small	Expected	$X^2$	df	P-value
'Calhoun Gray	y' x PI 49038	3					
$P_a$	3	1	2	-	-	-	-
$P_b$	5	5	0	-	-	-	-
$F_1$	13	9	4	-	-	-	-
$F_2$	142	78	63	N/A	-	-	-
$BC_1P_a$	43	26	17	N/A	-	-	-
$BC_1P_b$	29	17	11	N/A	-	-	-
'Allsweet' x PI	482283						
$P_a$	5	2	3	-	-	-	-
$P_b$	4	3	1	-	-	-	-
$F_1$	9	5	4	-	-	-	-
$F_2$	136	75	61	N/A	-	-	-
$BC_1P_a$	41	29	12	N/A	-	-	-
$BC_1P_b$	34	20	14	N/A	-	-	-

<sup>&</sup>lt;sup>2</sup> Data are ratings from two families of *Citrullus lanatus*: 'Calhoun Gray' x PI 490383 and 'Allsweet' x PI 482283

# Diagnostic Chloroplast DNA Haplotypes to Distinguish Cultivated from Citron Type Watermelon

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Plant molecular evolution has been dominated by studies of the chloroplast genome (cpDNA). There are several reasons for this focus on a single organelle which accounts for less than 0.1% of the genetic complement of plants. cpDNA is an abundant component of total cellular DNA, which has facilitated the early molecular characterization of its genome. The cp genome is small, being 155 kb in Citrullus (2) and most genes are essentially single copy. cpDNA has a conservative mode of nucleotide substitution and slow rate of molecular evolution which are ideal for the study of plant phylogenetic relationships. The high degree of sequence conservation has facilitated the use of PCR primers in unrelated species (7). Universal primers have been constructed on the basis of conserved sequences of cpgenes and used to amplify the DNA located between the primer binding sites (6, 7). This study was undertaken to detect phylogeographic patterns among a phenotypically and geographically diverse array of cultivated and wild types of Citrullus lanatus.

Methods: Seeds of more than 20 different Plant cultivated Introduction (PI) accessions of watermelon, C. lanatus var. lanatus, and more than 45 citron types, C. lanatus var. citroides PIs, originating from many different countries, were obtained from the S-9 PI collection in Griffin, GA or from The Cucurbit Network (Table 1). Seeds from the following watermelon cultivars: 'AU-Producer', 'Blackstone', 'Crimson Sweet', 'Dixielee', 'Ferrari', 'Mickylee', 'Jubilee'. 'Klondike'. 'Mardi Gras'. 'Royal Sweet', 'Peacock', 'Regency', 'Sangria', 'Starbrite', and 'Stone Mountain' were obtained from commercial breeding companies. Cultivars and accessions were selected based on dendrograms using polymorphism (4) or RAPDpolymorphisms (5). DNA was extracted from young leaves or seeds using the Qiagen plant DNeasy kit (Qiagen, Valencia, CA). Chloroplast specific primer pairs were used to amplify the corresponding regions PCR products were digested with several different restriction enzymes and informative regions were sequenced. DNA sequences of informative primer pairs and amplification reaction conditions are given in Table 2.

**Results:** Even though variability within *Citrullus* lanatus was very low, unique cultivar-specific patterns were detected using the following primer / restriction enzyme combination: ndhF 1955-607R and AluI for C. lanatus var. lanatus; ycf6-psbM and Taal for C. lanatus var. citroides. When these cpDNA regions were sequenced, the differences were due to single nucleotide substitutions at restriction enzyme recognition sites. No polymorphism was detected between phenotypically diverse C. lanatus var. lanatus or C. lanatus var. citroides sequences. C. lanatus var. citroides PI 179881, and C. lanatus var. lanatus PI 482251 (Zimbabwe), PI 494529 (Nigeria), and 'AU-Producer' showed identical sequences at *ycf6-psb*M and *ndh*F regions. Similarly, C. lanatus var. citroides PI 271769 (S. Africa) and PI 482252 (Zimbabwe) had homologous sequences at these regions.

Morphological characteristics of many of the citron types described in the GRIN database indicate high phenotypic variability. Fruit size varies from small (10x10 cm for PI 244018) to medium (30x30 cm for PI 270563), fruit shape from round to oblong, flesh color white to yellow, and seed size from 5 x 8 mm to 8 x 15 mm. Citron seeds generally lack the flatness of watermelon cultivar seeds. Several of the var. citroides PI accessions showed the C. lanatus var. lanatus haplotype and have medium-sized fruit with white, yellow or red flesh. Cultivated watermelons similarly have variable fruit types ranging from small fruit (10 x 10 cm for PI 494527) with white flesh and Egusi type edible seeds (5 x 27 mm), to the large round or oblong watermelon fruit with red or yellow flesh presently available on the U.S. market. Levi et al. (5) detected higher levels of genetic variation using RAPDs among C. lanatus var. citroides accessions as compared to C. lanatus var. lanatus. However, in this study no association between

most accessions). Table 1. List of investigated C. lanatus Plant Introductions and their geographical origin (see GRIN database for morphological characteristics of

C. lanatus var. lanatus	Origin	C. lanatus var. citroides	Origin
165451	Mexico	179881, 288316	India
176492	Turkey	189225, 532738	Zaire
185636, 271751	Ghana	244018, 255136, 255137, 270563, 271769, 271779, 295850	S. Africa
211011	Afghanistan	296334, 296335, 295842, 296341, 482293, 596665, 596667	S. Africa
241689	Chile	525081	Egypt
254742	Senegal	254744	Senegal
273481	Ethiopia	248774, TCN 1126	Namibia
295845, 271778	S. Africa	346082	Afghanistan
385964	Kenya	379243	Yugoslavia
482251	Zimbabwe	482246, 482259, 482261, 482279, 482303, 482311, 482319,	Zimbabwe
494527, 494529, 494531	Nigeria	482324, 482334, 482361	Zimbabwe
500314, 500324, 500353	Zambia	532664, 532667	Swaziland
507858	Hungary	532819	China
536453	Maldives	TCN 1360, TCN 1337	U.S.
549160	Chad	542114, 532669, 542123, 483583	Botswana
15 different cultivars	U.S. (see text)	512385, 512854	Spain

Table 2. DNA sequences of *C. lanatus* informative chloroplast specific primer pairs and PCR amplification reaction conditions.

Primer pair	Primer sequence	PCR condition
ndhF: 1955F-607R (6)	TAT ATG ATT GGT CAT ATA ATC G ACC AAG TTC AAT GTT AGC SAG ATT AGT C	4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min 55°C, 2 min at 65°C
yc/6 F-psbM R (3)	CTT GGG CTG CTT TAA TGG GTA AAT ATT CTT GCA TTT ATT GC	4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min 50°C, 2 min at 65°C 10 min at 65°C.

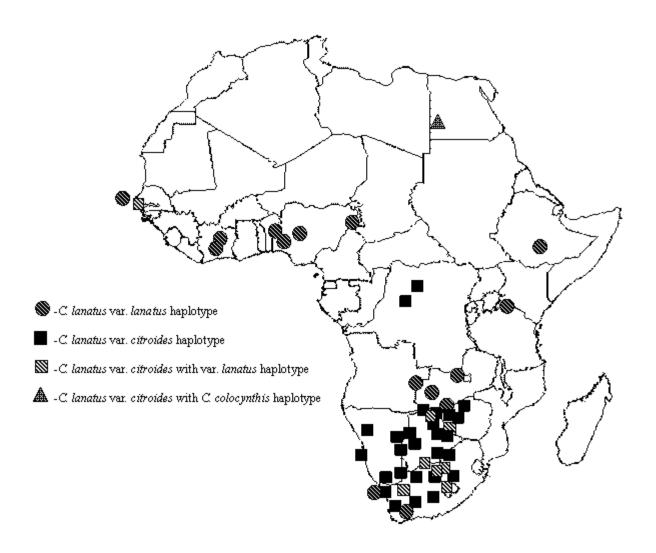


Figure 1. Geographical distribution of Citrullus lanatus haplotypes on the African continent.

phenotypic variability and chloroplast DNA patterns was detected.

It is interesting to note that several accessions classified as *C. lanatus* var. *citroides* showed the *C. lanatus* var. *lanatus* haplotype (PI 179881, PI 254744, PI 255136, PI 271779, PI 295850, PI 295842, PI 482319, PI 482293, and PI 482334), while two accessions (PI 386082 and PI 525081) showed the *C. colocynthis* haplotype. Since cpDNA is maternally inherited in *Citrullus* (2), only the maternal parent of these accessions can be identified. We are currently working on nuclear DNA markers (AFLPs) to detect the paternal origin.

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# Two Oriental Squash Varieties Resistant to Powdery Mildew Bred through Interspecific Crosses

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Powdery mildew disease is frequently found in fields of cucurbit crops (2). The causal agents of this disease are reported as Ervsiphe cichoracearum DC ex. Merat and Sphaerotheca fuliginea (Schlecht ex. Fr) Poll. by Sitterly (5). In Korea, Sphaerotheca fuliginea is the most prevalent pathogen based on conidiaphore studies (3). Korean varieties of Cucurbita moschata have good quality fruit, but are susceptible to powdery mildew. Cucurbita lundelliana is resistant to powdery mildew (6). Resistance is governed by a single dominant gene that can be transferred to *C. moschata* (4). Contin (1) concluded that C. martinezii has a single dominant gene for powdery mildew resistance. On the other hand, C. lundelliana and C. martinezii posses additional modifier genes influencing the level of resistance. In 1989 several seeds of C. martinezii were obtained from Dr. R. W. Robinson, Cornell University. The present paper reports development of novel types of C. moschata varieties through interspecific crosses with good quality, powdery mildew resistance, earliness, which produce slightly tapered cylindrical fruits.

**Method:** The lines were obtained through backcross and pedigree selection as shown in Fig. 1. The interspecific crosses were done between the cultivar Jecheoniaerae of C. moschata and C. martinezii in hybrids were backcrossed 1991. The 'Jecheonjaerae' in 1992. Progenies selected for resistance to powdery mildew and good fruit quality from the BC<sub>1</sub>F<sub>1</sub> population were again backcrossed to 'Jecheonjaerae'. Lines selected for these characters from the BC<sub>2</sub>F<sub>1</sub> population were crossed with another variety of C. moschata, 'Seoulmadi' in 1995 which has good fruit quality when immature fruits are steamed. The hybrid plants were sibbed once and then selfed. Two progenies selected from the BC<sub>3</sub>F<sub>2</sub> population were backcrossed with 'Seoulmadi' and selfed four times from the year 1998 to 2001. For the selection of progenies highly resistant to powdery mildew, both the seedling and field tests were carried out in each generation.

**Result:** Selections were made based on the resistance to powdery mildew and on the quality of fruit in each generation. The two lines selected showed slightly lower levels of resistance to powdery mildew than *C. martinezii*, but the quality of the fruits were almost similar with the recurrent parent *C. moschata*. One of the selected lines was named 'Sigol' with the other named 'Sangol'.

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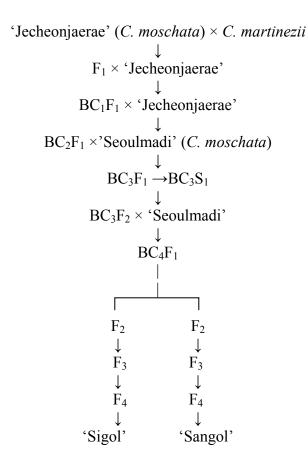


Fig. 1. Pedigree of the powdery mildew resistant *C. moschata* breeding lines, 'Sigol' and 'Sangol'. (S = Sibbed)

Table 1. Major characteristics of the two lines derived from the interspecific crosses C. moschata  $\times$  C. martinezii backcrossed to C. moschata.

Lines or Variety	Degree of resistance <sup>z</sup>	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	Fruit shape	Fruit bitterness
'Sigol' (Breeding line)	2	373	7.2	13.1	Cylindrical	No
'Sangol' (Breeding line)	2	333	6.5	15.2	Oblong	No
'Seoulmadi' (C. moschata)	9	319	4.2	27.0	Cylindrical	No
C. martinezii	1	233	6.8	7.5	Spherical	Yes

<sup>&</sup>lt;sup>2</sup> Resistance to powdery mildew: 1(resistant) - 9(susceptible)

# Seed Transmission of Zucchini Yellow Mosaic Virus on Cucurbita pepo conv. citrullinina var. styriaca (Oilseed Pumpkin)

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**Introduction**: Zucchini Yellow Mosaic Virus (ZYMV) remains one of the most widespread and destructive viral agents affecting Cucurbitaceae. The apparently rapid spread in many countries of different continents suggested a long distance distribution via infected seeds. However, this avenue dissemination is very difficult to conclusively prove. Our studies, involving large numbers of zucchini, summer squash and cucumber plants from seeds of infected fruits, have failed to prove seed transmission of ZYMV. The first data indicating that ZYMV could be transmitted via seed, was obtained in a study with hulless oilseed pumpkin (2). The objective of this research was to study seed transmission of ZYMV in naturally infected hulless oilseed pumpkin.

Materials and Methods: Naturally infected fruits of hulless oilseed pumpkin (*Cucurbita pepo* convar. *citrullinina* var. *styriaca*) showing mild, severe and very severe symptoms of ZYMV infection, were selected and weighed. The seeds were taken out of the fruit, dried, weighed and kept at 5° C. The seeds were sowed in insect proof greenhouses and the plants were visually observed. The plants with abnormal growth or virus symptoms were selected and tested by ELISA techniques (1). In ELISA serological tests *Zucchini Yellow Mosaic Virus* (Bioreba Art No. 161222), *Watermelon Mosaic Virus* 2 (Bioreba Art. No. 161122) and *Cucumber Mosaic Virus* (Bioreba Art. No. 160622) kits were used.

**Results:** The data and results are shown in Table 1. Out of the 6073 plants, 104 showed symptoms and were virus infected. According to the ELISA tests only ZYMV were detected in all cases and CMV or WMV-2 could not be identified. Virus infected plants were detected in 7 stocks out of 22. Virus transmission rates varied between 0.3% and 15.3%. There was no correlation between symptom severity of fruits and the virus transmission by seeds. Seeds from severely distorted fruits (3, 17, 25, 31 and 37 stock samples) were virus free just as seeds from mild distorted fruits (27, 28 and 46 stock samples) or distorted fruits (7, 22, 3672, 75 and Kákai stock samples). Seeds originated from distorted fruit (10 stock) and from severely distorted fruit (55 stock) showed almost the same rate of virus infection (13.14% and 15.34% respectively).

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Table 1. Seed transmission of ZYMV from naturally infected fruits of hull-less oilseed pumpkin.

Seed Sample	Severity of symptoms <sup>1</sup>	Weight of fruits (kg)	Number of seeds	Number of plants	Number of virus infected plants	
Stock 1.	***	2.1	240	228	1	0.44
3.	***	2.9	220	195	0	0.00
7.	**	2.3	288	261	0	0.00
10.	**	2.4	156	137	18	13.14
17.	***	2.6	275	266	0	0.00
18.	***	3.9	359	334	1	0.30
22.	**	3.1	220	203	0	0.00
25.	***	4.3	504	378	0	0.00
27.	*	3.5	380	354	0	0.00
28.	*	3.6	270	259	0	0.00
31.	***	3.3	580	557	0	0.00
33.	**	3.7	350	319	10	3.13
36.	**	3.9	330	271	0	0.00
37.	***	3.4	320	242	0	0.00
43.	***	3.3	310	283	2	0.71
45.	***	4.9	360	274	1	0.36
46.	*	3.6	60	52	0	0.00
55.	***	2.8	393	378	58	15.34
72.	**	3.5	427	401	0	0.00
75.	**	3.2	139	128	0	0.00
82.	***	3.0	250	238	13	5.46
Kákai	**	3.6	348	315	0	0.00
			6779	6073	104	1.71

<sup>&</sup>lt;sup>1</sup> \*\*\* - severely distorted fruit, \*\* - distorted fruit, \* - mild distortion

# Fruit Color Inheritance in a Cross of a Dark-Colored Accession with a Light-Colored Accession in *Cucurbita pepo*

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Thirteen loci, some of them multiple-allelic, have been identified as affecting fruit exterior color in *Cucurbita pepo* L. (2). Three major loci are *D* (*Dark stem*), *l-1* (*light coloration-1*), and *l-2* (*light coloration-2*) (5). These three loci were identified in a cross of 'Fordhook Zucchini' (*C. pepo* subsp. *pepo* Zucchini Group), which has intense-colored fruits and dark stems, with 'Vegetable Spaghetti' (*C. pepo* subsp. *pepo* Vegetable Marrow Group), which has light-colored fruits and light stems. 'Fordhook Zucchini' possesses genotype *D/D L-1/L-1 L-2/L-2* whilst 'Vegetable Spaghetti' possesses genotype *d/d l-1/l-1 l-2/l-2*.

