Cucurbit Genetics Cooperative

2004

ISSN 1064-5594

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The **Cucurbit Genetics Cooperative** (CGC) was organized in 1977 to develop and advance the genetics of economically important cucurbits. Membership to CGC is voluntary and open to 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*.

CGC Reports 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.

ISSN 1064-5594

NEWS & COMMENT

RESEARCH REPORTS

Cucumber (*Cucumis sativus*)

Melon (*Cucumis melo*)

Watermelon (*Citrullus lanatus*)

Comments:

Comments from the CGC Coordinating Committee - 2004: The Call for Papers for the 2005 Report (CGC Report No. 27) will be mailed in August 2004. Papers should be submitted to the respective Coordinating Committee members by 31 December 2004. The Report will be published by June/July 2005. As always, we are eager to hear from CGC members regarding our current activities and the future direction of CGC.

- David Wolff (melon)
- Todd C. Wehner (watermelon)
- Mark G. Hutton (other genera)
- Jack E. Staub (cucumber)
- Linda Wessel-Beaver (*Cucurbita* spp.)
- Timothy Ng, Chair

Comments from 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 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.

- Cucumber: Todd C. Wehner and Jack E. Staub
- Melon: Michael Pitrat and James D. McCreight
- Watermelon: Todd C. Wehner and Stephen R. King
- *Cucurbita* spp.: Harry Paris and Richard W. Robinson
- Other Genera: Mark Hutton and Deena Decker-Walters

Comments from the CGC Gene Curators: CGC has appointed curators for the four major cultivated groups: cucumber, melon, watermelon and *Cucurbita* spp.

Curators are responsible for collecting, maintaining and distributing upon request stocks of the known marker genes. CGC members are requested to forward samples of currently held gene stocks to the respective Curator.

- Cucumber: Todd C. Wehner and Jack E. Staub
- Melon: Michael Pitrat and James D. McCreight
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2003 CGC Business Meeting (Providence, Rhode Island)

Tim Ng, CGC Chair

The 2003 Business Meeting of the Cucurbit Genetics Cooperative was held on Friday, 3 October 2003, in the Rhode Island Convention Center in Providence, Rhode Island. Eighteen CGC members and guests were in attendance.

The meeting began with introductions around the room, then Tim Ng reported that CGC Report No. 25 (2002) had been printed and mailed, albeit almost a year late. As an apology for the delay, Tim had paid for the printing and mailing himself, not using any CGC funds, essentially giving every CGC member a free year of membership. He said that CGC Report No. 26 (2003) was being prepared, but that he was losing staff at the University of Maryland due to budget cuts, plus the university Print Shop he had used since 1988 was being closed. He did not yet have a target date for the printing and mailing of CGC 26. Tim then described some updates to the CGC website, including a javascript web-based application for membership applications.

Upcoming cucurbit-related meetings were then discussed. The 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, "Cucurbitaceae 2004," was scheduled for 12-17 July 2004 at Palacký University in Olomouc, Czech Republic, with Aleš Lebeda as one of the organizers. The webpage for the conference was already up, with links for deadlines, registration, and manuscript guidelines. Todd Wehner (North Carolina State University) announced that Cucurbitaceae 2006 would be held in Asheville, North Carolina, during the Fall of 2006, and that he would have more details at the next CGC Business Meeting. Tim

mentioned that there were still some copies of the proceedings from the Cucurbitaceae 2002 conference in Naples, Florida, available through the American Society for Horticultural Science (ASHS), and that although no copies appeared available at the ASHS website, they were actually holding a few in reserve for present and future CGC members. Tim also mentioned that he had recently received a number of copies of the proceedings from the Cucurbitaceae 1994 conference in Padre Island, Texas, and that these copies would be made available to CGC members.

With regard to gene list updates, Tim indicated that the next scheduled update was for Cucurbita. This would be an full update, unlike the updates from 4 and 8 years ago which only listed newly reported genes for that period. The comprehensive update would be prepared by Dick Robinson (Cornell University) and Rebecca Brown (Oregon State University). Todd requested that all new gene list updates be given a consistent name on the CGC website (i.e., without the year of the update in the URL) so that links would not have to be rewritten with each update. It was agreed that this would be implemented.

Tim then indicated that over the years, particularly with the expansion of the web and email communications as well as increased sophistication of the CGC Report, and also the increased complexity of fiduciary responsibilities, that the duties of the CGC chair were expanding even as Tim's available time was decreasing. He suggested that the CGC By-Laws be changed to allow more management participation by CGC members. For instance, the By-Laws state that the CGC Chair serves as "spokesman of the CGC, as well as its Secretary and Treasurer." These duties could be distributed among 2-3 members to help ease the load. Tim also said that the By-Laws should be amended to (1) reflect the web as a site for archival CGC publications and other activities, and (2) allow email balloting instead of only having a mail ballot. Tim handed out a page of proposed By-Laws changes and said that he and others would be working on this over the next several months.

With regard to membership dues, Tim indicated that he thought they could remain constant for the next few years if there were no extraordinary increases in printing or postage costs. Tim also mentioned that he was looking at the amazon.com website the other day, and that the CGC Report was listed there as a "Magazine Subscription" for $28.85 an issue, which is more than twice what CGC charges. Amazon listed this subscription as being provided by "Magazine Express, Inc." A similar entry was listed for The Cucurbit Network (TCN) Newsletter, for the same price. Apparently, a broker is now willing to accept orders and deal with CGC through Amazon, but Tim had not been contacted by this individual as yet. After this last item of business, the meeting was adjourned.

Cucurbitaceae 2006 - September 17-21, Asheville, NC

Dr. Gerald Holmes, Chair
Dr. Jonathan Schultheis
Dr. Todd Wehner
Jane Dove Long, Conference Coordinator

Cucurbitaceae 2006 will be held 17 to 21 September at the Grove Park Inn, Asheville, North Carolina USA. It will be hosted by North Carolina State University. There will be numerous opportunities for participants to share up-to-date research information and to discuss common concerns with colleagues from around the world. This exchange of information is vital to the continued improvement and international advancement of cucurbits.

The purpose of Cucurbitaceae 2006 is to bring together those working in cucurbits so we can share information on all aspects of cucurbit research, development and production. We welcome you to join us for this exciting and in-depth conference exploring the ever-changing face of cucurbit research and development. (We anticipate that the USA Cucurbitaceae meetings will continue on a four-year schedule, alternating every two years with the EUCARPIA meetings in Europe.)

Meetings of the following groups will also take place:

- Cucurbit Crop Genetics Committee
- Cucurbit Genetics Cooperative
- International Cucurbit Genomics Consortium
- National Melon Research Group
- Pickling Cucumber Improvement Committee
- Squash Breeders Group
- Watermelon Research Group

For registration, please visit: www.ncsu.edu/cucurbit2006

Watermelon Research and Development Working Group Report - 24th Annual Meeting

by Stephen R. King

The 24th Annual Meeting of the Watermelon Research and Development Working Group (WRDWG) was held on Sunday, February 15, 2004 in Tulsa, OK at the Adam's Mark Hotel in conjunction with the annual meetings of the Southern Association of Agricultural Scientists (SAAS) and the Southern Region American Society for Horticultural Sciences (SR:ASHS). We had an excellent meeting with approximately 70 people in attendance.

Refreshments were provided by Abbott & Cobb, so be sure to thank Pete Suddarth and all of the Abbott & Cobb gang for sponsoring our refreshments! A special thank-you also goes to Paul Smeal, Secretary-Treasurer for SR:ASHS for the long-term support we have received from SR:ASHS; without this support these meetings would not be possible.

The program began with a welcome and web page update (http://www.lane-ag.org/h2omelon/watermelon.htm) by Chairman Benny Bruton. Seed company updates immediately followed the introduction:

Don Dobbs introduced new varieties from Willhite Seed (www.willhiteseed.com) and he discussed his wilt screening nursery.

Tom Williams introduced new varieties from Syngenta (www.rogersadvantage.com) and discussed hard seed coats in seedless watermelon.

Fred McCuistion discussed new varieties coming from Seminis (www.seminis.com).

Pete Suddarth talked about new varieties coming from Abbott & Cobb (www.acseed.com).

Glenn Price introduced new varieties soon to be available from Sugar Creek Seeds (www.sugarcreekseed.com).

Following the morning break, cultivar evaluation trials were presented:

Don Maynard provided a review of the Florida watermelon trials for the spring 2003 (mailto:mdnma@ifas.ufl.edu).

Juan Anciso reviewed the Texas Statewide watermelon trials (janciso@ag.tamu.edu).

George Boyhan presented the Georgia watermelon variety trials (gboyan@uga.edu).

Warren Roberts talked about the Oklahoma watermelon cultivar evaluation (wroberts@lane-ag.org).

The featured speaker for the afternoon session was Michele Westley, Director of Quality Assurance for J-M Foods and J-M Farms, Inc. The title of her presentation was "Factors influencing quality and food safety in fresh watermelon processing." Ms. Westly gave an excellent presentation on the future of fresh cut watermelon. The afternoon session resumed with the following research reports and updates:

Amon Levi presented: "Constructing genetic linkage map: differences in distribution of AFLP, ISSR and RAPD markers on watermelon genome," by A. Levi, C. Thomas, Y. Xu, T. Wehenr, X. Zhang, A. Davis, U. Reddy. (alevi@saa.ars.usda.gov)

Johnathan Edelson presented: "Effects of squash bug feeding on watermelon growth and fruit production," by J. Edelson and W. Roberts. (jedelson-okstate@lane-ag.org)

Daniel Egel presented "Race determination in Fusarium oxysporum f. sp. niveum from southern Indiana." (egel@purdue.edu)

Ron Gitaitis presented: "A log-normal distribution of phytopathogenic bacteria in seed." (gitaitis@tifton.uga.edu)

Haejeen Bang presented: "Environmental effects on seedling and fruit quality of watermelon" by D.I. Leskovar and J. Bang. (d-leskovar@tamu.edu)

Marvin Miller presented: “Insensitivity of Didymella bryoniae to azoxystrobin in South Texas.” (m-miller@tamu.edu)

Warren Roberts presented: “Watermelon Seedling Survival Associated with Pythium aphanidermatum.” (wroberts-okstate@lane-ag.org)

Jonathan Schultheis presented: “Commercial pollinizers for triploid watermelon production.” (johathan_schultheis@ncsu.edu)

Todd Wehner presented: “Watermelon gene overview,” by Nihat Guner and Todd Wehner. (todd_wehner@ncsu.edu)

Penny Perkins-Veazie presented “Update on research on watermelon antioxidants,” by P. Perkins-Veazie and J. Collins. (pperkins-usda@lane-ag.org)

Steve King presented: “Current status of carotenoid gene cloning in watermelon.” (srking@tamu.edu)

The afternoon session ended with a discussion on Fusarium wilt differientials. Benny Bruton discussed maintaining seed sources for Fusarium wilt differentials, and whether a company was willing to maintain these sources for distribution. It was decided that the differentials should be maintained as part of the core collection as well as part of the gene collection maintained by the Cucurbit Genetics Cooperative. While these collections only maintain stocks, members can request small samples and increase seed of the stocks for their own use.

The Cucurbit Network

Tom Andres
Deena Decker-Walters

In 1994 Deena Decker-Walters and Tom Andres founded The Cucurbit Network (TCN). Since then, we have published two newsletters a year for a total of 24 issues. Our last issue included an announcement that Deena was resigning as its president at the end of the year. Now, TCN is being reorganized under the International Society for Horticultural Science (ISHS) and the newsletter will continue with two issues a year. This arrangement, with the guidance and expertise of the Cucurbitaceae Working Group at ISHS, is an ideal match. Not only will TCN be able to continue its newsletter across a broad range of disciplines, but it will also be able to grow by reaching a larger international audience.

TCN will strive to present articles on all aspects of cucurbit science as well as popular cultural aspects of cucurbits, while fostering communication among the diverse TCN members from horticulturalists, breeders, ethnobotanists to gourd artists, culinary artisans, and other enthusiasts. The Web site www.cucurbit.org will be updated to reflect the latest news and developments in cucurbit taxonomy, and provide other features including a small online store. Details will soon follow on how you can join and participate in the new TCN in 2006. Anyone interested in one or more of the approximately 825 cultivated and wild species in the plant family Cucurbitaceae is welcome.

Upcoming Meetings & News of Interest

Organization/Meeting	Dates	Location	Contact
26th Annual Meeting of the Watermelon Research & Development Working Group	February 5, 2006	In conjunction with the 103rd Annual Meeting of the Southern Association of Agricultural Scientists, Orlando, FL. USA	Benny Bruton bbruton-usda@lane-ag.org
Cucurbit Crop Germplasm Committee Meeting	September 19, 2006	In conjunction with Cucurbitaceae 2006, Asheville, North Carolina. USA	Jim McCreight jmccreight@pw.ars.usda.gov
Cucurbit Genetics Cooperative Report Business Meeting	July 30, 2006 2:00-4:00 PM	In conjunction with ASHS 2006, New Orleans, LA. USA	Todd Wehner todd_wehner@ncsu.edu
Cucurbit Genetics Cooperative Report Business Meeting	September 18, 2006	In conjunction with Cucurbitaceae 2006, Asheville, North Carolina. USA	Todd Whener todd_wehner@ncsu.edu
Cucurbitaceae 2006	September 17-21, 2006	Asheville, North Carolina. USA	Gerald Holmes gerald_holmes@ncsu.edu Jonathan Schultheis jonathan_schultheis@ncsu.edu Todd Wehner todd_wehner@ncsu.edu
Pickle Packers International	October 24-26, 2006	Las Vegas, Nevada. USA.	
Eucarpia 2008	May 22-25, 2008	France	

Harvest-Dependent Chemical Components in *Cucumis sativus L.* Fruits: I. Salad Cucumbers

Galina Pevicharova and Nikolay Velkov
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Introduction: Chemical composition is important to fruit quality in fresh market or salad cucumbers. Indirect information regarding the organoleptic and nutritive properties of fruits of different cultivars can be obtained by an analysis of dry matter, monosaccharides, and titratable organic acids. Monosaccharides improve the taste density of fruit, while their refreshing properties are mainly due to free organic acids. Ascorbic acid is a substance having antioxidant effect, and it is a significant component of a fruit's biological value (3, 4, 6). The abundance of these substances in the salad cucumbers intended for fresh consumption is of great importance.

The concentration of these basic substances in cucumber fruits, however, is comparatively low and varies among varieties (7). In order to form appropriate conclusions from chemical analyses, it is very important to clarify the effects of sampling time prior to analysis.

Cucumber possesses an extended, continuous fruiting habit. The picking of fruit is carried out on fruits taken from multiple harvests. At each harvest plants are in different stages of maturity, fruits are located at different distances from the root system, and are matured under different light, temperature, humidity, and soil nutrient supply. The purpose of the present study is to estimate the effects that the microclimate at different harvests has on the monosaccharides, titratable organic acid, and ascorbic acid accumulation in the fruit of salad cucumbers. This was investigated using a diverse array of breeding lines.

Materials and Methods: The experiment was performed during 2001 to 2002 in a polyethylene greenhouse. Six salad cultivars possessing different morphological characteristics were studied: *Bistrenski* – monoecious, fruit length 20 –24 cm; *Midori* F_1 – gynoecious, 15 –18 cm fruit length; *Desislava* F_1 – gynoecious, 22 – 25 cm fruit length; *Gergana* – monoecious, 28 – 30 cm fruit length; *Linia* 61 – monoecious, length of the fruit 28 – 30 cm; and *Lora* F_1 – parthenocarpic, gynoecious, length of the fruit 33 – 35 cm.