The D gene has almost no effect on the fruits during the first week after anthesis. The dominant D allele causes the fruits to darken during the second and third weeks after anthesis, with recessive d/d having no effect. The darkening caused by the dominant allele occurs independently of the allelic state of the l-l and l-l genes and masks their effect, that is, D is epistatic to l-l and l-l (5).

A fourth locus is pl ( $plain\ light$ ). This gene is of relatively minor effect, determining whether or not the 'Light type 1' phenotype is expressed (1). Plants of genotype  $d/d\ L-1/-\ l-2/l-2\ pl/-$  exhibit the 'Light type 1' fruit phenotype whilst those of genotype  $d/d\ L-1/-\ l-2/l-2\ pl/pl$  exhibit the 'Plain light' fruit phenotype.

'True French' (C. pepo subsp. pepo Zucchini Group) intense exterior fruit color throughout development but has light stems. An inbred of 'True French' was crossed with an inbred of 'Beirut' (C. pepo subsp. pepo Vegetable Marrow Group) in order to determine the allelic state of 'True French' in regard to these four genes. 'Beirut' has 'Plain light' fruit color phenotype; its genotype is  $d/d l - l^{iSt}/l - l^{iSt} l$ 2/l-2 pl/pl (3), that is, it carries the  $l-l^{iSt}$  allele for irregular striping, but striping is not expressed because both, *l-2* and *pl* are homozygous recessive (1). The source of the original seed stock of 'True French' was as described previously (4). Seeds of the original seed stock of 'Beirut' were kindly provided by Hazera' Seeds (Berurim, Israel). Seeds of parental, filial and backcross generations were sown in the field at Newe Ya'ar. Most of the data are from the spring-summer and summer-fall seasons of 2002, with supplemental data from previous springsummer seasons.

None of the parental-, filial-, and backcross-generation plants of either cross had dark stems. Therefore, 'True French' does indeed possess genotype d/d.

All  $F_1$  plants and all plants of the backcross to 'True French' bore intense-colored fruits. This was true when the fruits were young as well as when they attained intermediate age (Table 1). Thus, intense coloration is dominant to light coloration. The young fruits segregated to intense-colored and light-colored in accordance with a 9:7 ratio in the  $F_2$  and a 1:3 ratio in the backcross ( $\chi^2$ =0.053, P=0.81 and  $\chi^2$ =0.476, P=0.48, respectively), as expected for two complementary dominant genes conferring intense coloration of young fruits. Therefore, 'True French' possesses genotype L-1/L-1 L-2/L-2.

Both the  $F_2$  and the backcross to 'Beirut' segregated to three phenotypes of intermediate-age fruits. The fruits that had been of intense color when young remained so. The light-colored fruits developed into one of two light-colored phenotypes—'Light type 2'

Table 1. Intermediate-age fruit color in the cross of 'True French' and 'Beirut' (d/d l-1/l-1 l-2/l-2).

	Number	of plants						
Generation & Description	Total	Dark	Light type 1	Light type 2*	Plain light	Expected ratio	$\chi^2$	P
P <sub>1</sub> , True French	48	48	0	0	0	_	_	_
P <sub>2</sub> , Beirut	42	0	0	0	42	_	_	_
$F_1$ , $P_1 \times P_2$	10	10	0	0	0	_	_	_
$F_1, P_2 \times P_1$	48	48	0	0	0	_	_	_
$F_2$ , $(P_1 \times P_2) \otimes$	101	61	0	21	19	9:3:4	2.130	0.35
$F_2$ , $(P_2 \times P_1) \otimes$	101	51	0	23	27	9:3:4	1.588	0.45
F <sub>2</sub> , Total	202	112	0	44	46	9:3:4	1.415	0.48
$BC_1, P_1 \times F_1$	33	33	0	0	0	_	_	_
$BC_1, P_2 \times F_1$	280	75	0	70	135	1:1:2	0.536	0.76

<sup>\*</sup>includes 'Light type 2' and irregular-striped (3) individuals

(with or without irregular stripes) (3) and 'Plain light'—by intermediate age; no 'Light type 1' individuals were observed. The three fruit-color phenotypes segregated in accordance with a 9:3:4 ratio in the  $F_2$  and a 1:1:2 ratio in the backcross (Table 1). The lack of individuals of 'Light type 1' phenotype indicates that 'True French' possesses genotype pl/pl. For the four loci, the genotype of 'True French' is therefore d/d L-1/L-1 L-2/L-2 pl/pl.

**Acknowledgement:** Contribution No. 111/2003 from the Institute of Field & Garden Crops, Agricultural Research Organization, Bet Dagan, Israel.

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# Fruit Color Inheritance in Crosses of a Striped Accession with Two Light-Colored Accessions in *Cucurbita pepo*

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Stripes on the exterior of the fruit of *Cucurbita pepo* are conferred by alleles at the *l-1* locus (3, 4). When both an allele for striping and the dominant *L-2* allele occur in the same genotype, stripes are present on the fruit from anthesis through fruit maturity (5). In *l-2/l-2* plants, stripes are not visible on young fruits, but become visible at intermediate age (15 -18 days past anthesis) in the presence of the dominant *Pl* allele. Plants of genotype *l-2/l-2 pl/pl* have light-colored, non-striped fruits, even if they carry an allele for striping (2, 7).

'Cocozelle' is a well-known cultivar of C. pepo subsp. pepo Cocozelle Group and bears fruits that have broad, contiguous dark stripes alternating with narrow light stripes. Broad, contiguous stripes are conferred by the l-l<sup>BSt</sup> allele (3). As the stripes are clearly visible from anthesis through fruit maturity, 'Cocozelle' apparently carries the dominant L-2 allele (5), but direct evidence confirming this has not been presented. Also, it is not known if 'Cocozelle' carries the dominant or recessive allele of gene pl. In order to investigate these issues, 'Cocozelle' was crossed with two light-fruited accessions, 85a-30-45 (1), genotype *l-1/l-1 l-2/l-2 Pl/Pl*, and 'Sihi Lavan' (C. pepo subsp. pepo Vegetable Marrow Group), a cultivar from Israel which carries an allele for striping at the *l-1* locus and genotype l-2/l-2 pl/pl (2). All three accessions are d/d, that is, do not carry the dominant and epistatic allele D for dark stems and dark intermediate-age fruits (6).

The striping of 'Cocozelle' is dominant to the plain light color of accession 85a-30-45 (Table 1). The F<sub>2</sub> and backcross progenies segregated to four fruit-color phenotypes: broad, contiguous dark stripes alternating with narrow, light type 2 stripes; broad,

The  $F_2$  also segregated in accordance with a 36:9:12:7 ratio. This ratio would be expected if 'Cocozelle' was pl/pl rather than Pl/Pl (2). Thus, from the data presented in Table 1, it is not possible to determine the allelic state of 'Cocozelle' with regards to the pl gene.

When 'Cocozelle' was crossed with the plain light 'Sihi Lavan', the  $F_1$  plants were striped, as expected for dominance of striped over plain light (5). Of the 119  $F_2$  plants observed, 107 had striped fruits and 12 had plain light fruits. This result is not in accordance with the 3:1 one-gene ratio ( $\chi^2 = 14.12$ , P << 0.01). However, this result is in reasonable accordance with the 15:1 two-gene ratio ( $\chi^2 = 2.985$ , P = 0.08). 'Sihi Lavan' carries a gene for striping (7) but has genotype l-2/l-2 pl/pl (2) and this could account for the fit to the 15:1  $F_2$  ratio if 'Cocozelle' is L-2/L-2 Pl/Pl.

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Table 1. Intermediate-age (15—18 days past anthesis) fruit color in the cross of 'Cocozelle' and accession 85a-30-45 (*d/d l-1/l-1 l-2/l-2 Pl/Pl*).

	Numbe	r of plants						
Generation & Description	Total	Striped, D/LT2*	Striped, LT1/PL**	Light Type 2	Plain light	Expected ratio	$\chi^2$	P
Cocozelle	14	14	0	0	0			
85a-30-45	14	0	0	0	14			
$F_1, P_1 \times P_2$	25	25	0	0	0			
$F_1, P_2 \times P_1$	11	11	0	0	0			
$F_2, (P_1 \times P_2) \otimes$	51	31	7	9	4	9:3:3:1 36:9:12:7	1.113 0.670	0.77 0.88
$F_2$ , $(P_2 \times P_1) \otimes$	49	26	8	8	7	9:3:3:1 36:9:12:7	5.458 0.923	0.13 0.82
F <sub>2</sub> , Total	100	57	15	17	11	9:3:3:1 36:9:12:7	4.533 0.239	0.21 0.97
$BC_1, P_1 \times F_1$	21	21	0	0	0	_		_
$BC_1, P_2 \times F_1$	101	31	19	25	26	1:1:1:1	2.881	0.42

<sup>\*</sup>Striped: Dark on Light type 2 background

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<sup>\*\*</sup>Striped: Light type 1 on Plain light backg

## Inheritance of Immature Fruit Color in *Cucurbita maxima* var. Zapallito (Carrière) Millán

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**Introduction.** Cucurbita is characterized by its ample variation in forms and colors of fruits. In C. pepo the inheritance of both mature and immature fruit color has been revealed (2,5). In C. maxima, however, the studies of color have been concentrated in the mature state (1,6), the most popular form of consumption in North America and Europe. In southern Latin America C. maxima has been largely bred for immature fruit consumption given rise to the so-called zapallito varieties (4). This culinary use is very popular and has pre-Columbian cultural roots (4). Fruits which are green and round in shape are normally harvested, depending on air temperatures, 9 to 18 days past anthesis (dpa). In a segregant population of zapallito a plant bearing a new variant of light green color was recognized. The fruits were similar to those of plain light green color described for subspecies andreana by Millán (3). In this report a first approach is advanced in the inheritance of immature fruit color of zapallito.

**Material and Methods.** In 1996, in a segregant population of the Rosario National University zapallito breeding stock, a plant bearing light green color was recognized and selfed for three seasons; rendering in all instances uniform light green immature fruits. The line was identified as I-18. An advanced inbred line (Germ-4) derived from zapallito cultivar 'La Germinadora' was used as a parent of normal green fruits (Figure 1). During 1999 and 2000, F<sub>1</sub>, F<sub>2</sub> and backcrosses were advanced. These generations and parents were evaluated for immature fruit color during 2001 and 2002. In both seasons the normal planting grid of 1.4 m between lines and 0.80 m within plants in the line was used. Two weeks after emergence hills were thinned to one plant. Fruits

were visually examined for color when they reached 5-10 cm in diameter (commercial size). Each plant was assessed at least twice.

Results and Discussion. Results of the generation analysis for fruit color are presented in Table 1. Monogenic and digenic hypothesis (3:1, 9:7, 13:3 and 15:1) failed to explain the observed segregation. However, as in C. pepo for 15-18 dpa fruits (5), a three loci model fitted. In this model two genes interact with a third complementary independent locus. Light green color would be the result of recessive homozigosity in the third locus and, at least, in one of the two complementary genes. These give an expected 57:7 and 5:3 normal to light green ratio in F<sub>2</sub> and BC<sub>2</sub> respectively. In C. pepo the third locus (D) is responsible for stem color, with a pleiotropic effect on fruit color. The dominant allele renders dark green stems. In our experience, unfortunately, stem color was assessed only in plants of the light green color class, which were all classified as possessing light green stems.

In conclusion, the segregations observed for immature fruit color in zapallito is in agreement with a three loci model, like that proposed in *C. pepo* for 15-18 dpa fruits. A combined inheritance study with stem color should be conducted in order to verify if stem color has a pleiotropic effect on fruit color. Crosses to materials of *C. maxima* ssp. *andreana* bearing light green fruits should be advanced to test allelism for these loci.

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Table 1. Results of the two years analysis for normal green and light green fruits of zapallito.

			N	$N^{\circ}$ of Plants		Expected		
Year	Generation	Description	Normal green	Light green	Total	Ratio	$\chi^2$	P
2001	$\mathbf{P}_1$	Germ-4	20		20			
	$P_2$	1-18	ı	15	15			
	$\mathrm{F}_1$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2)$	22		22			
	$F_2$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2)$	142	19	161	57:7	0.12	0.97-0.90
	$BC_1$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2) \times \mathbf{P}_1$	21	1	21			
	$BC_2$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2) \times \mathbf{P}_2$	18	9	24	5:3	1.6	0.50-0.10
2002	$\mathbf{P}_1$	Germ-4	18	,	18			
	$P_2$	1-18	ı	15	15			
	$\mathbf{F}_1$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2)$	15		15			
	$\mathrm{F}_1$	$(\mathbf{P}_2\mathbf{x}\mathbf{P}_1)$	17		17			
	$F_2$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2)$ @	80	6	68	57:7	90.0	0.90-0.50
	$\mathbf{BC}_1$	$(P_1xP_2) \times P_1$	36	,	36			
	$BC_2$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2) \times \mathbf{P}_2$	13	9	19	5:3	0.28	0.90-0.50
2001-2002	$\mathrm{F}_2$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2)$	222	28	250	57:7	0.01	0.90-0.50
	$BC_2$	$(P_1xP_2) \times P_2$	31	12	43	5:3	1.68	0.50-0.10
Heterogeneity	$F_2$	$(\mathbf{P}_1\mathbf{x}\mathbf{P}_2)$ @				57:7	0.18	0.97-0.90
	$BC_2$	$(P_1xP_2) \times P_2$				5:3	0.19	0.50-0.10



Figure 1. Light green (left) and normal green (right) immature fruits of zapallito.

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## Ascorbate and Carotenoid Content in an Indian Collection of Pumpkin (*Cucurbita moschata* Duch. ex Poir.)

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Cucurbita moschata Duch. ex Poir. (pumpkin) is grown throughout tropical and sub tropical countries. In India, pumpkin is the principal ingredient of several culinary vegetables utilized at the immature and mature fruit stage. Pumpkin provides a valuable source of carotenoids and ascorbic acid that have a major role in nutrition in the form of provitamin A and vitamin C as antioxidants, when used at ripening stage or after storage. Content of caroteniods in Spanish pumpkin was higher than that of other pumpkin varieties and even higher than that of beta carotene in carrots (5). In India, consumers prefer dark yellow color, round shape fruit with thick and deep yellow internal flesh colour (personal communication). Indian farmers generally grow their own saved seeds of land races because commercially produced seed is not easily available. Therefore, exploration and collection of pumpkin germplasm was done from main pumpkin growing areas of India with the objective of screening the pumpkin lines having high ascorbic acid and caroteniod content for further improvement in pumpkin quality (6).

After collection, all the germplasm was maintained by hand sib mating/selfing through two seasons. Seeing the homozygosity in the populations, a total of seventy accessions of pumpkin were selected from the genetic pool and grown in a randomized block design with three replications during July to November 2002. All recommended agronomical practices were adopted for good growth of the crop. Most of the fruit and plant characters were also observed during the cropping period. All the lines were monoecious. Number of fruits per plant varied from 1 to 3.5 and weighed from 1.25 to 9 kg each. Polar circumference of fruit ranged from 43 to 85 cm. Similarly equatorial circumference ranged from 43.33 to 95 cm. Fruit shape varied from round, flat round oval and oblong type. The fruit flesh of mature fruit varied in colour as several shades of vellow, deep yellow to orange with 2-5 cm thick flesh. For analysis of ascorbic acid and caroteniod, fruits were harvested at the fully matured stage. The ascorbic acid (mg/100g) content was estimated titrimatically using 2, 6- dichlorophenol dye method of Ranganna (7). Total carotenoids were extracted and partitioned in acetone and petroleum ether, respectively and estimated spectrophotometrically as described by Gross (1).