Entries were arranged in a randomized complete block design with four replications at 100 and 50 x 45 cm planting scheme where the area of an experimental plot was 3.4 m^2 each containing 10 plants. The seeds were sown on 23 March, and the plants were maintained until 30 July.

Fruits from each of three harvests were analyzed where the harvest interval was every 15 days. The first harvest was carried out 66 – 73 days from the date of plant germination, the second one– 90 – 98 days, and the third – 110 – 117 days. The content of dry matter, monosaccharides and ascorbic acid was determined according to the method of Shoorl-Regenbogen (2) using the reaction of Tilmans. Titratable organic acid concentration was obtained by direct titration of juice with 0.1 n NaOH taking the average of 10 plants in each replication.

Results were analyzed by two-way analysis of variance (3), means were separated using Duncan's multiple range test (1), and correlations were made between varieties for each of the three substances (3).

Results and Discussion: The concentration of the substances studied varied depending on harvest date (Table 1). A systematic increase or

decrease in their amounts was not observed. Significant correlation coefficients for the dry matter content between II-nd and III-rd harvesting during the first experimental year and between 1st and 2nd harvesting during the second experimental year were greater than 0.5 (Table 2a). In the other treatments, correlation coefficient values were low. The relationship between the dry matter content in fruits taken over the three harvests was not unidirectional. General trends regarding the correlation coefficients of ascorbic acid, titratable organic acids and monosaccharides content were similar (Tables 2b, c, d). Thus, based these results it appears possible to predict the concentration of these

Table 1. Chemical components in salad cucumber fruits.

Cultivars	Time of harvesting	Dry matter (%)		Ascorbic acid (mg %)		Titratable organic acids (%)		Monosaccharides (%)	
		2001	2002	2001	2002	2001	2002	2001	2002
Midori	I	3.90 b	4.50 n.s.	13.14 n.s.	9.21 c	0.08 n.s.	0.14 a	1.79 b	1.90 n.s.
	II	4.64 a	4.78 n.s.	9.14 n.s.	11.52 b	0.08 n.s.	0.11 b	1.94 ab	2.04 n.s.
	III	4.62 a	4.75 n.s.	11.13 n.s.	13.48 a	0.09 n.s.	0.08 c	2.30 a	2.07 n.s.
Bistrenski	I	4.22 n.s.	4.57 ab	14.59 a	11.03 n.s.	0.14 a	0.09 n.s.	1.83 n.s.	1.92 n.s.
	II	4.20 n.s.	4.80 a	9.70 b	11.86 n.s.	0.07 b	0.10 n.s.	1.68 n.s.	2.01 n.s.
	III	4.30 n.s.	4.26 b	13.98 a	11.14 n.s.	0.10 b	0.08 n.s.	2.14 n.s.	1.76 n.s.
Gergana	I	4.04 b	4.46 n.s.	12.61 n.s.	8.58 b	0.10 n.s.	0.11 n.s.	1.74 b	2.22 n.s.
	II	4.74 a	4.56 n.s.	13.17 n.s.	11.30 a	0.10 n.s.	0.10 n.s.	2.09 a	2.24 n.s.
	III	4.70 a	4.90 n.s.	13.78 n.s.	11.20 a	0.10 n.s.	0.09 n.s.	2.14 a	2.02 n.s.
Desislava	I	4.54 n.s.	4.33 b	14.80 n.s.	9.56 b	0.08 n.s.	0.12 n.s.	2.02 n.s.	1.78 b
	II	4.84 n.s.	5.20 a	14.31 n.s.	12.64 b	0.09 n.s.	0.10 n.s.	2.09 n.s.	2.37a
	III	4.89 n.s.	4.52 b	12.34 n.s.	18.34 b	0.10 n.s.	0.10 n.s.	2.12 n.s.	1.94 b
Lora	I	4.34 n.s.	3.93 n.s.	12.15 n.s.	8.65 b	0.08 b	0.11 b	2.01 n.s.	1.62 c
	II	4.42 n.s.	4.46 n.s.	15.01 n.s.	12.14 ab	0.09 ab	0.10 c	1.98 n.s.	1.98 a
	III	4.54 n.s.	4.32 n.s.	14.43 n.s.	14.06 a	0.10 a	0.14 a	2.20 n.s.	1.84 b
Linia 61	I	3.89 b	4.30 n.s.	13.74 a	8.38 b	0.12 a	0.13 a	1.58 b	1.86 n.s.
	II	4.48 ab	4.90 n.s.	9.63 b	11.93 ab	0.08 b	0.10 c	1.92 ab	2.29 n.s.
	III	4.81 a	5.17 n.s.	11.42 b	17.26 a	0.10 ab	0.11 b	2.25 a	2.26 n.s.

a, b, c… - Duncan's multiple range test ($p<0.05$), n.s. – not significant

Table 2. Coefficients of correlations between studied chemical components

2002 ←

	I	II	III
I	♦	0.515	-0.060
II	-0.046	♦	0.025
III	0.115	0.599**	♦

2001 →

a) dry matter

2002 ←

	I	II	III
I	♦	-0.029	-0.352
II	-0.152	♦	0.351
III	-0.010	0.485	♦

2001 →

b) ascorbic acid

2002 ←

	I	II	III
I	♦	0.534	0.108
II	-0.446	♦	-0.466
III	0.131	-0.070	♦

2001 →

c) titratable organic acids

2002 ←

	I	II	III
I	♦	0.157	0.202
II	0.044	♦	0.429
III	0.176	-0.140	♦

2001 →

d) monosaccharides

Table 3. Two-way analysis of variance for studied chemical components in salad cucumber fruits depending on cultivar (factor A) and time of harvesting (factor B)

Experimental year	Chemical components	Factors influence (ŋ%)			
		Cultivar (A)	Time of harvesting (B)	A x B	Error
2001	Dry matter	21.34*	39.26***	18.51	20.89
	Ascorbic acid	25.70*	11.34*	40.53*	22.43
	Titratable org. acids	16.78*	13.35**	55.14***	14.63
	Monosaccharides	10.18	46.70***	21.22	21.90
2002	Dry matter	25.56**	33.82***	25.19*	15.43
	Ascorbic acid	12.74**	54.22***	24.13**	8.91
	Titratable org. acids	26.99***	16.31***	50.00***	6.70
	Monosaccharides	33.25***	27.25***	26.50**	13.01

substances based on information from a single harvest.

With rare exceptions, differences in the substances studied were recorded during the three harvests (Table 1). Given the significant mean treatment differences, it is possible to divide data for dry matter content and monosaccharide content into two groups. For some cultivars, three groupings for the ascorbic acid and titratable organic acids was possible for each of the harvests. Therefore, harvesting date is influential affecting the concentration of the chemical substances studied.

This hypothesis was confirmed by two-way analysis of variance of concentration differences (Table 3). The influence of factor B (time of harvesting) on the content of dry matter and titratable organic acids exceeds those of the factor A (cultivar). The results of ascorbic acid and monosaccharide concentration are variable. In fact, the effect of factor B for the whole experimental period is statistically significant, and is over 11 per cent. The influence of both factors was best expressed in the concentration of titratable organic acid.

The results indicate that the time of harvesting is an important factor in the accumulation of the substances studied and must be considered during data interpretation. It is impossible to make reliable conclusions from information obtained from one harvest. Likewise, it is impossible to predict a single optimal moment for carrying out chemical analyses of cucumber fruit. It is more correct to consider the designation of an optimal period of fruiting for the accurate determination of dry matter, ascorbic acid, titratable acids and monosaccharides. In the evaluation of salad cucumber breeding material it is very important to carry out obligatory chemical analyses several times over several harvests to increase the reliability of the results.