Data were averaged and statistically analyzed. The analysis of variance indicated highly significant differences between germplasm for ascorbic acid and carotenoids (3). This suggests that there is a considerable amount of variation among the germplasm. Mean, range, phenotypic (PCV) and genotypic (GCV) coefficient of variation is presented in Table 1. The variability estimate, in general, reveal that the phenotypic coefficient of variance (PCV) was higher than the genotypic coefficient of variance (GCV) but the differences was relatively low for ascorbic acid and beta-carotene (2). The estimates of PCV and GCV indicated the existence of a fairly high degree of variability for ascorbic acid and carotenoid content. The differences in PCV and GCV were minimum for beta carotene and ascorbic acid, indicating that these traits are least affected by environment. The range of ascorbic acid content was 1.53mg to 6.74mg with an overall average of 2.89 mg/100g of fresh weight. Similarly total carotenoid ranged from 2.34 mg to 14.85 mg with a population mean of 9.29 mg/100g of fresh weight. Carotenoid concentration in tropical pumpkin compares favorably with those summarized by Paris (4) for butternut squash. The highest ascorbic acid content was recorded in IVPK-222 (7.4mg) followed by IVPK-224-4 (4.61mg), SA-90(4.08mg), DR/SP-132(4.57mg) and IVPK-225(4.55 mg). germplasm SA-90 with the maximum content of carotenoid (14.85mg) was considered as a promising line for carotenoid followed by Pumpkin-111 (14.19mg), BP-14(13.61mg), IVPK-225(13.29mg) and IVPK-226(13.08mg). The two constituents under study generally constitute the major portion of pumpkin quality.

Table 1: Total carotenoids and ascorbate value of 5 most important accession out of 70, based on maximum value.

Characters	Ascorbic acid (mg/100g)	Beta carotene (mg/100g)
Range	1.53-6.74	2.34-14.85
Population mean	2.89	9.29
GCV	31.73	33.19
PCV	32.13	33.97
CV (%)	19.84	7.5
CD at 5%	0.98	1.07
High value accessions	IVPK-222, IVPK-224-4, SA-90, DR/SP-132, IVPK-225	SA-90, Pumpkin-111, BP-14, IVPK-225, IVPK-226

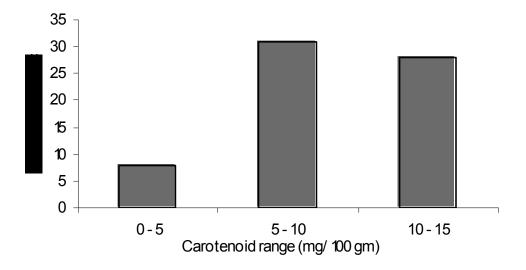


Figure 1. Carotenoid variability in the pumpkin collection.

Among the evaluated germplasm the genotype SA-90 was found to have superior beta carotene as well as ascorbic acid and favorable values for yield, equatorial and polar circumference of fruit, number of fruits per plant and average fruit weight. Therefore, SA-90 is the most promising genotype for beta carotene and ascorbic acid. The significant

aspect of this work is that the lines with high ascorbic acid and carotenoids may be utilized for further quality improvement of pumpkin for these traits during the development of hybrids and varieties. These materials may be also utilized for study of gene action. All materials used for this study are maintained as inbreds.

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# Combining Ability and Heterosis in Line x Tester Crosses of Summer Squash (*Cucurbita pepo* L.)

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**Introduction.** Cucurbits play a significant role in human nutrition, especially in tropical countries where their consumption is high. Cucurbit crops constitute a major portion of vegetables and are grown in different regions of the Sudan. Squash was ranked fourth among the world's most popular cucurbits preceded by watermelon, cucumber and melon (7). In Sudan squash production is confined to urban areas where there is a good potential for local market and export.

The mating design (Line x Tester) suggested by Kempthorne (4) has been extensively used to estimate GCA and SCA variances and their effects. Also, it is used in understanding the nature of gene action involved in the expression of economically important quantitative traits. Thus, GCA and SCA estimates, which are useful in devising breeding strategies, were reported in some cucurbits (2). Cucurbita pepo L. was reported to be adapted to hybrid production (8). Lopez-Anido et al. (5) reported that non-additive gene actions were of major relative importance for vegetative characters (leaf number, leaf diameter and plant height) and precocious fruit number. Also they reported the importance of additive and non-additive gene actions for total fruit number and days to first harvest. In early reports significant heterosis has been reported in crosses between certain varieties of C. pepo(1). Firpo et al. (3) reported superior heterosis for total fruit number and precocious fruit number in crosses between inbred lines derived from a summer squash (C. pepo) population. Also they concluded that inbreeding and crossing methods could be a useful tool in increasing the population means for yield traits through hybrid or synthetic variety production. Crosses between inbred lines derived from different populations (interpopulation crosses) were reported to increase heterosis (6). The objectives of this study were to measure and evaluate additive and nonadditive gene action, GCA, SCA and heterosis among 14 hybrids resulting from the crossing of seven lines with two testers.

Materials and Methods. The experimental material consisted of seven inbred lines, which have been designated as 1,2,3,4,5,10,12 derived from a summer squash (C. pepo) population. The population developed from a cross between the commercial cultivar "Eskandarany" and breeding lines obtained from Cornell University (USA), which are segregating for Zucchini vellow mosaic virus resistance (ZYMR) and powdery mildew resistance (PMR). The two testers are cv. "Eskandarany" (E) and ZYMR and PMR cv. "Whitaker" (W) obtained from Cornell University. The seven lines and the two testers were crossed in a line x tester (L x T) design. The resulting 14 hybrids with their nine parents were evaluated in a randomized complete block design with three replications. The following characters were evaluated on a plot (10 plants) basis: number of days from sowing to the first pistillate flower (DFL), Ovary length at anthesis (OL), total number of marketable fruits per plant (TNF) and plant vigor (PLV). For PLV a scale of 1=small, 2= intermediate and 3=vigorous was used. Statistical analysis was performed according to Kempthorne (4) and software MSTAT-C Version 4.1 was used. Heterosis over mid parent was computed for all characters measured.

**Results and Discussion**. The mean squares for the four characters are shown in Table 1. Variability among genotypes was highly significant ( $P \le 0.01$ ) for the four characters. No significant difference was observed among lines, while variability among testers was significant for TNF ( $P \le 0.01$ ). Crosses and lines x testers showed significant differences for OL and TNF ( $P \le 0.01$ ). High significant differences were observed among parents for DFL and OL, while parents versus crosses showed significant differences for all characters.

Table 1. Mean squares of the four characters: number of days to flowering (DFL), ovary length (OL), total number of marketable fruits (TNF) and plant vigor (PLV).

		MEAN SQU	MEAN SQUARES				
Sources of var.	df	DFL	OL	TNF	PLV		
Replications	2	198.3	0.494	0.012	2.8		
Genotypes	22	278.87**	1.79**	24.3**	0.33*		
Lines (L)	6	39.9 ns	0.478 ns	5.23 ns	0.20 ns		
Testers (T)	1	26.88 ns	0.945 ns	62.4**	0.09 ns		
LxT	6	29.9 ns	0.433**	10.12**	0.103 ns		
Crosses (C)	13	34.29 ns	0.493**	11.9**	0.15 ns		
Parents (P)	8	277.21**	2.98**	0.57 ns	0.23 ns		
P vs C	1	3471.6**	9.09**	375.2**	3.44**		
Error	44						

<sup>\*, \*\*</sup> Significant at 0.05 and 0.01 level of probability, respectively

Table 2: Average heterosis (expressed as above mid parent) for the 14 crosses.

Cross	OL (%)	DFL (%)	TNF (%)	PLV (%)
1xW	-16.1	-36.7	115.3	32.8
2xW	-5.3	-26.6	126.8	28.9
3xW	-5.5	-30.2	182.4	33.3
4xW	-10.8	-24.6	90.0	3.7
5xW	-10.8	-38.5	182.2	15.9
10xW	-4.4	-15.8	52.6	2.1
12xW	-7.5	-25.9	99.2	14.1
1xE	9.7	-16.6	92.0	50.0
2xE	19.8	-6.6	260.4	42.9
3xE	1.9	-19.9	65.7	20.7
4xE	13.9	-5.8	102.8	31.7
5xE	15.1	-20.5	140.2	27.1
10xE	24.5	-18.0	120.3	13.2
12xE	25.1	-5.4	67.6	34.5

The ratio of  $\sigma^2$  GCA to  $\sigma^2$  SCA was less than one for the four characters indicating the importance of non-additive gene action for these characters. Lopez-Anido et al. (5) reported the importance of non-additive gene actions for vegetative characters, leaf number, leaf diameter and plant height. Also they reported the importance of additive and non-additive gene actions for total fruit number and precocious production.

None of the female parents or testers showed significant estimates of GCA or SCA for DFL. However, the tester Whitaker was late (51 days) compared to Eskandarany (36 days) while the average of the lines is (58 days). But, the crosses were earlier, with an average of 40 days. The contribution of lines to total variance is 53.7% and that for L x T is 40.3%.

The female parent (10) is a good combiner for long ovary as shown by its significant GCA estimate. Among the testers Whitaker showed a significant negative GCA although it has a relatively long ovary (7.8 cm), while Eskandarany, which has a small ovary (5.4cm) showed a significant positive GCA estimate. However, the lines have an average ovary length of 4.8 cm. The contribution of lines to total variance is 44.7% and that for LxT is 40.6%.

The tester Whitaker is a good combiner for yield as shown by its significant positive GCA estimate, while Eskandarany showed a significant negative GCA estimate. The contribution of lines to total variance is 20.3%, 40.4% for testers, and 39.3% for Lx T.

None of the lines or testers showed significant GCA or SCA for plant size. Nevertheless, the contribution to total variance of lines, testers and LxT is 63.2%, 4.7% and 32.1% respectively.

Heterosis was superior for total fruit number, but low for the other characters (Table 2). Similar results regarding superior heterosis for total fruit number were reported (3). Mean separation showed that the five top crosses with respect to yield involved Whitaker as a common parent. Thus, they can be used as high yielding commercial F1 hybrids.

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# On the Genetics and Histology of the Hull-less Character of Styrian Oil-Pumpkin (*Cucurbita pepo* L.)

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**Introduction:** The seeds of *Cucurbita pepo* possess normally a thick, leathery seed coat (hull) due to the strong lignification of some of its testa layers. The mutant Styrian oil-pumpkin exhibits a complete lack of lignification of the testa. The proto-chlorophyll content of the fifth testa, chlorenchym, layer gives the seeds an olive-green color. This mutation emerged probably in the 1880's in the South-East of the then Austro-Hungarian-Monarchy (8).

Several studies on the seed coat character and on its genetics were carried out in the early 1950's (1, 2, 3, 4, 5, 6). There is a general agreement on the existence of a major dominant gene responsible for a strong lignification of some of the testa layers of the hulled seed type. If both alleles in this locus are homozygous recessive, the seeds are basically of hull-less type. Nevertheless, they show a varying amount of lignification from a complete lack up to a clearly lignified margin and a thin lignified layer on the seed surface. For this variation different genetic interpretations were put forward, from a single gene with modifiers (6) up to 9 minor genes (8).

Histological investigations demonstrate that seed-coat development is identical in both seed types up to 15 to 20 days post-anthesis, resulting in five clearly distinguishable cell layers. The effect of the mutation becomes visible when lignification starts. While in the hulled type the second, third and fourth cell layers become strongly lignified, in the hull-less type, due to the lack of lignin deposition, these cell layers collapse (4, 7).

In this report we are reexamining the variation of the seed coat character of the hull-less segregants in three different crosses, using visual and histological investigations.

#### **Materials and Methods**

Crosses: The three crosses were: "SZG1 x True French", "Retzer Gold x Tigress" and "Lady Godiva x Bianco friulano". We used two Austrian and a French oil-pumpkin varieties as the hull-less parents, and two zucchini varieties and one crock-neck genotype, as the hulled parents. Crosses were made in the green house in the winter 1999/00. In summer 2000 F1 plants from each cross were grown and selfed. From a single F1 fruit per cross more than 100 F2 seeds were planted in summer 2001 and selfed to produce F3 seeds. To further follow segregation of the hull-less character, 10 F3 seeds from each of the F2 hull-less plants from the first cross (SZG1 x True French) were planted in the summer 2002 to produce F4 seeds. It should be noted that the seed coat of a F(n) plant can only be evaluated after harvesting the F(n+1) seeds, since the seed coat is of maternal origin.

Segregation and statistical analysis: Ripe seeds of F2-plants from each of the three crosses were harvested and dried. The hull-less seed types were then categorized based on the visual appearance of the seed coat: Category 1: completely hull-less phenotypes, similar to the hull-less parents (Fig. 1b). Category 3: the most lignified seeds among the hull-less segregants, with a thin but definitely lignified layer on the surface of the seed (Fig. 2b). Category 2: all the other seed coat phenotypes: seeds with margin but clearly less than in category 3 or even without margin, generally with a thin layer on the surface clearly less then in category 3, not necessarily

covering the whole seed surface (Fig. 2a as an example). To check Mendelian segregation, results were submitted to a  $\chi^2$  test.

Histological observation: Seeds from the parents and from the different categories established visually were fixed right after opening the fruit in the field in a formaldehyde (40%) - glacial acetic acid - ethanol (70%) mixture (2:1:7) for three days, then transferred to 96% ethanol. The material was then embedded in technovit (embedding solution). Cross sections of 5 µm from the seeds were mounted on slides, stained specifically for lignin with safranine-astrablue and toluidine blue which gives blue-greenish staining in colored pictures. Investigation was done under the light microscope.

#### **Results and Discussion**

Segregation ratios: In the three crosses, the seed coat of the F1 plants was completely hulled. Seed coats of the F2s segregated 3:1 hulled versus hull-less (Table 1). According to the data in Table 1, complete lignification of the seed coat is basically controlled by a single major gene with two alleles, with the genotypes "HH" and "Hh" for the completely hulled seed type (Fig. 1a) and "hh" for the hull-less seed type (Fig. 1b) as was postulated first by Schöniger (4). Nevertheless, the F2 hull-less progenies include a number of segregants the seeds of which exhibit a residual lignification, that appear to vary between the F2-segregants.

The segregation pattern of the three categories in the three crosses did not fit a 1:2:1 ratio (Table 2), assuming that an incompletely dominant secondary major gene is responsible for this residual lignification (5). This finding supports the critics on the two genes hypothesis of Schöniger (4, 5) by Grebenščikov (1) and Prym von Becherer (3). We studied the F3 progeny (10 plants) derived from the hull-less F2-plants of the cross (SGZ1 X True French) for further segregation of the three categories. If a secondary major gene is responsible for the phenotype of these categories, category 1 and 3 will not further segregate but category 2 will produce all three phenotypes (5). This was not the case, and from the 26 hull-lees F2-progenies only 5 progenies (1 from category 3, and 4 from category 1)

appeared to be non-segregating. All other progenies showed a seemingly random segregation.

Histological analysis: The five layers described in the literature namely, epidermis (E), hypodermis (H), sclerenchyma (S), aerenchyma (Ae) and chlorenchyma (C) are present in the testa of the ripe parental hulled seeds, the layers H, S and Ae being strongly lignified on the seed surface (Fig. 3a) and in the margin (Fig. 3b). In the parental hull-less ripe seed testa, the four upper layers are collapsed into a hyaline without any trace of lignin (Fig. 4a and b).

The different degrees of the residual lignification observed in the testa of the hull-less seeds are shown in Figures 5 and 6. While the visual classification gave rise to three categories, the histological observations show a more or less continuous variation of lignin deposition.

In the testa of category 1 seeds like in the hull-less parent, only a thick hyaline of the four collapsed layers can be seen without any trace of lignin even in the margin (not shown). The seed coat of category 2 shows sclerenchyma cells in a single continuous (Fig. 5a and b) or discontinuous layer (Fig. 5c and d), the shape of the sclerenchyma cells is either normal (Fig. 5c) or half collapsed as if the amount of lignin would not be enough to support cell architecture (Fig. 5a and d). Category 3 representing the most lignified hull-less progeny, showed a clear lignin deposition in the sclerenchyma which consisted of one to three continuous cell layers (Fig. 6a and c) with characteristic elongated cells in the margin (Fig. 6b and d).

Further extended histological studies comprising all F2-segregants of hull-less type from the three crosses will disclose whether residual lignification is controlled by several genes with minor effects producing a continuous variation or a discontinuity controlled by a manageable number of genes.