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Harvest-Dependent Chemical Components in *Cucumis sativus L.* Fruits: II. Pickling Cucumbers

Galina Pevicharova and Nikolay Velkov
Maritsa Vegetable Crops Research Institute, Plovdiv 4003, 32 Brezovsko shosse Str., Bulgaria

Introduction: A basic purpose in pickling cucumber breeding is development of hybrids suitable for sterilized processing. For the production of high quality processing products (cans) it is necessary to consider the interests both of growers and consumers. The processing industry has specific requirements for fruit appearance and taste characteristics of raw material (greenstock), while the consumer would like higher nutritive and biological value in the product. A part of the nutritive value of cucumber fruits is determined by the content of dry matter, titratable organic acids and monosaccharides. In contrast, biological value of fruit is primarily determined by the ascorbic acid content. Many vegetable crop breeding programs have become focused on the development of germplasm with increased ascorbic acid content, a proven antioxidant (1,3,4). Pickling cucumbers, which pass through thermal processing and the break down of the ascorbic acid during processing. Breeding of germplasm with high ascorbic acid content is a strategy that has potential assisting with this impediment.

In order to develop breeding strategies for this purpose, it is very important to establish optimal harvest times for the analysis of selected chemical components. The aim of the present study was to be determined the optimal sampling times for chemical analysis for dry matter, titratable organic acids , monosaccharides, and ascorbic acid in pickling cucumbers.

Materials and Methods: The experiment was performed during 2001 to 2002 in a polyethylene greenhouse. Pickling cultivars Toni F_1, Iren F_1 and Pobeda F_1 are of gynoecious type and indeterminate growth, and were use in this study. Treatments were arranged in randomized complete block design with four replications at 100 and 50 x 35 cm planting scheme where each experimental plot was given 2.6-m^2 area with 10 plants. The seeds were sown on 23 March, and the plants were maintained until 30 July.
The analysis of the fruits and data processing were according to methods used in the experiment with salad cucumbers (this volume, Part I).

Results and Discussion: Dry matter content in the fruits of pickling cucumbers during 2001 was highest in the second harvest (Table 1). The correlation (r) between the values of this character for three harvests were significant at $-0.738<r<0.935$ (Table 2). Thus, with each harvest dry matter content changed in the three cultivars examined over the harvest periods.

During 2002, the highest content in the parameters studied was recorded at third harvest. However, the correlation coefficients between harvests differed, and they suggest that cultivar differences exist in the second and third harvests.

In content of ascorbic acid, titratable organic acids and monosaccharide concentrations also elicited non-unidirectional changes at each harvests (Tables 1 and 2). Similar to salad cucumbers (this volume, Part I), it can be concluded that only in one harvest is it impossible to be predicted their amount in other harvests during fruit development in pickling cucumbers.

The importance of the time of harvest for the values of chemical characters is expressed in Table 1. In treatments with significant mean differences, the presence of two or three groups

within one cultivar corresponding to any particular harvest was observed.

In contrast from salad cucumbers, the influence of time of harvesting (factor B) on the studied chemical components was more extreme than the effect of the cultivar (factor A) (Table 3). The weaker influence of the factor A is probably due to the fact that the hybrid cultivars examined share the same parent female inbred line.

Considerable differences concerning the ascorbic acid content particularly were established in our previous investigation where a more diverse array of

Table 1. Chemical components in pickling cucumber cultivars

Cultivars	Time of harvesting	Dry matter (%)		Ascorbic acid (mg %)		Titratable organic acids (%)		Monosaccharides (%)	
		2001	2002	2001	2002	2001	2002	2001	2002
Toni	I	5.64 b	5.46 n.s.	10.96 b	12.07 b	0.10 b	0.12 a	2.06 n.s.	1.44 b
	II	5.95 a	5.37 n.s.	16.29 a	13.82 b	0.15 a	0.10 b	2.05 n.s.	1.92 a
	III	5.42 b	5.48 n.s.	15.27 a	19.68 a	0.10 b	0.10 b	1.91 n.s.	1.91 a
Iren	I	5.33 n.s.	5.42 ab	12.96 c	14.03 b	0.10 c	0.14 a	1.86 n.s.	1.42 b
	II	5.64 n.s.	5.36 b	19.55 a	15.42 b	0.15 a	0.11 b	1.82 n.s.	2.06 a
	III	5.32 n.s.	5.48 a	14.84 b	18.56 a	0.12 b	0.14 a	1.88 n.s.	1.98 a
Pobeda	I	5.24 b	5.28 b	12.68 c	13.40 b	0.10 b	0.13 a	1.89 a	1.42 b
	II	5.70 a	5.33 b	18.28 a	16.60 a	0.14 a	0.11 b	1.82 ab	1.93 a
	III	5.20 b	5.50 a	15.27 b	17.94 a	0.12 a	0.11 b	1.80 b	1.80 ab

a, b, c… - Duncan's multiple range test ($p<0.05$), n.s. – not significant

Table 2. Coefficients of correlations between studied chemical components

2002 ←

	I	II	III
I	♦	0.734	-0.208
II	0.898	♦	0.120
III	0.935	0.738	♦

2001 →

a) dry matter

2002 ←

	I	II	III
I	♦	0.008	0.634
II	-0.592	♦	0.176
III	0.951**	-0.516	♦

2001 →

b) ascorbic acid

2002 ←

	I	II	III
I	♦	0.000	0.784
II	-0.400	♦	0.439
III	0.054	-0.542	♦

2001 →

c) titratable organic acids

2002 ←

	I	II	III
I	♦	0.643	-0.507
II	0.788	♦	0.138
III	0.228	0.607	♦

2001 →

d) monosaccharides

Table 3. Two-way analysis of variance for studied chemical components in pickling cucumbers depending on cultivar (factor A) and time of harvesting (factor B).

Experimental year	Chemical components	Factors influence (ŋ %)			
		Cultivar (A)	Time of harvesting (B)	A x B	Error
2001	Dry matter	11.87	50.45*	2.05	35.63
	Ascorbic acid	7.08**	84.91***	6.00**	2.02
	Titratable org. acids	2.67	71.92***	14.64	10.77
	Monosaccharides	44.13*	6.49	10.13	39.25
2002	Dry matter	8.47	31.34	12.37	47.82
	Ascorbic acid	1.91	69.91***	9.15	19.03
	Titratable org. acids	37.23***	37.23***	19.05	6.49
	Monosaccharides	2.73	85.96***	2.04	9.27

cultivars was tested (2). The presence of significant differences between the individual cultivars indicates that chemical components in the cucumber fruits could be increased through breeding. Also of importance is the effect of harvest time on chemical concentration of the characters examined and this should be considered during the breeding process. In the evaluation of breeding material parental components should be characterized or a selection is made. In this case, the average arithmetical value from the chemical analysis performed over a minimum of two harvests should be used as a basis for selection. Multiple determinations of chemical components in cucumber fruit will make the breeding process difficult to a certain extent, but this will guarantee more precise results from which conclusions can be drawn.

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Notes on the Change of the Causal Species of Cucurbit Powdery Mildew in the U.S.

James D. McCreight
U.S. Department of Agriculture, Agricultural Research Service, 1636 E. Alisal St., Salinas, California 93905 U.S.A.