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Fig. 1: Seed types of the hulled (a) and hull-less (b) parents





Fig. 2: A sample of category 2 seeds exemplifying heterogeneity (a) and seeds typical for category 3 (b)

Table 1: The segregation of the seed coat character in the F2s in the three crosses

	Total	Hulled	Hull-less	Ratio	P
SZG1 x True French	195	148	47	3.1:0.9	0.96
Lady Godiva x Bianco friulana	190	140	50	2.8:1.2	0.90
Retzer Gold x Tigress	117	84	33	2.6:1.4	0.72

Table 2:  $\chi^2$  test of segregation ratios of the three categories in the three crosses.

	Total	Categ. 1	Categ. 2	Categ. 3	P
SZG1 x True French	25	9	10	6	0.52
Lady Godiva x Bianco friulana	25	12	10	3	0.02
Retzer Gold x Tigress	25	13	12	1	0.003

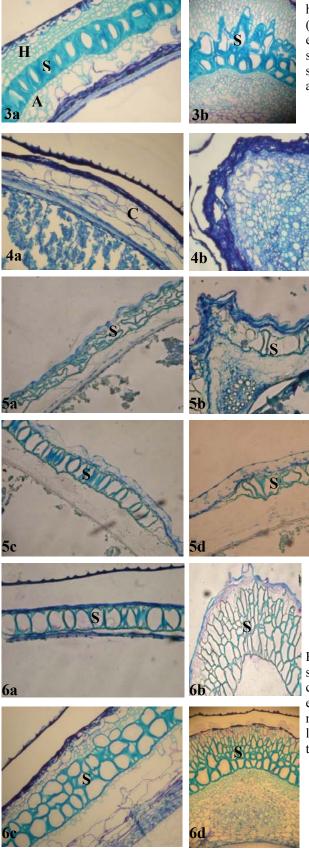


Fig. 3: Cross sections of testa of a hulled zucchini seed on the surface (a) and in the margin with typically elongated sclerenchyma cells (b). The strong lignification of the sclerenchyma (S), hypodermis (H) and aerenchyma (A) is clearly visible.

Fig. 4: Cross sections of the testa of a hull-less seeded Styrian oil pumpkin. Hypodermis, sclerenchyma and aerenchyma above chlorenchyma (C) on the seed surface (a) and in the margin (b) are collapsed to a thin hyaline. Cross sections of seeds category 1 are undistinguishable from those of the hull-less parent.

Fig. 5: Cross sections of category 2 seeds, showing sclerenchyma cells in a single continuous (a, b) or discontinuous (c and d) layer, the shape of the cells is either normal (c) or half collapsed (a).

Fig. 6: Cross sections of category 3 seeds with one or more sclerenchyma cell layers (a and c) and with elongated cells characteristic for the margin (b and d). The degree of lignification appears to be higher in the seeds c and d.

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# Comparison of RAPD Fragment Separation in Agarose and Polyacrylamide Gel by Studying *Cucurbita* Species

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Introduction: Random Amplified Polymorphic DNA (RAPD), first reported by Welsh and McClelland (7) and Williams et al. (8), is one of the most popular DNA marker systems owing to its simple and straightforward protocol. It is fast and very cost efficient. It does not require prior sequence knowledge, it needs only nanogram amounts of template DNA and a minimum of laboratory equipment. It is highly suitable for quick fingerprinting, for analysing genetic relationships, tagging traits for use in marker-assisted selection, for QTL mapping, or for the rapid construction of a genetic linkage map. In a standard protocol amplification, products are separated in a 1.5% - 2% agarose gel, which effectively separates linear DNA molecules of 0.5-2 kb. Bands are stained with ethidium bromide (6). It is well known, however, that resolution of amplified fragments is much higher in polyacrylamide gel (PAA) visualized autoradiography or silver staining (1, 3, 4, 5). Nevertheless, despite this obvious advantage of PAA, in publications reporting the application of RAPD markers, fragment separation is almost exclusively done in agarose..In this communication we report on a comparison of the two amplified fragment separation methods studying different Cucurbita genotypes.

#### **Materials and Methods**

**Plant material and DNA extraction**: Three *C. moschata* genotypes: "Menina" (1) and "Bolina" (6) from Portugal, "Nigerian Local" (2) from Nigeria, three *C. pepo* varieties: the zucchini varieties "Jaguar" (3) and "Tigress" (4), and the Austrian oilpumpkin variety "Gleisdorfer Ölkürbis" (5) have been used.. For DNA extraction the Wizard<sup>®</sup> Genomic DNA Purification Kit, supplied by Promega Corp., WI, USA (www.promega.com) was used.

#### **DNA** amplification:

*Primers:* 10mer RAPD primers were used, supplied by ROTH, Karlsruhe, Germany (<u>www.carl-roth.de</u>).

*PCR conditions:* 40 ng of genomic DNA were used in 15 μl amplification reactions containing 0.3 μM 10mer random primer, 1x reaction buffer, 1.5 mM  $MgCl_2$ , 200 μM dNTP and 0.5 U Taq polymerase. For amplification we used a MWG Primus 96plus, MWG Biotech AG, Ebersberg, Germany (www.mwg-biotech.com).

Temperature program: Initial denaturation of 60 seconds at 94°C followed by 34 cycles of 60 seconds at 94°C, 45 seconds at 36°C, 30 seconds at 72°C, finished with a final extension step of 5 minutes at 72°C.

## **Electrophoresis:**

Agarose technique: Fragments were separated by standard electrophoresis methods on 1.5% agarose gels in 1 x TBE in a BIO-RAD (BIO-RAD Laboratories Inc., CA, USA, <a href="https://www.bio-rad.com">www.bio-rad.com</a>) submarine system. Gels were stained after separation with 0.1% ethidium bromide. Gel image was captured under UV-illumination by a Polaroid camera.

Polyacrylamide technique: Non-denaturing 10% polyacrylamide gels were prepared according to standard protocols. Fragment separation was done in a vertical C.B.S. Scientific (C.B.S. Scientific, CA, USA, <a href="https://www.cbsscientific.com">www.cbsscientific.com</a>) electrophoresis unit. Silver staining was done according to a modified protocol of Bassam and Caetano-Anolés (2) as follows.

#### Silver staining

Solutions: All solutions (except stopping solution) are prepared as a five-fold stock solution. They are all made with distilled water. Our gels have a size of 16 x 33 cm, 0.75-mm thick, and we use a staining tray made of stainless steel. Routinely two gels will be stained in one tray.

<u>5x Fixing Solution</u>: This is a 3.0% benzene sulphonic acid solution in 24% ethanol. For two liters of a five-fold stock solution dissolve 60 g benzene sulphonic

acid in 480 ml of ethanol absolute. This yellow colored solution is filled up to two liters with water. Remaining particles do not disturb the staining procedure. Solution can be kept at room temperature. (acc. to the protocol from Pharmacia Biotech DNA silver staining kit: Amersham-Pharmacia Biotech, cat.nr. 17-6000-30). For staining mix 100 ml of this 5x fixing solution with 400 ml of 24% ethanol.

<u>5x Silver Solution</u>: Prepare a 2% silver nitrate solution by dissolving 10 g AgNO<sub>3</sub> in 500 ml A.dest. Keep this solution in a dark bottle at room temperature. <u>For staining</u> take 50 ml of 2% AgNO<sub>3</sub>, add 11.66 ml 5x fixing solution and fill up to 500 ml with water.

5x Developer: Dissolve 312.5 g sodium carbonate in 1.5 l water and fill up to 2 l. For a faster dissolving of the powder stir it on a hot magnetic stirring plate. Keep the solution at room temperature, over 15°C. At lower temperatures, the sodium carbonate will cristallize. For staining take 100ml of the 5x Developer Solution and fill it up to 500 ml with water. Cool this solution to +4°C in the fridge. Just before use, add to this solution 1.000 μl 37% formaldehyde and 1.000μl 2% sodium thiosulphate. 2% sodium thiosulphate is made by dissolving 2 g sodium thiosulphate in water and filled up to 100 ml.

Stopping solution: It is a 7.5% acetic acid solution. To 925 ml water add 75 ml acetic acid 99%. Keep this Solution at +4°C.

Additional water will be needed for washing steps. Shaking of gels during the staining procedure is carried out with an orbital Shaker such as HOEFER Red Rotor (Amersham-Pharmacia Biotech, cat.nr. 80-6097-10, http://www.apbiotech.com).

*Method:* The staining protocol is shown in Table 1. The times are given for 0.75-mm thick gels and may vary for gels with other thickness. Avoid touching the gels by hand or unwashed, powdered gloves. Otherwise brown spots or dots will appear on the gel. Never let the gel dry out during the staining.

The developing solution, containing formaldehyde is unstable and therefore the formaldehyde should be added immediately before use. During development it is necessary to watch the gel. Do not use warm developer, this will lead to a too fast development and strong background. We recommend to prepare during the development a second tray with the stopping solution. Take out the properly developed

gel, put it into the stop solution and stop the development of each gel individually. For an immediate stop of developing reaction it is necessary to use a cold (+4°C) stopping solution.

Storage of the gels: put the gel on a sheet of normal white paper and cover it with a piece of an overhead foil. This sandwich is dried in a HOEFER SE 1160 Drygel Sr. Vacuum Gel Dryer System (Amersham-Pharmacia Biotech, Cat. No. 80-6119-33). Drying of two gels normally needs 1.5 hours. Carefully dried gels can be stored for years.

To recover the silver of the silver nitrate solution we precipitate the used solution with NaCl (VWR, cat.nr. 106404, for analysis), then filter it through Whatman 1 (pore size  $11\mu m$ , Cole-Parmer, Vernon Hills, IL 60061-1844, U.S.A.). Collect all used solutions and dispose them properly.

Dried gels were scanned for further evaluation.

**Results and Discussion:** In Figure 1 two examples, comparing the two techniques, are given. Comparison of the size marker shows the different distances in which fragments are separated in the two media, resulting in a better resolution of the bands in PAA. It has to be observed that the proportion of distances are not comparable. Duplex DNA migrates at rates that are inversely proportional to the log 10 of their size. However, while in PAA electrophoretic mobility is also affected by base composition and sequence, DNAs of exactly the same size can differ in mobility by up to 10%, in agarose only the number of base pairs matters (5).

In summary, separation of RAPD amplification products in PAA instead of agarose results in the following advantages: (1) More bands are produced, even though reproducibility of weak bands is not improved. (2) Band separation is clearer: single bands are often resolved in two bands, leading to an increase of polymorphic bands.(3) Less DNA and PCR reaction ingredients are needed. (4) Better cost efficiency due to the lower price for PAA compared to agarose (Calculation of the cost per lane revealed an almost 60% lower price for PAA than for agarose). (5) The use of mutagenic ethidium bromide is avoided. (6) The original PAA gels can be stored indefinitely.

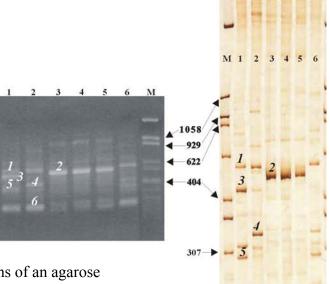
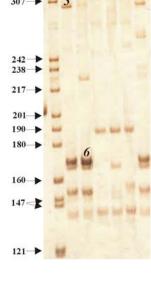


Fig.1: Corresponding sections of an agarose and an acrylamide gel loaded with the same amplification products 1 to 6 as listed in material and methods. Size marker is in both cases: pBR322/MspI + BstNI. Bands are numberd (*italic*) correspondingly to enable a better comparison.

A: The resolution in PAA is much higher especially below 400bp. The single polymorphic band in lane 3, 4 and 5 at 190bp is not visible on the agarose gel. Fragment 6 seems to be a single band on agarose, in PAA it is resolved in two, the weaker band being polymorphic in lane 3 and 5.

B: On PAA there are clear double bands at  $\sim$ 1000bp and at  $\sim$ 400bp (1,4) which are not visible on the agarose gel.



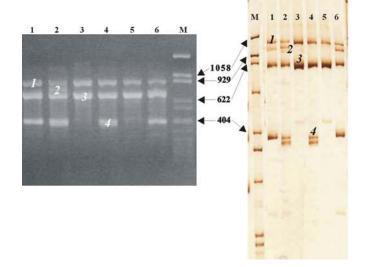


Table 1. Silver staining protocol.

Step	Solution	Time
Fixing	Fixer Solution	30 min
Staining	Staining Solution	30 min
Wash	A.bidest	1 min
Developing	Developer	4-10 min
Stop	Stopping Solution	3 min
Rinse	A.bidest	3 min
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## The Cultivation and Cucurbitacin Content of *Ecballium elaterium* (L.) A. Rich.

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**Introduction**: Although *Ecballium elaterium* (L.) A. Rich., is considered as a minor crop in the Cucurbitaceae family. However, its medicinal virtues (3, 8) and its resistance to pests and diseases (6, 7), have made the plant a suitable candidate to improve crop quality in Cucurbitaceae species. Cultivation studies were aimed at assessing the growth of the plant and the variation of cucurbitacins in the plant tissue with a change in the seasons.

Materials and Methods: Cultivation Studies. Squirted E. elaterium seeds were obtained from mature fruit collected from the Southern region of Malta. The seeds (n=50) were washed with distilled water, and seed coat cracked slightly (4) and placed overnight in a beaker with distilled water. The treated seeds were placed in seed trays and allowed for several weeks to germinate. The germinated seedlings were placed in Jiffy® pots (Sigma) and placed in a growth chamber at 24°C and a relative humidity of about  $95 \pm 5$  %. When root tips emerged from the Jiffy® pots repotting was performed in normal pots. The plants were routinely watered twice daily. When an apical height of 90 - 100 mm was reached, the plants were planted in soil. They were allowed to grow for one year from September 1998 till August 1999.

Elaterium and Cucurbitacin Contents. At monthly intervals, two plants were sacrificed and the fruits, stems and leaves were gathered. The juice was extracted from each plant part by homogenization and filtration. In the case of stems and leaves, distilled water was added to aid the extraction of the juice. The juice obtained was dried in an oven at 40 °C, to obtain the dried elaterium. The cucurbitacin content was determined as described previously (1) using cucurbitacin E as the reference for the total cucurbitacin content

Statistical Analysis. The results were analysed statistically by the one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test for equality of means. Only p $\leq$ 0.05 were considered statistically significant.

Results and Discussion: Cultivation Studies. Although dormancy is the main problem with the seeds this was overcome by slightly cracking the seed coat (5) and then immersed in water overnight. It was noted that germination was two-staged (figure 1), with 40 % of the seeds germinating within the first 38 days. There was an average of 1 seed germinating per day for the first 45 days and 2 seeds per day for the following 10 days. The highest percentage of seed germination was 73.8 % achieved after 55 days, which persisted over the last 14 days of the experiment. Costich (5) determined that the maximum germination was 61 % by three months. The latter lacked stratification treatment. From figure 2, it was observed that there was constant growth for the plantlets with slight peaking at 21 and 63 days from seed germination. This peaking indicated a change in medium volume to accomodate better the growing roots. On transfer of the seedlings to the compost pots after 14 days, there was a rapid increase in growth, followed by a slowing down, probably related to root expansion. After 63 days, the roots reached maximum capacity in the medium, and so transfer to larger pots was necessary. This further enhanced plant growth. There was exponential growth between days 63 and 91, after which there was retarded growth. This indicated a change in the volume of medium. Ecballium elaterium is a plant that has a proliferative rooting system, therefore root expansion is essential for this plant.