Beginning with the first report of powdery mildew (PM) on melon in Imperial Valley in 1925 (Jagger, 1926) through 1967, the causal organism of powdery mildew on melon and other cucurbit species in the U.S. was generally regarded as *Erisyphe cichoracearum* (Ec). Walker (1952) identified both Ec and *Sphaerotheca humuli* var. *fuliginea*, which is synonymous with *Sphaerotheca fuliginea* (Sf) (Braun, 1995), as causal organisms of PM, but stated that Ec "is the prevalent pathogen in the United States and apparently the most common generally." Moreover, the cleistothecial stage of Ec had been observed on cucumber in the U.S. (Randall and Menzies, 1956). In 1968, while Sf was named as the cause of PM without mention of Ec in a semi-popular article on control of PM on cucumber and squash (Paulus et al., 1968), two reports on genetic resistance to PM referred only to Ec (Harwood and Markarian, 1968a; Harwood and Markarian, 1968b). Two subsequent peer-reviewed reports on chemical control of PM on cucurbits referred only to Sf as the causal organism of PM (Paulus et al., 1972; Paulus et al., 1969). Two abstracts from U.S. researchers referred to Sf as the causal organism on cucumber, melon and *Cucurbita* spp. without specifying criteria for the identification of the pathogen (Sowell and Clark, 1971; Sowell and Corley, 1969). Shanmugasundaram et al. (1971) recognized both species as causal agents of PM in the introduction of their report of a genetic resistance to PM in cucumber, but they did not clearly state the criteria used to identify Sf as the pathogen in their research. In a review on breeding cucurbits for disease resistance, Sitterly (1973) referred only to Ec as the cause of PM. In the first comprehensive review of the described genes of cucurbits, Robinson et al. (1976) referred only to Sf as the causal organism of PM on cucumber, melon, watermelon and *Cucurbita*. In 1978, Thomas stated that Sf was "…a production-limiting factor throughout all cantaloup-growing areas of the United States." There was an abrupt change in the identity of the causal organism of PM on cucurbits, melons in particular, across the U.S. without any apparent scientific documentation. The change from Ec to Sf should not be confused with the nomenclatural changes for these two species (Jahn et al., 2002).

Pertinent literature on this problem first appeared in 1937. Homma (1937) distinguished the fibrosin bodies in Ec and Sf and their utility in distinguishing the two species. Hirata (1955) found differences in the germ tubes of Ec (single, inconspicuous appressoria) and Sf (some are forked) useful to distinguish the two species. Zaracovitis (1965) was also examining conidial characters for distinguishing powdery mildew fungi.

In 1961, G.W. Bohn and T.W. Whitaker reported a new host of cucurbit mildew, and after a concise review of the literature available to them concluded "The fungus reported here is considered to be a conidial clone of an unknown species of the Erysiphaceae since it has not been observed to produce perithecia." Moreover, they cited the conflicting host-range reports in the literature, the presence of two biological races in southern California (Jagger et al., 1938), strains with different temperature requirements (Yarwood et al., 1954), absence of heterothallism on cucurbits in the U.S., and wide host range as reasons for

research to determine the "…true identity or identities…" of the causal organism(s) of PM of cucurbits (Bohn and Whitaker, 1961).

Ballantyne reviewed the PM situation in Australia including the observations of fibrosin bodies in all specimens from cucurbits in New South Wales, Queensland and western Australia. She cited the statement by Weiss (1960) of the lack of any report of Sf from the U.S., and the 1961 report by Bohn and Whitaker. She noted that the reported reactions of various melon cultigens to PM in the U.S. were similar to those observed in Australia, and concluded that "such close agreement would be of considerable significance in the field of plant breeding if Sf were the only cucurbit PM present in Australia and Ec the only such fungus in the U.S.A." (Ballantyne, 1963)

At about this same time, Sf was being recognized as the dominant species causing PM on cucurbits in The Netherlands (Boerema and Kesteren, 1964) and India (Jhooty, 1967).

Kable and Ballantyne first reported a positive identification of Sf in the U.S. on cucurbits in Ithaca, N.Y. based on the presence of fibrosin bodies (Kable and Ballantyne, 1963). Yarwood and Gardner (1964) stated that PM on cucurbits they examined "…usually contain fibrosine bodies and perithecia are not formed…," and they further stated that the host ranges of Ec and Sf overlap. Sowell, Corley and Clark identified Sf as the pathogen in screening tests of cucurbit germplasm for host plant resistance to PM in Georgia and Iowa (Clark, 1975; Sowell and Clark, 1971; Sowell and Corley, 1969), but did not clearly state their criteria for identifying the pathogen. These few reports appear insufficient for a complete change in the identity of the causal species of PM in the U.S., but Sitterly (1978) noted in his review that either Ec or Sf predominates, or is the only species present.

Correspondence between G.W. Bohn, formerly Research Geneticist and Plant Pathologist, USDA-ARS, La Jolla and Brawley, Calif., and two colleagues provides insight into the change reflected in the literature cited above. A letter from Barbara Ballantyne, which followed the report by her and Kable (Kable and Ballantyne, 1963), and dated 10 September 1963 continued a sequence of interchanges on breeding melons for resistance to powdery mildew, but with a new focus on the identity of the pathogen in the U.S.: "Do you know if the California mildew resembles *Sphaerotheca fuliginea* in the characteristics mentioned in this paper?" (Fig. 1). In a letter dated 25 October 1963, Bohn stated that "Conidia of our fungus mounted in aceto-carmine (after Homma) and in dilute iodine-potassium iodide appear to contain large fibrosin bodies." (Fig. 2). He then surmised that "Perhaps it is all *Sphaerotheca fuliginea* and *Erisyphe cichoracearum* does not occur on cucurbits." A letter to Ballantyne dated 8 November 1963 indicated that Bohn was going to examine PM conidia in KOH, and included two color photographic slides of PM conidia, one in iodine-potassium iodide, the other in aceto-carmine (Fig. 3). Dr. Ballantyne replied on 14 November 1963 and stated that the iodine-potassium iodide slide showed fibrosin bodies that resembled *Sphaerotheca fuliginea* in New South Wales, Australia (Fig. 4). Bohn in a letter dated 15 November 1963 stated that PM samples from South Carolina and Texas had fibrosin bodies (Fig. 5). A letter dated 6 December 1963 from Bohn stated "Powdery mildew conidia from cantaloupe in California, Texas, and South Carolina, and from the wild squash *Cucurbita digitata* in the southern California desert, all look like those of the N.S.W. fungus. All exhibit fibrosin bodies in 3% KOH and all have the same shape." (Fig. 6).

Correspondence between Bohn and D.M. McLean, formerly Research Plant Pathologist, USDA-ARS, Charleston, S.C.,

in 1969 shed additional light on the situation. McLean stated in a letter on 9 April 1969, "I am having difficulty finding *Erisyphe cichoracearum* and most collections about Charleston are *Sphaerotheca fuliginea* " (Fig. 7). On 14 April 1969 Bohn wrote, "I have examined specimens of cucurbit powdery mildew on muskmelons collected at several sites in California, Arizona, and Texas, and single sites in South Carolina and Michigan. Although I was unable to find the perfect stage, the conidia of all collections had inclusion bodies characteristic of *Sphaerotheca fuliginea*. In addition to that evidence, our breeding lines resistant to race 2 are resistant at nearly all sites tested in various parts of the world including those where *Sphaerotheca fuliginea* has been described. Therefore, our race 2 is *Sphaerotheca fuliginea* and that species is the one prevalent on muskmelons everywhere. Intended to publish a brief paper on it this year but I haven't written it yet. Would you like to join me in the effort?" (Fig. 8). In a final letter on 18 April 1969 McLean replied, "I have been waiting for someone to admit that part of the mildew problem in the U.S. was caused by *Sphaerotheca fuliginea*. Now I am satisfied that we are on the right track. I have examined specimens from Texas, California, and Beltsville and have tried paring different isolates hoping to get a perfect stage. I have failed to find *Erysiphe cichoracearum* in any of these studies. I most certainly would like to join you in describing race 2 and will help where I can." (Fig. 9). In a 13 May 1970 request for a sample of Ec, McLean mentioned a request from an unnamed person at Cornell University for a sample of Ec (Fig. 10). McLean mentions difficulty in finding Ec. Bohn sent a sample of Ec found on sunflower to McLean on 26 October 1970 (Fig. 11).