Elaterium and Cucurbitacin Contents. High elaterium contents were mainly obtained in leaves compared to that in stems and fruit. Also accumulation of elaterium in leaves is temperaturedependent. In fact, the higher the temperature, the higher the accumulation of elaterium in the leaves, indicating that the metabolic and photosynthetic activities in the leaves are directly correlated with temperature. However, the extraction of elaterium from leaves is much more laborious and destructive. So the fruit still remain the plant part of choice for elaterium collection. In fruit, elaterium production was low in the flowering periods (March and

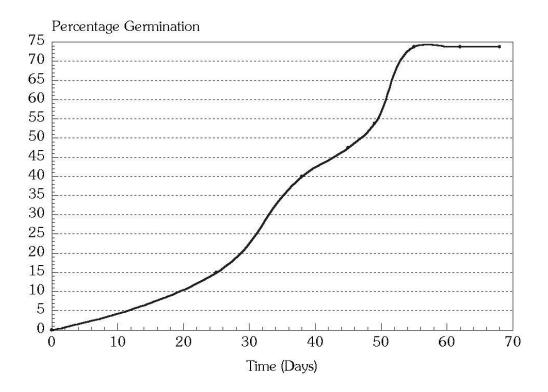


Figure 1. The percentage germination of E. elaterium seeds with time

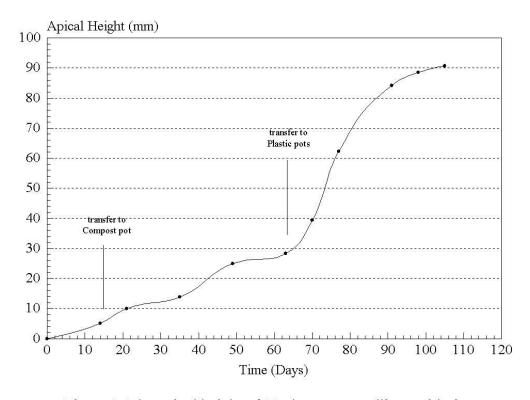


Figure 2. The apical height of *E. elaterium* seedlings with time

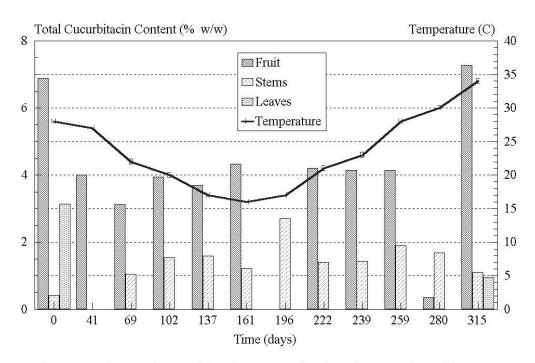


Figure 3. The total cucurbitacin content in elaterium produced from E. elaterium fruit, stems and leaves with time and temperature.

September, 0 and 0.297 % w/w respectively) but high in the fruiting periods (May-August and October-January, 1.4206 and 1.255 % w/w respectively). The constant but low yields of elaterium in the stem suggest that the elaterium is only transported through the stem to the different plant parts.

The total cucurbitacin content (figure 3) in elaterium produced from fruit, stems and leaves was also studied over the 315-day period. The fruit showed the highest mean total cucurbitacin content (3.84 % w/w), followed by the stems (1.34 % w/w) and then by the leaves (0.34 % w/w). The stems are involved in the transport of the cucurbitacins but only the fruit are associated with storage. Although the leaves contained a low concentration of cucurbitacins or none at all, the role of cucurbitacins is still important as antifeedants. For example, the bitterness of cucurbitacin E is experienced at a concentration of 10 ppb, (5), a concentration that is not detectable by the quantitative methods used. Balbaa and co-workers (2) determined a cucurbitacin content in the fruit 40 times greater than that in the leaves. In the present study, considering the fresh plant material, the cucurbitacin content in the fruit is about 22 times greater than that of the leaves. Therefore, the content of cucurbitacins in the leaves is adequate to promote the antifeedant and antimicrobial properties of the plant.

The elaterium and cucurbitacin contents of ripe and unripe fruit did not vary significantly (p>0.1, v=11). Lower elaterium contents in ripe fruit indicate a higher water content, a factor leading to the build-up of pressure inside the fruit and hence juice expression.

From this study, it can be concluded that the production of elaterium and particularly cucurbitacins reaches a peak during the active growth of the plant in the summer months, which coincides with the active growth and fruiting of several cucurbitaceous plants. As a result, *E. elaterium* can be cultivated

adjacent to edible cucurbitaceous plants in order to protect them from several pest and diseases.

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## Habitat Studies for the Wild Stocks of *Ecballium elaterium* (L.) A. Rich.

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**Introduction**: *Ecballium elaterium* (L.) A. Rich. (Cucurbitaceae), is a wild Mediterranean medicinal plant which has been described to thrive in drastic environmental conditions. In one study, it has been described to be frost-tolerant as compared to other wild species of the Cucurbitaceae family (4). This study was conducted on *E. elaterium* wild stocks growing in Malta (Central Mediterranean), on several soil types in order to determine the soil conditions ideal for the growth of this disease-resistant wild species (1).

**Materials and Methods**: Four sites representative of the different soil types in Malta were selected for the habitat studies of *E. elaterium* wild stocks. The localities are illustrated in Figure 1, while the description of the soil types is given in Table 1. The experiments were subdivided into two groups:

- (a) The soil physical characteristics. The soil moisture content was determined by air-drying the soil until a constant dry weight was obtained. The percentage weight loss corresponded to the moisture content. The gravel and soil contents were determined by using a 2-mm sieve to separate the large stones from the fine soil particles.
- (b) The soil chemical characteristics. The pH and conductivity were determined using a pH meter (Dulcometer, from Prominent, Germany) and a conductivity meter (Jenway, U.S.A.). The organic matter was determined by using the dichromate oxidation test (3) while the carbonate content was determined using the sodium hydroxide-hydrochloric acid titration.

The results were analysed statistically by the one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test for equality of means. Only  $p \le 0.05$  were considered statistically significant.

**Results and Discussion**: Soil Physical Characteristics. The moisture, gravel and soil contents are shown in Table 1. There was no

statistically significant difference in the moisture contents of the four soil types (p>0.1, v=19), while a statistical significance was recorded for the gravel and soil contents (p<0.0001, v=19). The highest difference was found in the carbonate raw soil that contained the highest soil content and the lowest gravel content, than the other soil types.

Soil Chemical Characteristics. The results for the four chemical parameters studied are shown in Table 2. Most Maltese soils have a pH of normally above 7.5 to 9. The mean soil pH values for the four E. elaterium habitats range between 8.07 and 8.65. The ANOVA analysis shows a significant difference between the soils (p<0.0001, v=19) even though their pH's lie within the same range. The results obtained suggested that E. elaterium thrives on soils or disturbed land with an alkaline nature. As regards the electrical conductivity, the Terra type and the brown rendzina type different significantly from the other two (p<0.0001, v=19). The readings suggest that the sites at Marsascala, Mellieha and Rabat are practically saline-free while that at Siggiewi is slightly saline.

The results indicate that the plant lives on soils with a very low salinity or none at all. The different sites exhibited a great variability in the organic matter content. Terra soils such as that at Marsascala (Terra rossa type) have a high organic matter content of about 3.1 % (5). For the site studied, the mean organic matter content was of 1.847 %. This may be due to the fact that the soil was rather disturbed containing rubble and hence decreasing the organic matter in it. The same problem took place with the carbonate raw soil at Rabat (Fiddien series), where the organic matter content was about one-third the value stated earlier by Sacco (5). In the case of rendzina soils, i.e. Siggiewi and Mellieha, these gave a reasonable organic matter content (3.829 and 2.001 %) as compared to those in the mentioned study (5), i.e. a mean of 2.0 %. The organic matter content does not affect the ability of the plant to grow. The plant produces its own organic material. Underneath the

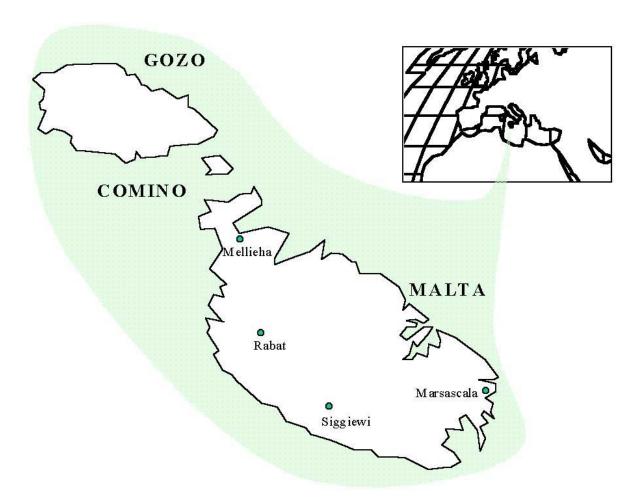


Figure 1. The localities chosen for soil sample collection.

Table 1. The moisture, gravel and soil contents of the different soils on which E. elaterium grows.<sup>z</sup>

Localities	Soil Description	% Moisture Conten	t % Gravel Content	% Soil Content
M'Scala	Terra soil (Terra rossa type)	18.6600 ± 1.7106 (2.9629)	$46.8467 \pm 0.9192$ $(1.5921)$	$53.1533 \pm 0.9192$ $(1.5921)$
Siggiewi	Rendzina soil (Xerorendzina type)	$17.9300 \pm 2.7900$ $(3.9457)$	$29.9750 \pm 0.1150$ (0.1626)	$70.0250 \pm 0.1150$ $(0.1626)$
Meffieha	Rendzina soil (Brown rendzina type)	$10.5000 \pm 1.0800$ $(1.5274)$	$36.1600 \pm 0.5200$ $(0.7354)$	$63.8400 \pm 0.5200$ $(0.7354)$
Rabat	Carbonate raw soil (Fiddien series)	$17.7900 \pm 2.2303$ (3.8629)	$6.5033 \pm 1.6734 \\ (2.8985)$	$93.4967 \pm 1.6734$ $(2.8985)$
ANOVA		p>0.1232	p<0.0001 p<0.0001	

<sup>&</sup>lt;sup>z</sup> Values represent means  $\pm$  S.E.M. of 3 determinations. ANOVA results are also tabulated (v=19).

Table 2. Soil pH, electrical conductivity, the organic matter and carbonate contents of the different soils on which *P. elaterium* grows.<sup>2</sup>

Localities	рН	Conductivity (mS/cm)	Organic Matter	Carbonate
M'Scala	$8.6483 \pm 0.0210$ (0.0515)	$1.1083 \pm 0.0260$ $(0.0637)$	$1.8447 \pm 0.0025$ $(0.0061)$	$53.8583 \pm 0.4696$ (1.1502)
Siggiewi	$8.3000 \pm 0.0141$ $(0.0283)$	$6.1175 \pm 0.1091$ $(0.2182)$	$3.8297 \pm 0.3024$ (0.6048)	$53.6925 \pm 0.4760$ (0.9521)
Mellieha	$8.0750 \pm 0.0222$ $(0.0443)$	$1.2500 \pm 0.0147$ $(0.0294)$	$2.0019 \pm 0.0966$ (0.1932)	$54.2450 \pm 0.1444$ (0.2887)
Rabat	$8.1667 \pm 0.0169$ $(0.0413)$	$3.5100 \pm 0.0605$ $(0.1482)$	$0.5443 \pm 0.0011$ (0.0026)	$83.3067 \pm 0.3091$ (0.7571)
ANOVA	p<0.0001	p<0.0001	p<0.0001	p<0.0001

<sup>&</sup>lt;sup>z</sup> Values represent means  $\pm$  S.E.M of 4-6 determinations. Standard deviation values are indicated in brackets. ANOVA results are also tabulated (v=19).

vegetative canopy, it is separated from the soil by dead material from the plant itself.

Although terra soils have a low carbonate content (<15 %) (2), in the case of the Marsascala site, the carbonate content was excessively high (i.e. 53.86 %). This might be reflected in the fact that this soil was loaded with limestone rubble (as it is a wasteland) and hence retaining a high amount of carbonate even with weathering. On the other hand, since the sites at Siggiewi and Mellieha are derived from fields with 'no limestone contaminants', the levels of carbonate were on the lower scale (53.69 and 54.25 %, respectively) of the range (55 - 80%) (2). For the carbonate raw soil at the Rabat site, the carbonate content (83.31 %) fell within the range of 80 to 90 % (2).

From the results obtained, it was concluded that E. elaterium grows on calcerous soils (53.86 – 83.31 %) that are salinity free or slightly saline (0.497 – 2.807 ppm), and with a high pH (8.08 – 8.65) and variable

organic matter content (0.54 - 3.83 %). This study consolidates the resistance of *E. elaterium* to alkaline and calcerous soils.

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## In vitro Propagation of Pointed Gourd (Trichosanthes dioica Roxb.)

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Pointed gourd (*Trichosanthes dioica* Roxb.) is one of the most nutritive cucurbit vegetables holds a coveted position in the Indian market during summer and rainy season. It is a perennial crop highly accepted due to its availability for eight months in a year (February–September). Being very rich in protein and vitamin A, it has certain medicinal properties and many reports are available regarding its role in circulatory system especially in lowering blood sugar and serum triglycerides (5). The fruits are easily digestible and diuretic in nature. It is also known to have antiulcerous effects (6).

Traditionally *T. dioica* is multiplied through seeds, stem cuttings and root cuttings. Propagation through seeds is not desirable due to poor germination and imbalanced male-female ratio. Seed based populations have a tendency to give more male then female plants and in some cases the ratio goes upto 85:15 (6), limiting their use as their utility ends with pollination. Additionaly, due to dioecy and resulting cross-pollinated, the maintenance of true to type plant is another major problem. Stem and root cutting are labour intensive and also requires bulk amount of vines/roots, which restricts their multiplication at commercial level. In vitro multiplication of elite clones will be an attractive approach in order to meet the requirement of quality propagules at large scale for commercial cultivation. Hence the present investigation was carried out to establish rapid in vitro propagation of a superior line of pointed gourd.

Materials and Methods: Young vines of *T. dioica*-VRPG 101 (an advance breeding line) were collected from polyhouse grown plants. Shoot tip and nodal portions of vine were excised and washed with 1.0 % Cetrimide solution followed by surface sterilization with 0.1% HgCl<sub>2</sub> for 5 minutes. Finally explants were rinsed 4-5 times with sterile double distilled water. For establishment of cultures, explants were inoculated onto hormone free half strength MS medium (3). All the cultures including shoot initiation, and shoot growth cultures were kept in culture room maintained at 25±2°C with 16 hour

photoperiod provided, The established shoots were served as mother stock. For further multiplication, shoot tip and nodal portions were excised from mother stock and cultured onto MS medium supplemented with different BA concentrations (8.88, 17.76, 26.64, 35.52 and 44.44 µM). Multiplication rate per explant was determined at 4 week interval excluding the basal nodes which was cultured initially and the data was analyzed statistically using Analysis of variance (RBD). For root induction, in vitro raised shoots were cultured onto MS medium supplemented with different concentrations of IBA or NAA. Shoots with primary and secondary roots were transferred to pots containing soil and sand mixture. Plantlets were kept under controlled condition and irrigated with ¼ strength MS medium. Finally they were transferred to field for evaluation.

Results and Discussion: Development of efficient reproducible regeneration protocol from cells/tissues is a pre-requisite for the successful application of recent cellular manipulation techniques for the improvement of crop plants. In this direction the choice of explants is of cardinal importance and makes an absolute difference between success and failure in inducing regeneration in vitro. The prime objective in this study was to get true to type plants using shoot tips and nodal portions as explants. Also the establishment of aseptic culture is must in any in vitro study, in T. dioica, the aseptic cultures were obtained by washing the explant in 1.0 % Cetrimide solution, followed by 0.1% mercuric chloride solution for 5 minutes as surface sterilization. Shoot bud development was visible within a week following inoculation of explants. Multiple shoot induction was observed on all the media tested except medium devoid of any growth regulator. Significantly higher number of shoots per explant (5.78), number of nodes/shoot (6.11) and longest shoot (3.57 cm) were observed on the medium containing 8.88 µM BA. This is an improvement of 5-6 times in the multiplication rate as compared to earlier reports. Higher concentrations of BA (i.e. 17.76, 26.64, 35.82 and 44.44 µM) produced large

amount of callus, which suppressed shoot elongation. Josekutty et al (2) reported direct organogenesis in *Coccinia indica* on the medium containing 11.0  $\mu$ M BA, 2.32  $\mu$ M Kinetin and 0.49 $\mu$ M IBA. Similar kind of results were also reported by Hoque et al. (3) in which, shoot formation was achieved in seedling explants of *T. dioica* on MS medium supplemented with 4.44  $\mu$ M BA. However, Hoque et al (1) used cotyledons rescued from physiologically matured and immature seeds. In pointed gourd multiplication through seeds is not feasible.

Among the media tested for root induction a very low concentration of IBA (0.49  $\mu$ M) produced significantly higher number of roots per shoot. Higher concentrations of IBA favored formation of malformed and thick roots. In most of the cucurbits the root induction was achieved on either basal MS medium alone or with very low level of auxin (4). In the present study, rooting on medium containing even a very low level of NAA (0.27  $\mu$ M) induced formation of thick, hairy and malformed roots, which were not suitable for pot transfer. The rooted platelets were transferred to pots for acclimatization and hardening and finally they were transferred to field for further growth.

The significant aspect of this work is that as compared to 8-10 plants produced by conventional root cutting, it gives a much higher multiplication rate (5.78 times in 4 weeks) from small portion of tissue with true to type plants. Another point is that

shoots continue to elongate even after transferring them to root regenerating medium. Further, a large number of plants are being produced for distribution to the farmers to fulfill local demands of quality propagules.

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#### **Gene List for Watermelon**

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This is the latest version of the gene list for watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai). The watermelon genes were originally organized and summarized by Poole (1944). ). The list has been expanded by Robinson et al. (1976), the Cucurbit Gene List Committee (1979, 1982, and 1987), Henderson (1991 and 1992), Rhodes and Zhang (1995), and Rhodes and Dane (1999). The current list provides an update of the known genes of watermelon. This year, the list has 162 total mutants, grouped into seed and seedling mutants, vine mutants, flower mutants, fruit mutants, resistance mutants, protein (isozyme) mutants, DNA (RFLP and RAPD) markers, and cloned genes.

Researchers are encouraged to send reports of new genes, as well as seed samples of lines containing the gene mutant to the watermelon gene curator (Todd C. Wehner), or to the assistant curator (Stephen R. King). Please inform us of omissions or errors in the gene list. Scientists should consult the list as well as the rules of gene nomenclature for the Cucurbitaceae (Cucurbit Gene List Committee, 1982; Robinson et al., 1976) before choosing a gene name and symbol. Please choose a gene name and symbol with the fewest characters that describes the recessive mutant, and avoid use

of duplicate gene names and symbols. The rules of gene nomenclature were adopted in order to provide guidelines for naming and symbolizing genes. Scientists are urged to contact members of the gene list committee regarding rules and gene symbols. The watermelon gene curators of the Cucurbit Genetics Cooperative are collecting seeds of the type lines for use by interested researchers, and would like to receive seed samples of any of the lines listed.

This gene list has been modified from previous lists in that we have 1) added or expanded the description of the phenotypes of many of the gene mutants, 2) added descriptions for phenotypes of interacting gene loci, 3) identified the type lines that carry each form of each gene, 4) identified the gene mutant lines that are in the curator collections, and 5) added genes that have not previously been described (cr. Ctr. dw-3, eg. ms2, Ti, ts and zvm). Initially, we had intended to include a review of gene linkage, but few reports were found except for sets of molecular markers in wide crosses of Citrullus. Additional work is needed in this area to measure linkages and clarify gene interactions. Finally, we attempted to correct some of the errors in gene descriptions or references from previous lists.

Table 1. The genes of watermelon.

Gene	Syno nym	Character	References <sup>z</sup>	Supplemental references <sup>z</sup>	Avai lable
а	-	andromonoecious; recessive to monoecious; a from 'Angeleno' (black seeded); A from cultivars 'Conqueror' and 'Klondike'.	Rosa, 1928	Porter, 1937; Poole, 1944	С
Aco-1	-	Aconitase-1.	Navot et al., 1990	_	M
Aco-2	-	Aconitase-2.	Navot et al., 1990	-	M
Adh-1	-	Alcohol dehydrogenase-1; one of five codominant alleles, each regulating one band	Navot and Zamir 1986, 1987; Zamir et al., 1984	-	M
Adh- 1 <sup>1</sup>	-	Alcohol dehydrogenase-1 <sup>1</sup> ; one of five codominant alleles, each regulating one band; found in <i>C. lanatus</i> var. <i>citroides</i> and <i>C. colocynthis</i> .	Navot and Zamir 1986, 1987; Zamir et al., 1984	-	M
Adh- 1 <sup>2</sup>	-	Alcohol dehydrogenase-1 <sup>2</sup> ; one of five codominant alleles, each regulating one band; found in <i>C. lanatus</i> var. <i>citroides</i> and <i>C. colocynthis</i> .	Navot and Zamir 1986, 1987; Zamir et al., 1984	-	M
Adh- I <sup>3</sup>	-	Alcohol dehydrogenase-1 <sup>3</sup> ; one of five codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot and Zamir 1986, 1987; Zamir et al., 1984	-	M
Adh- 1 <sup>4</sup>	-	Alcohol dehydrogenase-1 <sup>4</sup> ; one of five codominant alleles, each regulating one band; found in Acanthosicyos naudinianus.	Navot and Zamir 1986, 1987; Zamir et al., 1984	-	M
Af	-	Aulacophora faveicollis resistance; resistance to the red pumpkin beetle; dominant to susceptibility; Af from Sl.72 and Sl.98 inbreds; af from 'Sugar Baby'.	Vashishta and Choudhury, 1972	-	?
Aps-1	Acph -A	Acid phosphase-1.	Navot et al., 1990; Navot and Zamir 1986, 1987; Zamir et al., 1984	-	M
Aps- 2 <sup>1</sup>	-	Acid phosphatase-2 <sup>1</sup> ; one of two codominant alleles, each regulating one band; found in <i>C. lanatus</i> and <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir 1986, 1987	-	M
Aps- 2 <sup>2</sup>	-	Acid phosphatase-2 <sup>2</sup> ; one of two codominant alleles, each regulating one band; found in Acanthosicyos naudinianus.	Navot et al., 1990; Navot and Zamir 1986, 1987	-	M
Ar-1	B,Gc	Anthracnose resistance to races 1 and 3 of Glomerella cingulata var. orbiculare (Colletotrichum lagenarium); Ar-1 from 'Africa 8'*, 'Africa 9'*, and 'Africa 13'* and 'Charleston Gray'**; ar-1 from 'Iowa Belle 476', 'Iowa Belle 487'* and N.C.9-2, N.C.11, and 'New Hampshire Midget'**.	Layton 1937*	Hall et al., 1960; Robinson et al., 1976; Winstead et al., 1959**	С
$Ar-2^{I}$	-	<i>Anthracnose resistance to race 2</i> of	Winstead et al., 1959*	Love and	P

			T	I	1
		Colletotrichum lagenarium; Ar-2- <sup>1</sup> from W695 citron* and PI 189225, PI 271775, PI 271779, and PI 299379**; ar-2-1 from		Rhodes, 1988***, 1991; Sowell et al.,	
		'Allsweet', 'Charleston Gray', and 'Florida		1980**;	
		Giant'; resistance in <i>Citrullus colocynthis</i> is due to other dominant factors; resistance from R309***; susceptibility from 'New		Suvanprakorn and Norton, 1980	
В	Y	Hampshire Midget'.  Yellow flesh; Wf is epistatic to B (Y renamed B by Henderson); WfWf BB or WfWf bb white fleshed; wfwf BB yellow fleshed; wfwf bb red fleshed; B from	Shimotsuma, 1963	Henderson, 1992	?
		breeding line V.No.3 and <i>b</i> from V.No.1; flesh color segregated into 12 white, 3 yellow and 1 red in the F2.			
С	-	Canary yellow flesh; dominant to pink; i inhibitory to C, resulting in red flesh; in the absence of i, C is epistatic to Y; CC from 'Honey Cream'*, cc from 'Dove'*; CC YY I-C I-C from 'Yellow Baby' F1**	Poole, 1944*	Henderson et al., 1998**	С
		and 'Yellow Doll' F1**; cc y <sup>o</sup> y <sup>o</sup> I-C I-C from 'Tendersweet Orange Flesh'**; cc yy I-C I-C from 'Golden Honey'**; cc YY i-C i-C from 'Sweet Princess'**.			
Ctr		Cool temperature resistance; Ctr from line PP261-1 (a single plant selection of PI 482261 from Zimbabwe); ctr from 'New Hampshire Midget'; resistant to leaf mosaic injury when grown at air temperature below 20°C.	Provvidenti, 1992	Provvidenti, 2003	P
cr		cracked seed coat; recessive to Cr (non-cracked) seed coat; cr from 'Leeby' and Cr from 'Kaho' and 'Congo'.	El-Hafez et al., 1981	-	?
d	-	dotted seed coat; black dotted seeds when dominant for color genes $r$ , $t$ , and $w$ ; $d$ is a specific modifier of black seed coat color wherein $RR$ $TT$ $WW$ $DD$ is solid black and $RR$ $TT$ $WW$ $dd$ is dotted black seed coat; $d$ from 'Klondike' and 'Hope Giant'; $D$ from 'Winter Queen'.	Poole et al., 1941	Poole, 1944; Kanda, 1951	С
db	-	Resistance to gummy stem blight caused by Didymella bryoniae; db from PI 189225; Db from 'Charleston Gray'.	Norton, 1979	-	P
dg	-	delayed green; cotyledons and young leaves are initially pale green but later develop chlorophyll; first reported to be hypostatic to <i>I-dg</i> ; more recent evidence indicates a simple recessive; <i>dg</i> from breeding line 'Pale 90'; <i>Dg</i> from 'Allsweet'.	Rhodes, 1986	-	?
Dia-1	_	Diaphorase-1	Navot et al., 1990	-	M
dw-1	-	dwarf-1; short internodes, due to fewer and shorter cells than normal forms; allelic	Mohr, 1956	Liu and Loy, 1972	С

		to dw-1 <sup>s</sup> ; dw-1 from 'Bush Desert King'; Dw-1 from 'Sugar Baby' and 'Vine Desert King'.			
dw-1 <sup>s</sup>	-	short vine; allelic to dw-1; vine length intermediate between normal and dwarf; hypocotyl somewhat longer than normal vine and considerably longer than dwarf; dw-1 <sup>s</sup> recessive to normal; dw-1 <sup>s</sup> from 'Somali Local' (All-Union Research Institute of Plant Growing No.4641).	Dyutin and Afanas'eva, 1987	-	?
dw-2	-	dwarf-2; short internodes, due to fewer cells; dw-1 from inbred line WB-2; Dw-2 from 'Sugar Baby' and 'Vine Desert King'.	Liu and Loy, 1972	Mohr and Sandhu, 1975	?
dw-3		dwarf-3; dwarf with fewer leaf lobes (intermediate between normal leaf and non-lobed leaf); dw-3 from 'Dwarf Male-Sterile Watermelon (DMSW)'; Dw-3 from 'Changhui', 'Fuyandagua', and 'America B'.	Hexun et al., 1998	-	?
e	t	explosive rind; thin, tender rind, bursting when cut; e from 'California Klondike'; E from 'Thurmond Gray'.	Porter, 1937	Poole, 1944	?
eg	-	egusi seed; immature seeds with fleshy pericarp, becoming normal at maturity; eg from PI 490383 selection NCG-529 and PI 560006; Eg from 'Calhoun Gray' and 'Charleston Gray'.	Gusmini et al., 2003	-	С
Est-1	-	Esterase-1; one of six codominant alleles, each regulating one band; found in C. lanatus.	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-1	-	Esterase-1 <sup>1</sup> ; one of six codominant alleles, each regulating one band; found in <i>C. lanatus</i> var. <i>citroides</i> and <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-1 <sup>2</sup>	-	Esterase-1 <sup>2</sup> ; one of six codominant alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-1 <sup>3</sup>		Esterase-1 <sup>3</sup> ; one of six codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-1 <sup>4</sup>	-	Esterase-1 <sup>4</sup> ; one of six codominant alleles, each regulating one band; found in <i>C. ecirrhosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-1 <sup>5</sup>	-	Esterase-1 <sup>5</sup> ; one of six codominant alleles, each regulating one band; found in Acanthosicyos naudinianus.	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-2	-	Esterase-2; one of five codominant alleles, each regulating one band; found in C. lanatus.	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-2	-	Esterase-2 <sup>1</sup> ; one of five codominant alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est- $2^2$	-	Esterase- $2^2$ ; one of five codominant	Navot et al., 1990;	-	M

		alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot and Zamir, 1986, 1987		
Est-2 <sup>3</sup>	-	Esterase-2 <sup>3</sup> ; one of five codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Est-2 <sup>4</sup>	-	Esterase-2 <sup>4</sup> ; one of five codominant alleles, each regulating one band; found in Acanthosicyos naudinianus.	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
f	-	furrowed fruit surface; recessive to smooth; type inbreds not given; f like 'Stone Mountain' or 'Black Diamond'; F like 'Mickylee'.	Poole, 1944	-	?
Fdp-1	-	Fructose 1,6 diphosphatase-1.	Navot et al., 1990; Navot and Zamir, 1986	-	M
Fo-1	-	Fusarium wilt resistance for race 1; dominant gene for resistance to race 1 of Fusarium oxysporum f. sp. niveum; Fo-1 from 'Calhoun Gray' and 'Summit'; fo-1 from 'New Hampshire Midget'.	Henderson et al., 1970	Netzer and Weintall, 1980	С
For-1	-	Fructose 1,6 diphosphatase-1.	Navot et al., 1990	-	M
Fwr	-	Fruit fly resistance caused by Dacus cucurbitae; dominant to susceptibility; Fwr from breeding lines J 18-1 and J 56-1; fwr from 'New Hampshire Midget', 'Bykovski', 'Red Nectar' and breeding line 'J 20-1'.	Khandelwal and Nath, 1978	-	?
g	d	light green fruit rind pattern; light green fruit recessive to dark green ( <i>G</i> ) and striped green ( <i>g</i> <sup>s</sup> ); <i>g</i> from 'Thurmond Gray' and <i>G</i> from 'California Klondike'.	Weetman, 1937	Poole, 1944; Porter, 1937	?
g <sup>s</sup>	ď	striped green fruit rind pattern; recessive to dark green but dominant to light green skin; $g^s$ from 'Golden Honey'; $G$ from 'California Klondike'.	Weetman, 1937	Poole, 1944	С
Gdh- 1	-	Glutamate dehydrogenase-1; isozyme located in cytosol.	Navot and Zamir, 1986	-	M
Gdh- 2	-	Glutamate dehydrogenase-2; isozyme located in plastids.	Navot et al., 1990; Navot and Zamir, 1986	-	M
gf	-	light green flower color; gf from 'KW-695' and 'Dalgona'; Gf from Korean watermelon accession 'SS-4'.	Kwon and Dane, 1999	-	?
gms	$ms_g$	glabrous male sterile; foliage lacking trichomes; male sterile caused by chromosome desynapsis (named glabrous male sterile by Robinson*); gms from 'Sugar Baby' irradiated with gamma rays.	Watts, 1962, 1967	Robinson et al., 1976*; Ray and Sherman, 1988	?
L	+	golden yellow color of older leaves and	Barham, 1956	Robinson et al.,	С