Bohn was at that time largely occupied with research on resistance to the mosaic virus complex of the southwest desert U.S. and the proposed manuscript was never written. He apparently had not been aware of the work by Homma (1937) on conidial characteristics of Ec and Sf as indicated by his handwritten note at the top margin of the 10 September 1963 letter from Ballantyne that he had requested a copy of that work from the National Agriculture Library (Fig. 1). He also was not aware of the differences in germ tubes between Ec and Sf described by Hirata (1965) as indicated by his request for an outline of the technique for germinating conidia in his letter of 6 December 1963 (Fig. 6). He was probably also unaware of the more contemporary report on using conidial characters to identify PM fungi (Zaracovitis, 1965)

The last publication authored by Bohn in which the causal organism of PM was attributed to Ec was in 1967 in a semi-popular article on control of PM on melon (Paulus et al., 1967). When he determined experimentally to his satisfaction that Sf was the only species causing PM on cucurbits following the 1961 paper (Bohn and Whitaker, 1961), Bohn changed the U.S. literature beginning in 1968 (Paulus et al., 1968), but did not publish his experimental evidence. Positive identification of race 3 as Sf was based on presence of fibrosin bodies and forked germ tubes (Thomas, 1978). Thus, the change in identity of the predominant causal organism of PM in the U.S. followed a similar course of discovery and verification, but it was not so clearly documented as in Australia (Ballantyne, 1963), The Netherlands (Boerema and Kesteren, 1964), and India (Jhooty, 1967).

Erisyphe cichoracearum may still play an important role in causing PM on cucurbits in the U.S., but there has been little evidence in the past 40 years. Limited attempts in my laboratory to infect melon with Ec from lettuce (*Lactuca sativa*) in Salinas Valley, Calif. were negative (unpublished data). One North American isolate of Ec, UCSC1, recovered from *Arabidopsis thaliana*, was demonstrated to be pathogenic on melon,

cucumber, watermelon, *Cucurbita pepo*, and *Cucurbita maxima* (Adam et al., 1999).

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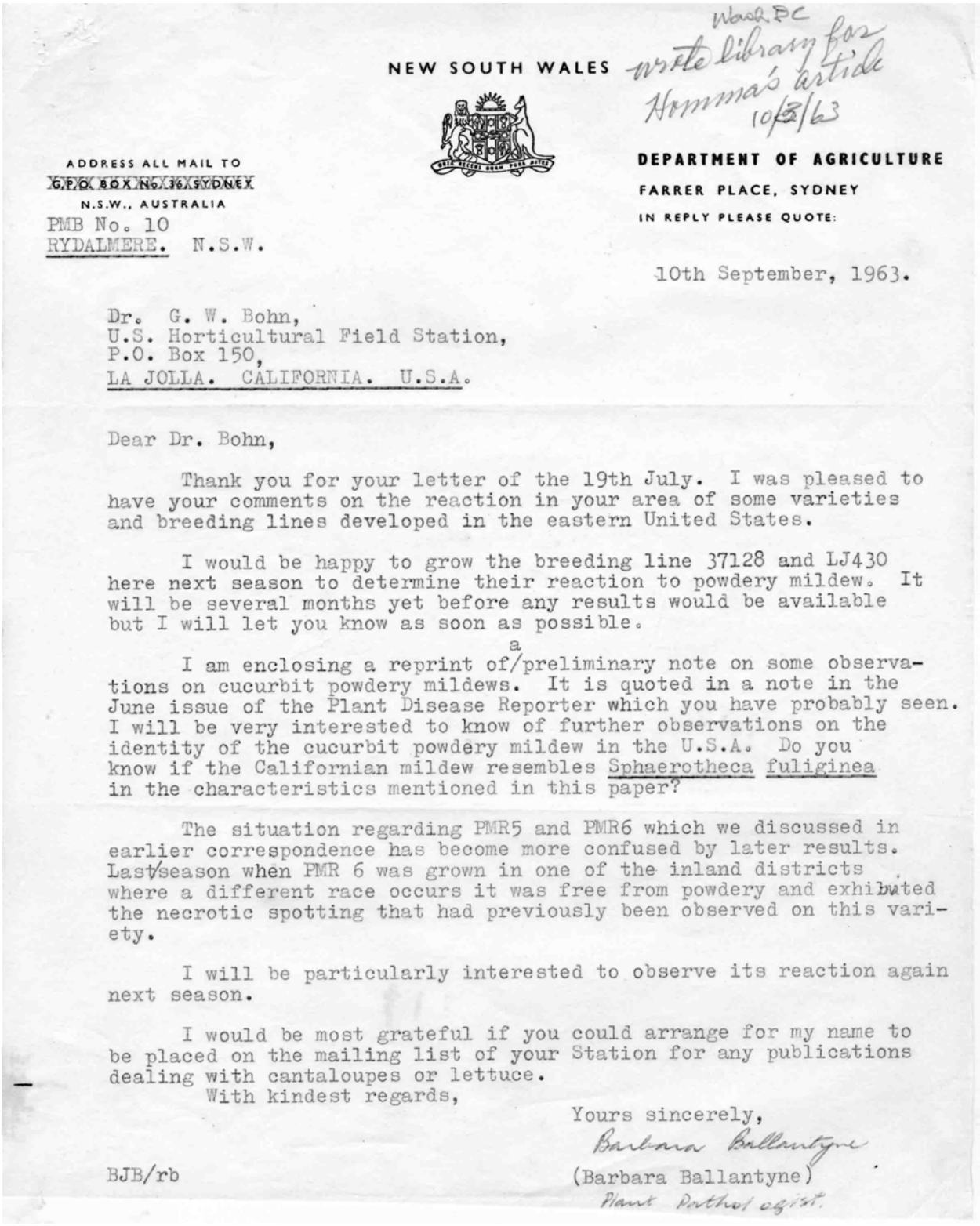


NEW SOUTH WALES

Wash DC wrote library for Homma's article 10/8/63

ADDRESS ALL MAIL TO
G.P.O. BOX No. 36, SYDNEY
N.S.W., AUSTRALIA
PMB No. 10
RYDALMERE. N.S.W.

DEPARTMENT OF AGRICULTURE
FARRER PLACE, SYDNEY
IN REPLY PLEASE QUOTE:

10th September, 1963.

Dr. G. W. Bohn,
U.S. Horticultural Field Station,
P.O. Box 150,
LA JOLLA. CALIFORNIA. U.S.A.

Dear Dr. Bohn,

Thank you for your letter of the 19th July. I was pleased to have your comments on the reaction in your area of some varieties and breeding lines developed in the eastern United States.

I would be happy to grow the breeding line 37128 and LJ430 here next season to determine their reaction to powdery mildew. It will be several months yet before any results would be available but I will let you know as soon as possible.

I am enclosing a reprint of a preliminary note on some observations on cucurbit powdery mildews. It is quoted in a note in the June issue of the Plant Disease Reporter which you have probably seen. I will be very interested to know of further observations on the identity of the cucurbit powdery mildew in the U.S.A. Do you know if the Californian mildew resembles Sphaerotheca fuliginea in the characteristics mentioned in this paper?

The situation regarding PMR5 and PMR6 which we discussed in earlier correspondence has become more confused by later results. Last season when PMR 6 was grown in one of the inland districts where a different race occurs it was free from powdery and exhibited the necrotic spotting that had previously been observed on this variety.

I will be particularly interested to observe its reaction again next season.

I would be most grateful if you could arrange for my name to be placed on the mailing list of your Station for any publications dealing with cantaloupes or lettuce.