		mature fruit; (named golden by		1976*	
		Robinson*); go from 'Royal Golden'; Go from 'NC 34-9-1' and 'NC 34-2-1'.			
Got-1	-	Glutamate oxaloacetate transaminase-1;	Navot et al., 1990;	_	M
		one of four codominant alleles, each	Navot and Zamir,		
		regulating one band; found in <i>C. lanatus</i> .	1986, 1987;		
			Zamir et al., 1984		
Got-	-	Glutamate oxaloacetate transaminase-1;	Navot et al., 1990;	-	M
$I^{I}$		one of four codominant alleles, each	Navot and Zamir,		
		regulating one band; found in <i>C</i> .	1986, 1987; Zamir et		
		colocynthis and Praecitrullus fistulosus.	al., 1984		
Got-	-	<i>Glutamate oxaloacetate transaminase-<math>1^2</math>;</i>	Navot et al., 1990;	-	M
$I^2$		one of four codominant alleles, each	Navot and Zamir,		
		regulating one band; found in <i>C. lanatus</i>	1986, 1987; Zamir et		
		var. citroides.	al., 1984		
Got	_	Glutamate oxaloacetate transaminase-1 <sup>3</sup> ;	Navot et al., 1990;	_	M
$I^3$		one of four codominant alleles, each	Navot and Zamir,		
		regulating one band; found in	1986, 1987; Zamir et		
		Acanthosicyos naudinianus.	al., 1984		
Got-2	_	Glutamate oxaloacetate transaminase-2;	Navot et al., 1990;	1_	M
G01-2	_	one of five codominant alleles, each	Navot and Zamir,		141
		regulating one band; found in <i>C. lanatus</i> .	1986, 1987; Zamir et		
		regulating one band, round in C. tanatus.	al., 1984		
Got-		Glutamate oxaloacetate transaminase-2 <sup>1</sup> ;	Navot et al., 1990;		M
$2^{l}$	_		1	-	1V1
2		one of five codominant alleles, each	Navot and Zamir,		
		regulating one band; found in C.	1986, 1987; Zamir et		
Cat		colocynthis.	al., 1984		M
<i>Got-</i> 2 <sup>2</sup>	-	Glutamate oxaloacetate transaminase-2 <sup>2</sup> ;	Navot et al., 1990;	<del>-</del>	IVI
2		one of five codominant alleles, each	Navot and Zamir,		
		regulating one band; found in C.	1986, 1987; Zamir et		
Cat		ecirrhosus.	al., 1984		M
<i>Got-</i> 2 <sup>3</sup>	-	Glutamate oxaloacetate transaminase-2 <sup>3</sup> ;	Navot et al., 1990;	-	M
2		one of five codominant alleles, each	Navot and Zamir,		
		regulating one band; found in	1986, 1987; Zamir et		
<i>C</i> .		Praecitrullus fistulosus.	al., 1984		3.6
Got-	-	Glutamate oxaloacetate transaminase-2 <sup>4</sup> ;	Navot et al., 1990;	-	M
$2^4$		One of five codominant alleles, each	Navot and Zamir,		
		regulating one band; found in	1986, 1987; Zamir et		
<i>C</i> . 2		Acanthosicyos naudinianus.	al., 1984		3.6
Got-3	-	Glutamate oxaloacetate transaminase-3.	Zamir et al., 1984	-	M
Got-4	-	Glutamate oxaloacetate transaminase-4.	Navot et al., 1990;	-	M
			Zamir et al., 1984		
hen		heat shock protein 70; one gene	Wimmer et al., 1997		M
hsp-	-	1	willing et al., 199/	_	IVI
70		presequence 72-kDa hsp70 is modulated			
. 1		differently in glyoxysomes and plastids.	D1 1 1007		т
i-dg	-	inhibitor of delayed green; Epistatic to dg;	Rhodes, 1986	-	L
		<i>I-dg I-dg dgdg</i> plants are pale green; and <i>i-</i>			
		dg i-dg dgdg plants are normal; dg from			
		breeding line Pale 90; <i>Dg</i> from 'Allsweet';			

		i-dg gene was lost when advanced inbreds			
Idh-1		were made.  Isocitrate dehydrogenase-1	Zamir et al., 1984		M
i-C	i	inhibitor of canary yellow, resulting in red flesh (renamed by Rhodes and Dane*); CC YY I-C I-C from 'Yellow Baby' F1 and 'Yellow Doll' F1; cc y <sup>0</sup> y <sup>0</sup> I-C I-C from 'Tendersweet Orange Flesh'; cc yy I-C I-C from 'Golden Honey'; cc YY i-C i-C from 'Sweet Princess'.	Henderson et al., 1998	Rhodes and Dane, 1999*	С
ja	-	juvenile albino; chlorophyll in seedlings, leaf margins, and fruit rind reduced when grown under short days; ja from 'Dixielee mutant' and 'G17AB' F2; Ja from 'Sweet Princess' and '20J57'.	Zhang et al., 1996b	-	?
l	-	long (or large) seeds; interacts with s; long recessive to medium or short; LL SS for medium, ll SS for long, and LL ss or ll ss for short seed; ll SS from 'Peerless'; LL SS from 'Klondike'; LL ss from 'Baby Delight'.	Poole et al., 1941	-	?
Lap-1	-	Leucine aminopeptidase-1.	Navot et al., 1990; Navot and Zamir, 1986	-	M
m	-	<i>mottled skin</i> ; greenish white mottling of fruit skin; <i>m</i> from 'Iowa Belle' and <i>M</i> from 'Japan 4'.	Weetman, 1937	Poole, 1944	?
Mdh- I	-	Malic dehydrogenase-1; one of two codominant alleles, each regulating one band; found in C. lanatus.	Navot and Zamir, 1987; Zamir et al., 1984	-	M
<i>Mdh-</i> 1 <sup>1</sup>	-	Malic dehydrogenase-1 <sup>1</sup> ; one of two codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
Mdh- 2	-	Malic dehydrogenase-2; one of three codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot and Zamir, 1987	-	M
<i>Mdh-</i> 2 <sup>1</sup>	-	<i>Malic dehydrogenase-2</i> <sup>1</sup> ; one of three codominant alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot and Zamir, 1987	-	M
<i>Mdh-</i> 2 <sup>2</sup>	-	Malic dehydrogenase-2 <sup>2</sup> ; one of three codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot and Zamir, 1987	-	M
Me-1	-	Malic enzyme-1; one of three codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Me-1 <sup>1</sup>	-	Malic enzyme-1 <sup>1</sup> ; one of three codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Me-1 <sup>2</sup>	-	Malic enzyme-12; one of three codominant	Navot et al., 1990;	-	M

		alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot and Zamir, 1986, 1987; Zamir et al., 1984		
<i>Me-2</i>	-	Malic enzyme-2.	Zamir et al., 1984	-	M
ms-1	ms	male sterile; plants with small, shrunken anthers and aborted pollen; ms-1 from 'Nongmei 100'; Ms from most cultivars, e.g. 'Allsweet'.	Zhang and Wang, 1990	Zhang et al., 1994b	?
ms <sup>dw</sup>	-	male sterile, dwarf; ms <sup>dw</sup> from 'Dwarf Male-Sterile Watermelon (DMSW)'; non- dwarf fertile from 'Changhui', 'Fuyandagua', and 'America B'.	Huang et al., 1998	-	?
ms-2		<i>male sterile</i> with high seed productivity; <i>ms-2</i> from 'Kamyzyakskii'; <i>Ms-2</i> from cultivars like 'Allsweet'.	Dyutin, and Sokolov, 1990	-	?
nl	-	nonlobed leaves; leaves lack the typical lobing; incomplete dominance; (named nonlobed by Robinson*); nl from spontaneous mutation of 'Black Diamond'.	Mohr, 1953	Robinson et al., 1976*	С
0	-	Elongate fruit; incompletely dominant to spherical, so that <i>Oo</i> is oval; <i>O</i> from 'Long Iowa Belle'; <i>o</i> from 'Round Iowa Belle', 'China 23', 'Japan 4', and 'Japan 6'.	Weetman, 1937	Poole and Grimball, 1945	?
p	-	pencilled lines on skin; inconspicuous stripes; greenish-white mottling* (called pencilled by Robinson**); recessive to netted fruit; p from 'Long Iowa Belle' and P from 'Japan 6'.	Weetman, 1937*	Poole and Grimball, 1945; Robinson et al., 1976**	?
Pgd-1	6 Pgdh -1	6-Phosphogluconate dehydrogenase-1; one of three codominant alleles, each regulating one plastid band; found in <i>C. lanatus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgd- 1	6 Pgdh -1	6-Phosphogluconate dehydrogenase-1 <sup>1</sup> ; one of three codominant alleles, each regulating one plastid band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgd- 1 <sup>2</sup>	6 Pgdh -1 <sup>2</sup>	6-Phosphogluconate dehydrogenase-1 <sup>2</sup> ; one of three codominant alleles, each regulating one plastid band; found in <i>Acanthosicyos naudinianus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgd-2	6 Pgdh -2	6-Phosphogluconate dehydrogenase-2; one of five codominant alleles, each regulating one cytosolic band; found in <i>C. lanatus</i> .	Navot and Zamir, 1986; Zamir et al., 1984	-	M
Pgd- 2 <sup>I</sup>	6 Pgdh -2 <sup>1</sup>	6-Phosphogluconate dehydrogenase-2 <sup>1</sup> ; one of five codominant alleles, each regulating one cytosolic band; found in <i>C. ecirrhosus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
<i>Pgd-</i> 2 <sup>2</sup>	6 Pgdh	6-Phosphogluconate dehydrogenase-2 <sup>2</sup> ; one of five codominant alleles, each	Navot and Zamir, 1987; Zamir et al.,	-	M

	-2 <sup>2</sup>	regulating one cytosolic band; found in <i>Praecitrullus fistulosus</i> .	1984		
Pgd- 2 <sup>3</sup>	6 Pgdh -2 <sup>3</sup>	6-Phosphogluconate dehydrogenase-2 <sup>3</sup> ;	Navot and Zamir, 1987; Zamir et al., 1984	-	M
Pgd- 2 <sup>4</sup>	6 Pgdh -2 <sup>4</sup>	6-Phosphogluconate dehydrogenase-2 <sup>4</sup> ; one of five codominant alleles, each regulating one cytosolic band; found in <i>Acanthosicyos naudinianus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
Pgi-1	-	Phosphoglucoisomerase-1; one of three codominant alleles, each regulating one plastid band; found in <i>C. lanatus</i>	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	М
Pgi-1 <sup>1</sup>	-	Phosphoglucoisomerase-1 <sup>1</sup> ; one of three codominant alleles, each regulating one plastid band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Pgi-1 <sup>2</sup>	-	Phosphoglucoisomerase-1 <sup>2</sup> ; one of three codominant alleles, each regulating one plastid band; found in <i>Acanthosicyos naudinianus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Pgi-2	-	Phosphoglucoisomerase-2; one of six codominant alleles, each regulating one cytosolic band; found in <i>C. lanatus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgi-2 <sup>1</sup>	-	Phosphoglucoisomerase-2 <sup>1</sup> ; one of six codominant alleles, each regulating one cytosolic band; found in <i>C. lanatus and C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgi-2 <sup>2</sup>	-	Phosphoglucoisomerase-2 <sup>2</sup> ; one of six codominant alleles, each regulating one cytosolic band; found in <i>C. ecirrhosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgi-2 <sup>3</sup>	-	Phosphoglucoisomerase-2 <sup>3</sup> ; one of six codominant alleles, each regulating one cytosolic band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgi-2 <sup>4</sup>	-	Phosphoglucoisomerase-2 <sup>4</sup> ; one of six codominant alleles, each regulating one cytosolic band; found in <i>C. lanatus</i> var. <i>citroides</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgi-2 <sup>5</sup>	-	Phosphoglucoisomerase-2 <sup>5</sup> ; one of six codominant alleles, each regulating one cytosolic band; found in <i>Acanthosicyos naudinianus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgm- 1	-	Phosphoglucomutase-1; one of four codominant alleles, each regulating one plastid band; found in <i>C. lanatus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgm- 1	-	Phosphoglucomutase-1 <sup>1</sup> ; one of four codominant alleles, each regulating one plastid band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M

Pgm- 1 <sup>2</sup>	-	<i>Phosphoglucomutase-1</i> <sup>2</sup> ; one of four codominant alleles, each regulating one	Navot et al., 1990; Navot and Zamir,	-	M
I		plastid band; found in <i>Acanthosicyos</i> naudinianus.	1986, 1987; Zamir et al., 1984		
Pgm- I³	-	Phosphoglucomutase-1 <sup>3</sup> ; one of four codominant alleles, each regulating one plastid band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Pgm- 2	-	<i>Phosphoglucomutase-2</i> ; one of four codominant alleles, each regulating one cytosolic band; found in C. <i>lanatus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
Pgm- 2 <sup>1</sup>	-	Phosphoglucomutase-2 <sup>1</sup> ; one of four codominant alleles, each regulating one cytosolic band; found in <i>Acanthosicyos naudinianus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
Pgm- 2 <sup>2</sup>	-	Phosphoglucomutase-2 <sup>2</sup> ; one of four codominant alleles, each regulating one cytosolic band; found in <i>C. lanatus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
Pgm- 2 <sup>3</sup>	-	Phosphoglucomutase-2 <sup>3</sup> ; one of four codominant alleles, each regulating one cytosolic band; found in <i>Praecitrullus fistulosus</i> .	Navot and Zamir, 1987; Zamir et al., 1984	-	M
pm	-	powdery mildew susceptibility; susceptibility to Sphaerotheca fuliginea is recessive; pm from PI 269677; Pm from 'Sugar Baby' and most cultivars.	Robinson et al., 1975	-	P
Prx-1	-	Peroxidase-1; one of seven codominant alleles, each regulating one band; found in C. lanatus.	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Prx- 1 <sup>1</sup>	-	Peroxidase-1 <sup>1</sup> ; one of seven codominant alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
<i>Prx- 1</i> <sup>2</sup>	-	Peroxidase-1 <sup>2</sup> ; one of seven codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
<i>Prx- 1</i> <sup>3</sup>	-	Peroxidase- $1^3$ ; one of seven codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
<i>Prx- 1</i> <sup>4</sup>	-	Peroxidase-1 <sup>4</sup> ; one of seven codominant alleles, each regulating one band; found in <i>C. ecirrhosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
<i>Prx- 1</i> <sup>5</sup>	-	Peroxidase-1 <sup>5</sup> ; one of seven codominant alleles, each regulating one band; found in <i>C. lanatus and C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
<i>Prx-</i> 1 <sup>6</sup>	-	Peroxidase- $1^6$ ; one of seven codominant alleles, each regulating one band; found in <i>Acanthosicyos naudinianus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Prx-2	-	Peroxidase-2.	Navot and Zamir, 1987	-	M
Prx-3	-	Peroxidase-3.	Navot and Zamir, 1987	-	M

74		and good coat: gones a tond winters at to	Doolo et al. 1041		9
r	-	red seed coat; genes r, t and w interact to produce seeds of different colors; black	Poole et al., 1941	-	?
		from 'Klondike' ( <i>RR TT WW</i> ); clump from 'Sun Moon and Stars' ( <i>RR TT ww</i> ); tan			
		from 'Baby Delight' ( <i>RR tt WW</i> ); white			
		with tan tip from 'Pride of Muscatine' (RR			
		tt ww); red from citron (rr tt WW); white			
		with pink tip from 'Peerless' (rr tt ww).			
S	-	short (or small) seeds; epistatic to l; long	Poole et al., 1941	-	?
		recessive to medium or short; LL SS for			
		medium, ll SS for long, and LL ss or ll ss			
		for short seed; <i>ll SS</i> from 'Peerless'; <i>LL SS</i>			
		from 'Klondike'; <i>LL ss</i> from 'Baby			
C 4		Delight'.	Saita at al. 1007		M
Sat	_	Serine acetyltransferase; catalyzes the formation of O-acetylserine from serine	Saito et al., 1997	-	M
		and acetyl-CoA.			
Skdh-	_	Shikimic acid dehydrogenase-1.	Zamir et al., 1984	_	M
1		simume ded denym ogendee 1.	Zumii		1.12
Skdh-	-	Shikimic acid dehydrogenase-2; one of six	Navot et al., 1990;	-	M
2		codominant alleles, each regulating one	Navot and Zamir,		
		band.	1986, 1987		
Skdh- 2 <sup>1</sup>	-	Shikimic acid dehydrogenase-2 <sup>1</sup> ; one of	Navot et al., 1990;	-	M
2		six codominant alleles, each regulating	Navot and Zamir,		
Skdh-	_	one band; found in <i>C. colocynthis</i> . <i>Shikimic acid dehydrogenase-2</i> <sup>2</sup> ; one of	1986, 1987 Navot et al., 1990;	_	M
$2^2$	_	six codominant alleles, each regulating	Navot et al., 1990, Navot and Zamir,	-	IVI
2		one band; found in <i>C. colocynthis</i> .	1986, 1987		
Skdh-	-	Shikimic acid dehydrogenase-2 <sup>3</sup> ; one of	Navot et al., 1990;	-	M
$2^{3}$		six codominant alleles, each regulating	Navot and Zamir,		
		one band; found in Acanthosicyos	1986, 1987		
		naudinianus.			
Skdh- 2 <sup>4</sup>	-	Shikimic acid dehydrogenase-2 <sup>4</sup> ; one of	Navot et al., 1990;	-	M
2'		six codominant alleles, each regulating	Navot and Zamir,		
		one band; found in <i>C. ecirrhosus</i> .	1986, 1987		
Skdh-	-	<i>Shikimic acid dehydrogenase-2</i> <sup>5</sup> ; one of	Navot et al., 1990;	-	M
$2^{5}$		six codominant alleles, each regulating	Navot and Zamir,		
		one band; found in <i>Praecitrullus</i>	1986, 1987		
-		fistulosus.	D 11 1 1004		-
slv	-	seedling leaf variegation; conferred by a	Provvidenti, 1994	-	P
		single recessive gene in PI 482261; linked or pleiotropic with a dominant allele for			
		resistance to cool temperature injury			
		(20°C for greenhouse-grown plants); slv			
		from PI 482261 (resistant to ZYMV-FL);			
		Slv from 'New Hampshire Midget'.			
Sod-1	-	Superoxide dismutase-1; one of three	Navot et al., 1990;	-	M
		codominant alleles, each regulating one	Navot and Zamir,		
		band; found in C. lanatus.	1986, 1987; Zamir et		
			al., 1984		