With kindest regards,

Yours sincerely,

Barbara Ballantyne

BJB/rb

(Barbara Ballantyne)
Plant Pathologist.

Fig. 1. Letter from B. Ballantyne to G.W. Bohn, 10 Sept. 1963.

U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92038

October 25, 1963

Dr. Barbara Ballantyne
PMB No. 10
RYDALMERE. N.S.W., Australia

Dear Dr. Ballantyne:

Many thanks for your good letter of September 10, 1963 and your very interesting note on the identity of cucurbit powdery mildews. Conidia of our fungus mounted in aceto-carmine (after Homma) and in dilute iodine-potassium iodide appear to contain numerous, large fibrosin bodies. I have not observed germinating spores, but this and the resistance of American varieties in Australia both suggest that we have the same fungus. Perhaps it is all Sphaerotheca fuliginea and Erysiphe cichoracearum does not occur on cucurbits. The performances of 37128 and LJ430 should add some information on this matter, or you may want to test the 8 powdery mildew resistant lines mentioned in my 1961 paper on the nectarless gene and in a paper on resistance now in press.

Have I sent you any papers? I have placed your name on our mailing lists for melon and lettuce papers but I don't want to duplicate any that you have.

With best regards,

Sincerely yours,

G. W. Bohn
Pathologist

GWB:ds

Fig. 2. Letter from G.W. Bohn to B. Ballantyne, 25 Oct. 1963.

U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92038

November 8, 1963

AIR MAIL

Dr. Barbara Ballantyne
Division of Science Services
Department of Agriculture
RYDALMERE. N.S.W.
AUSTRALIA

Dear Dr. Ballantyne:

In response to your letter of October 30, 1963 and Aerogramme dated November 4, 1963: I am sending under separate cover 500 seeds each of powdery mildew resistant inbreds - $P_2 - F_{10}$, 36478 bulk selfs; $P_3 - F_{10}$, 36486 bulk selfs; $P_4 - F_{10}$, 36301 bulk selfs; $P_5 - F_{10}$, 37438 bulk selfs; $P_6 - F_{17}$, 36523 bulk selfs; $P_7 - F_{11}$, 36611 bulk selfs; and ± 200 seeds of $P_8 - F_{13} + F_{14}$, 36424 and its bulk selfs; $P_{10} - F_{16}$, 36607 bulk selfs. P_1 and P_9 are omitted because they are the cultivars PMR 45 and PMR 6, respectively. Incidentally, 37128 is $P_3 - F_{10}$.

I will look up seeds of P.I, 179260 and 181910 when I visit our seed file in Imperial Valley next week.

Many thanks for the reprint of your paper, Observations on the cucurbit powdery mildew in the Ithaca district. I will compare conidia in KOH with your figure 1. You may be interested in the enclosed kodachrome of our cucurbit powdery mildew mounted in iodine-potassium iodide, and one in aceto-carmine.
I will review our reprint file and send any that are not on the list that you kindly furnished.

With kindest regards,

Sincerely yours

G. W. Bohn
Plant Pathologist

ARS:CR:GWBOHN:ds 11/8/63

Fig. 3. Letter from G.W. Bohn to B. Ballantyne, 8 Nov. 1963.

NEW SOUTH WALES

ADDRESS ALL MAIL TO
G.P.O. BOX No. 36, SYDNEY
N.S.W., AUSTRALIA

DIVISION OF SCIENCE SERVICES,
DEPARTMENT OF AGRICULTURE
RYDALMERE. N.S.W.
~~FARRER PLACE, SYDNEY~~

IN REPLY PLEASE QUOTE: B.B.662.
BJB/MN.
14th November, 1963.

Dr. G.W. Bohn,
Plant Pathologist,
U.S. Horticultural Field Station,
P.O. Box 150,
LA JOLLA,
CALIFORNIA 92038, U.S.A.

Dear Dr. Bohn,

I was very pleased to receive the seed of the powdery mildew resistant inbreds - P_2, P_3, P_4, P_5, P_6, P_8 and P_{10}. I was also interested to receive the kodachrome slides of your powdery mildew which you sent with your letter of 8th November. I will let you know of the results as soon as possible.

The iodine-potassium iodide mount shows the fibrosin bodies clearly and as far as I can tell your fungus certainly does resemble that present in New South Wales. When you compare conidia in KOH with the figure 1 of the Plant Disease Reporter note you may be interested to compare it also with the photo I am enclosing of the N.S.W. mildew in 3% KOH.

I have followed the method of Hirata in using onion epidermis strips for germinating powdery mildew conidia, and have found this quite satisfactory. If you are interested I could send the details to you.

Please accept my warmest thanks for your help.

With kindest regards.

Yours sincerely,

Barbara Ballantyne

BARBARA BALLANTYNE,
Plant Pathologist.

Fig. 4. Letter from B. Ballantyne to G.W. Bohn, 14 Nov. 1963.

U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92038

November 15, 1963

AIR MAIL

Dr. Barbara Ballantyne
Division of Science Services,
Department of Agriculture
Private Mail Bag No. 10
RYDALMERE. N.S.W. AUSTRALIA.

Dear Dr. Ballantyne:

In response to your request of November 4. 1963: You will find, enclosed, seed samples of Cucumis sativus, Plant Introductions 179260 from Turkey and 181910 from Syria. Seed increase plots were grown at Ames, Iowa without controlled inoculations so the stocks probably contain some mixture.

A single selection of powdery mildew from South Carolina and another from Texas produced conidia with large fibrosin bodies. The South Carolina culture looked slightly different from the Texas and California cultures but the slight differences in plant structure could have resulted from environmental variation.

With best regards,

Sincerely yours,

G. W. Bohn
Plant Pathologist

ARS:CR:GWBohn:ds 11-15-63

Enc.

Fig. 5. Letter from G.W. Bohn to B. Ballantyne, 15 Nov. 1963.

U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92038

December 6, 1963

AIR MAIL

Dr. Barbara Ballantyne
Division of Science Services
Department of Agriculture
Private Mail Bag No. 10
RYDALMERE. N.S.W. AUSTRALIA

Dear Dr. Ballantyne:

Many thanks for your letter of November 14, 1963 and the very excellent photograph of conidia of the N.S.W. cantaloup powdery mildew fungus. Powdery mildew conidia from cantaloupes in California, Texas, and South Carolina, and from the wild squash Cucurbita digitata in the southern California desert, all look like those of the N.S.W. fungus. All exhibit fibrosin bodies in 3% KOH mounts and all have the same shape.

I would appreciate an outline of Hirata's technique for conidia germination.

P_7 was omitted from your list of seeds received. A packet of P_7 (LJ 36611 bulk selfs) is herewith enclosed to complete the supply.

With kindest regards,

Sincerely yours,

G. W. Bohn
Plant Pathologist

ARS:CR:GWBohn:ds 12-6-63

Enc.

Fig. 6. Letter from G.W. Bohn to B. Ballantyne, 6 Dec. 1963.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
CROPS RESEARCH DIVISION
P. O. BOX 3348
CHARLESTON, SOUTH CAROLINA 29407

U. S. Vegetable Breeding Laboratory

April 9, 1969

Dr. G. W. Bohn
Geneticist
USDA, ARS, CR
U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92037

Dear Dr. Bohn:

The powdery mildew on cantaloupe in the envelope arrived in the morning and the specimens of spuash (boxed) in the afternoon of the same day. Have you identified the species? I am having difficulty finding Erysiphe cichorocearum and most collections about Charleston are Sphaerotheca fuliginea.

Both of the above specimens arrived in good shape, I prefer the boxed because of the larger quantity of the specimen.

Will you kindly send me a copy of your article describing race II of the powdery mildew organism.