Sod- 1 <sup>1</sup>	-	Superoxide dismutase-1 <sup>1</sup> ; one of three codominant alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Sod- 1 <sup>2</sup>	-	Superoxide dismutase-1 <sup>2</sup> ; one of three codominant alleles, each regulating one band; found in Acanthosicyos naudinianus.	Navot et al., 1990; Navot and Zamir, 1986, 1987; Zamir et al., 1984	-	M
Sod-2	-	Superoxide dismutase-2; one of two codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot and Zamir, 1987	-	M
Sod- 2 <sup>1</sup>	-	Superoxide dismutase-2 <sup>1</sup> ; one of two codominant alleles, each regulating one band; found in Acanthosicyos naudinianus.	Navot and Zamir, 1987	-	M
Sod-3	-	Superoxide dismutase-3; one of two codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot and Zamir, 1987	-	M
<i>Sod- 3</i> <sup>1</sup>	-	Superoxide dismutase-3 <sup>1</sup> ; one of two codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot and Zamir, 1987	-	M
Sp	-	Spotted cotyledons, leaves and fruit; dominant to uniform foliage and fruit color; Sp from 'Sun, Moon and Stars'* and 'Moon and Stars'*; sp from 'Allsweet'.	Poole, 1944*	Rhodes, 1986**	С
Spr-1	-	Seed protein-1.	Navot and Zamir, 1986	-	M
Spr-2	-	Seed protein-2.	Navot and Zamir, 1986	-	M
Spr-3	-	Seed protein-3.	Navot and Zamir, 1986	-	M
Spr-4	Sp-4	Seed protein-4.	Navot et al., 1990; Navot and Zamir, 1986	-	M
Spr-5	Sp-5	Seed protein-5.	Navot et al., 1990; Navot and Zamir, 1986	-	M
su	Bi, su <sup>Bi</sup>	suppressor of bitterness; (su named by Robinson*); non-bitter fruit; su from 'Hawkesbury'; Su from bitter-fruited mutant of 'Hawkesbury'; bitterness in C. colocynthis is due to Su Su genotype.	Chambliss et al., 1968	Robinson et al., 1976*	?
t	$b^t$	tan seed coat; genes r, t and w interact to produce seeds of different colors; black from 'Klondike' (RR TT WW); clump from 'Sun Moon and Stars' (RR TT ww); tan from 'Baby Delight' (RR tt WW); white with tan tip from 'Pride of Muscatine' (RR tt ww); red from citron (rr tt WW); white with pink tip from 'Peerless' (rr tt ww).	McKay, 1936	Poole et al., 1941	?

Ti	-	<i>Tiny seed;</i> dominant over medium seed ( <i>ti</i> ); <i>Ti</i> from 'Sweet Princess'; <i>ti</i> from 'Fujihikari'.	Tanaka et al., 1995	-	?
tl	bl	tendrilless (formerly called branchless*), after 4th or 5th node, vegetative axillary buds are transformed into flower buds and leaf shape is altered; tl from 'Early Branchless'; Tl from breeding lines 'G17AB', 'ASS-1', 'YF91-1-2', and S173 breeding line.	Rhodes, Zhang, Baird and Knapp, 1999; Zhang, Rhodes, Baird and Skorupska, 1996a	Lin, Tong, Wang, Zhang and Rhodes, 1992*	?
Трі-	-	Triosephosphatase isomerase-1. one of four codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Tpi-1 <sup>1</sup>	-	Triosephosphatase isomerase-1'; one of four codominant alleles, each regulating one band; found in <i>C. colocynthis</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Tpi-1 <sup>2</sup>	-	Triosephosphatase isomerase-1 <sup>2</sup> ; one of four codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Tpi-1 <sup>3</sup>	-	Triosephosphatase isomerase-1 <sup>3</sup> ; one of four codominant alleles, each regulating one band; found in <i>Acanthosicyos</i> naudinianus.	Navot et al., 1990; Navot and Zamir, 1986, 1987	-	M
Tpi-2	-	<i>Triosephosphatase isomerase-2</i> ; one of three codominant alleles, each regulating one band; found in <i>C. lanatus</i> .	Navot and Zamir, 1987	-	M
Tpi-2 <sup>I</sup>	-	Triosephosphatase isomerase-2 <sup>1</sup> ; one of three codominant alleles, each regulating one band; found in <i>Acanthosicyos naudinianus</i> .	Navot and Zamir, 1987	-	M
Tpi-2 <sup>2</sup>	-	Triosephosphatase isomerase-2 <sup>2</sup> ; one of three codominant alleles, each regulating one band; found in <i>Praecitrullus fistulosus</i> .	Navot and Zamir, 1987	-	M
ts	tss	tomato seed; seeds smaller than short (LLss or llss), almost the size of a tomato seed; ts from tomato seed Sugar Baby mutant; Ts from 'Gn-1'.	Zhang et al., 1994a	Zhang, 1996	С
Ure-1	-	Ureaase-1.	Navot and Zamir, 1987	-	M
w	-	white seed coat; genes r, t and w interact to produce seeds of different colors; black from 'Klondike' (RR TT WW); clump from 'Sun Moon and Stars' (RR TT ww); tan from 'Baby Delight' (RR tt WW); white with tan tip from 'Pride of Muscatine' (RR tt ww); red from citron (rr tt WW); white with pink tip from 'Peerless' (rr tt ww).	Poole et al., 1941	-	?
Wf	W	White flesh; (named white flesh by Robinson*); Wf is epistatic to B (Y renamed B by Henderson); WfWfBB or	Shimotsuma, 1963	Robinson et al., 1976*	?

		WfWf bb white fleshed; wfwf BB yellow fleshed; wfwf bb red fleshed; B from breeding line V.No.3 and b from V.No.1; flesh color segregated into 12 white, 3 yellow and 1 red in the F2.			
У	rd	yellow flesh; recessive to red flesh; y from 'Golden Honey'; Y from 'Angeleno' (black seeded).	Porter, 1937	Poole, 1944; Henderson, 1989; Henderson et al., 1998;	С
yo	-	orange flesh; allelic to y; Y (red flesh) is dominant to $y^0$ (orange flesh) and y (salmon yellow flesh); $y^0$ (orange flesh) is dominant to y (yellow flesh); $cc\ y^0y^0\ I-C$ I-C from 'Tendersweet Orange Flesh'; $cc\ yy\ I-C\ I-C$ from 'Golden Honey'; $cc\ YY\ i-C$ i-C from 'Sweet Princess'.	Henderson, 1989; Henderson et al., 1998	Poole, 1944; Porter, 1937	С
Yl	-	Yellow leaf; incompletely dominant to green leaf (yl); Yl from 'Yellow Skin'.	Warid and Abd-El- Hafez, 1976	-	?
zym- FL	zym	Resistance to zucchini yellow mosaic virus (ZYMV-FL); resistance is specific to the Florida strain; zym-FL from PI 482322, PI 482299, PI 482261, and PI 482308.	Provvidenti, 1991	-	P

Z Asterisks on cultigens and associated references indicate the source of information for each.

y C = Mutant available from Cucurbit Genetics Cooperative watermelon gene curator; M = molecular marker or isozyme; P = mutants are available as standard cultivars or accessions from the plant introduction collection; ? = availability not known; L = mutant has been lost.

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## Gene Nomenclature for the Cucurbitaceae

- 1. Names of genes should describe a characteristic feature of the mutant type in a minimum of adjectives and/or nouns in English or Latin.
- 2. Genes are symbolized by italicized Roman letters, the first letter of the symbol being the same as that for the name. A minimum number of additional letters are added to distinguish each symbol.
- 3. The first letter of the symbol and name is capitalized if the mutant gene is dominant,. All letters of the symbol and name are in lower case if the mutant gene is recessive, with the first letter of the symbol capitalized for the dominant or normal allele. (Note: For CGC *research articles*, the normal allele of a mutant gene may be represented by the symbol "+", or the symbol of the mutant gene followed by the superscript "+", if greater clarity is achieved for the manuscript.)
- 4. A gene symbol shall not be assigned to a character unless supported by statistically valid segregation data for the gene.
- 5. Mimics, i.e. different mutants having similar phenotypes, may either have distinctive names and symbols or be assigned the same gene symbol, followed by a hyphen and distinguishing Arabic numeral or Roman letter printed at the same level as the symbol. The suffix "-1" is used, or may be understood and not used, for the original gene in a mimic series. It is recommended that allelism tests be made with a mimic before a new gene symbol is assigned to it.
- 6. Multiple alleles have the same symbol, followed by a Roman letter or Arabic number superscript. Similarities in phenotype are insufficient to establish multiple alleles; the allelism test must be made.
- 7. Indistinguishable alleles, i.e. alleles at the same locus with identical phenotypes, preferably should be given the same symbol. If distinctive symbols are assigned to alleles that are apparent re-occurrences of the same mutation, however, they shall have the same symbol with distinguishing numbers or letters in parentheses as superscripts.
- 8. Modifying genes may have a symbol for an appropriate name, such as intensifier, suppressor, or inhibitor, followed by a hyphen and the symbol of the allele affected. Alternatively, they may be given a distinctive name unaccompanied by the symbol of the gene modified.
- 9. In cases of the same symbol being assigned to different genes, or more than one symbol designated for the same gene, priority in publication will be the primary criterion for establishing the preferred symbol. Incorrectly assigned symbols will be enclosed in parentheses on the gene lists.
- 10. The same symbol shall not be used for nonallelic genes of different *Cucurbita* species. Allelic genes of compatible species are designated with the same symbol for the locus.

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## **Covenant and By-Laws of the Cucurbit Genetics Cooperative**

## **ARTICLE I. Organization and Purposes**

The Cucurbit Genetics Cooperative is an informal, unincorporated scientific society (hereinafter designated "CGC") organized without capital stock and intended not for business or profit but for the advancement of science and education in the field of genetics of cucurbits (Family: Cucurbitaceae). Its purposes include the following: to serve as a clearing house for scientists of the world interested in the genetics and breeding of cucurbits, to serve as a medium of exchange for information and materials of mutual interest, to assist in the publication of studies in the aforementioned field, and to accept and administer funds for the purposes indicated.

#### **ARTICLE II. Membership and Dues**

- 1. The membership of the CGC shall consist solely of active members; an active member is defined as any person who is actively interested in genetics and breeding of cucurbits and who pays biennial dues. Memberships are arranged by correspondence with the Chairman of the Coordinating Committee.
- 2. The amount of biennial dues shall be proposed by the Coordinating Committee and fixed, subject to approval at the Annual Meeting of the CGC. The amount of biennial dues shall remain constant until such time that the Coordinating Committee estimates that a change is necessary in order to compensate for a fund balance deemed excessive or inadequate to meet costs of the CGC.
- 3. Members who fail to pay their current biennial dues within the first six months of the biennium are dropped from active membership. Such members may be reinstated upon payment of the respective dues.

### **ARTICLE III. Committees**

- 1. The Coordinating Committee shall govern policies and activities of the CGC. It shall consist of six members elected in order to represent areas of interest and importance in the field. The Coordinating Committee shall select its Chairman, who shall serve as a spokesman of the CGC, as well as its Secretary and Treasurer.
- 2. The Gene List Committee, consisting of at least five members, shall be responsible for formulating rules regulating the naming and symbolizing of genes, chromosomal alterations, or other hereditary modifications of the cucurbits. It shall record all newly reported mutations and periodically report lists of them in the Report of the CGC. It shall keep a record of all information pertaining to cucurbit linkages and periodically issue revised linkage maps in the Report of the CGC. Each committee member shall be responsible for genes and linkages of one of the following groups: cucumber, *Cucurbita* spp., muskmelon, watermelon, and other genera and species.
- 3. Other committees may be selected by the Coordinating Committee as the need for fulfilling other functions arises.

#### **ARTICLE IV. Election and Appointment of Committees**

1. The Chairman will serve an indefinite term while other members of the Coordinating Committee shall be elected for ten-year terms, replacement of a single retiring member taking place every other year. Election of a new member shall take place as follows: A Nominating Committee of three members shall

be appointed by the Coordinating Committee. The aforesaid Nominating Committee shall nominate candidates for an anticipated opening on the Coordinating Committee, the number of nominees being at their discretion. The nominations shall be announced and election held by open ballot at the Annual Meeting of the CGC. The nominee receiving the highest number of votes shall be declared elected. The newly elected member shall take office immediately.

- 2. In the event of death or retirement of a member of the Coordinating Committee before the expiration of his/her term, he/she shall be replaced by an appointee of the Coordinating Committee.
- 3. Members of other committees shall be appointed by the Coordinating Committee.

#### **ARTICLE V. Publications**

- 1. One of the primary functions of the CGC shall be to issue an Annual Report each year. The Annual Report shall contain sections in which research results and information concerning the exchange of stocks can be published. It shall also contain the annual financial statement. Revised membership lists and other useful information shall be issued periodically. The Editor shall be appointed by the Coordinating Committee and shall retain office for as many years as the Coordinating Committee deems appropriate.
- 2. Payment of biennial dues shall entitle each member to a copy of the Annual Report, newsletters, and any other duplicated information intended for distribution to the membership. The aforementioned publications shall not be sent to members who are in arrears in the payment of dues. Back numbers of the Annual Report, available for at least the most recent five years, shall be sold to active members at a rate determined by the Coordinating Committee.

#### **ARTICLE VI. Meetings**

An Annual Meeting shall be held at such time and place as determined by the Coordinating Committee. Members shall be notified of time and place of meetings by notices in the Annual Report or by notices mailed not less than one month prior to the meeting. A financial report and information on enrollment of members shall be presented at the Annual Meeting. Other business of the Annual Meeting may include topics of agenda selected by the Coordinating Committee or any items that members may wish to present.

#### **ARTICLE VII. Fiscal Year**

The fiscal year of the CGC shall end on December 31.

#### **ARTICLE VIII. Amendments**

These By-Laws may be amended by simple majority of members voting by mail ballot, provided a copy of the proposed amendments has been mailed to all the active members of the CGC at least one month previous to the balloting deadline.

#### **ARTICLE IX. General Prohibitions**

Notwithstanding any provisions of the By-Laws or any document that might be susceptible to a contrary interpretation:

- 1. The CGC shall be organized and operated exclusively for scientific and educational purposes.
- 2. No part of the net earnings of the CGC shall or may under any circumstances inure to the benefit of any individual.
- 3. No part of the activities of the CGC shall consist of carrying on propaganda or otherwise attempting to influence legislation of any political unit.
- 4. The CGC shall not participate in, or intervene in (including the publishing or distribution of statements), any political campaign on behalf of a candidate for public office.
- 5. The CGC shall not be organized or operated for profit.
- 6. The CGC shall not:
  - a. lend any part of its income or corpus without the receipt of adequate security and a reasonable rate of interest to;
  - b. pay any compensation in excess of a reasonable allowance for salaries or other compensation for personal services rendered to;
  - c. make any part of its services available on a preferential basis to;
  - d. make any purchase of securities or any other property, for more than adequate consideration in money's worth from;
  - e. sell any securities or other property for less than adequate consideration in money or money's worth; or
  - f. engage in any other transactions which result in a substantial diversion of income or corpus to any officer, member of the Coordinating Committee, or substantial contributor to the CGC.

The prohibitions contained in this subsection (6) do not mean to imply that the CGC may make such loans, payments, sales, or purchases to anyone else, unless authority be given or implied by other provisions of the By- Laws.

#### **ARTICLE X. Distribution on Dissolution**

Upon dissolution of the CGC, the Coordinating Committee shall distribute the assets and accrued income to one or more scientific organizations as determined by the Committee, but which organization or organizations shall meet the limitations prescribed in sections 1-6 of Article IX.