Sincerely yours,

D. M. McLean

D. M. McLean
Res. Plant Pathologist

Fig. 7. Letter from D.M. McLean to G.W. Bohn, 9 Apr. 1969.

CROPS RESEARCH DIVISION
U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92037

April 14, 1969

Dr. D. M. McLean
U. S. Vegetable Breeding Laboratory
P. O. Box 3348
Charleston, South Carolina 29407

Dear Mac:

I have examined specimens of cucurbit powdery mildew on muskmelons collected at several sites in California, Arizona, and Texas, and single sites in South Carolina and Michigan. Although I was unable to find the perfect stage, the conidia of all collections had inclusion bodies characteristic of Sphaerotheca fuliginea.

In addition to that evidence, our breeding lines resistant to race 2 are resistant at nearly all sites tested in various parts of the world including those where Sphaerotheca fuliginea has been described. Therefore, our race 2 is Sphaerotheca fuliginea and that species is the one prevalant on muskmelons everywhere. Intended to publish a brief paper on it this year but I haven't written it yet. Would you like to join me in the effort?

With best regards,

Sincerely,

G. W. Bohn
Research Geneticist

Fig. 8. Letter from G.W. Bohn to D.M. McLean, 14 Apr. 1969.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
CROPS RESEARCH DIVISION
P. O. BOX 3348
CHARLESTON, SOUTH CAROLINA 29407

U. S. Vegetable Breeding Laboratory

April 18, 1969

Dr. G. W. Bohn
Research Geneticist
U. S. Horticultural Field Station
P. O. Box 150
La Jolla, California 92037

Dear Wes:

I have been waiting for someone to admit that part of the mildew problem in the U. S. was caused by Sphaerotheca fuliginia. Now I am satisfied that we are on the right track. I have examined specimens from Texas, California, Beltsville and have tried paring different isolates hoping to get a perfect stage. I have failed to find Erysiphe cichoracearum in any of these studies.

Today I found a specimen affecting watermelon fruits in the green-house. I have observed infection on Citrullus fistulosis plants but never on the fruit.

I most certainly would like to join you in describing race 2 and and will help where I can.

Best regards.

Sincerely yours,

D. M. McLean

D. M. McLean
Plant Pathologist

Fig. 9. Letter from D.M. McLean to G.W. Bohn, 18 Apr. 1969.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
CROPS RESEARCH DIVISION
P. O. BOX 3348
CHARLESTON, SOUTH CAROLINA 29407

U. S. Vegetable Breeding Laboratory

May 13, 1970

Dr. G. W. Bohn
U. S. Horticultural
Field Station
P. O. Box 150
La Jolla, California 92037

Dear Wes:

Can you send me a culture of Erysiphe cichoracaerum? I am having a little problem finding it among our cultures. Send it on any plant that you choose.

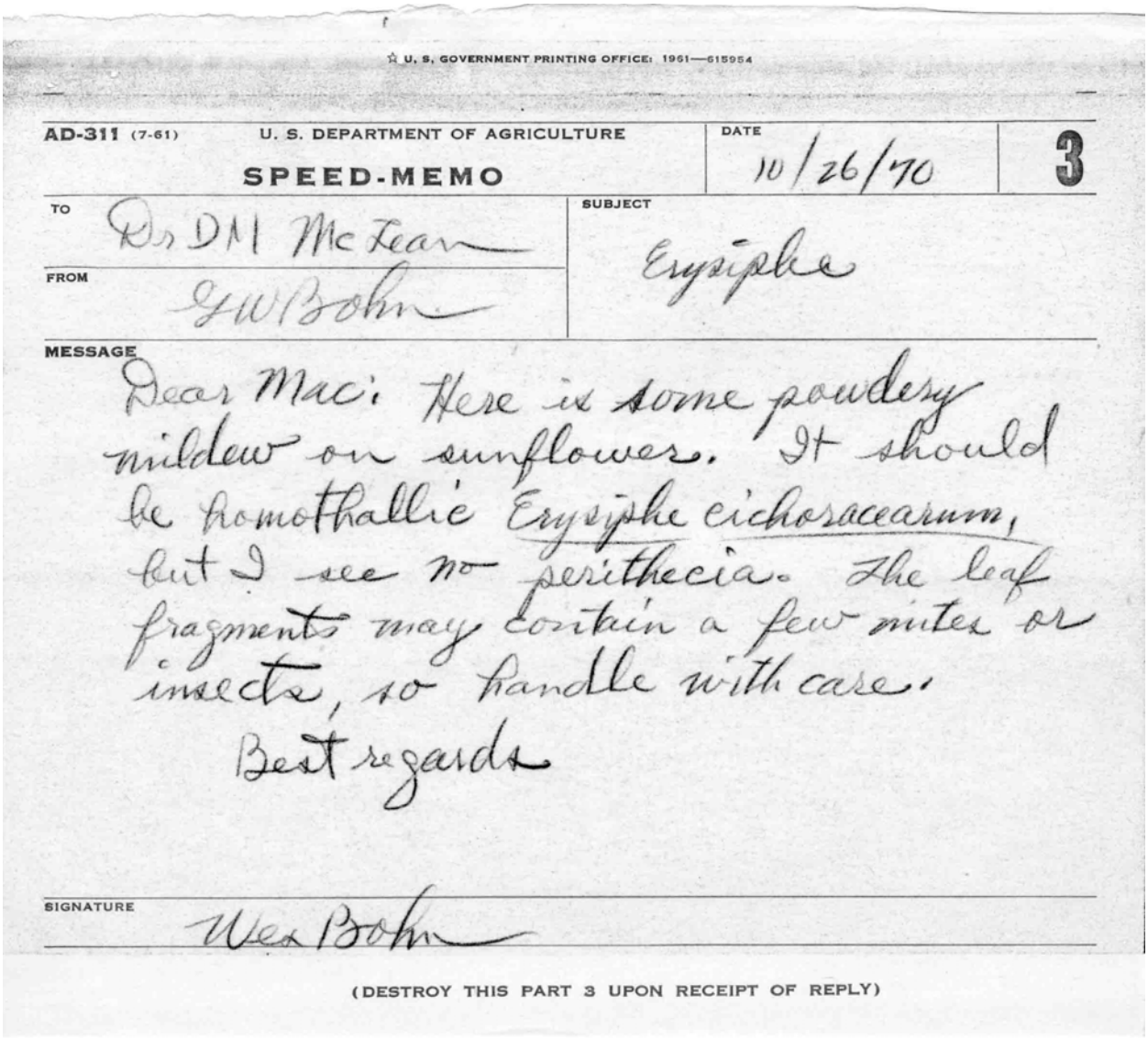
Cornell University asked us to let them know when I find E. cichoracaerum. Apparently, they have difficulty locating it also.

Sincerely,

D. M. McLean

D. M. McLean
Plant Pathologist

Fig. 10. Letter from D.M. McLean to G.W. Bohn, 13 May 1970.



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AD-311 (7-61) U. S. DEPARTMENT OF AGRICULTURE

SPEED-MEMO

DATE 10/26/70

3

TO Dr DM McLean

FROM G W Bohn

SUBJECT Erysiphe

MESSAGE

Dear Mac: Here is some powdery mildew on sunflower. It should be homothallic Erysiphe cichoracearum, but I see no perithecia. The leaf fragments may contain a few mites or insects, so handle with care.

Best regards

SIGNATURE Wes Bohn

(DESTROY THIS PART 3 UPON RECEIPT OF REPLY)

Fig. 11. Memo from G.W. Bohn to D.M. McLean, 10 Oct. 1969.

Qualitative Genes for Use in Development of Elite Watermelon Cultivars

Todd C. Wehner and Nihat Guner
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Plant breeders interested in developing elite cultivars of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) need to make use of qualitative genes to provide top performance. For example, a cultivar similar to 'Allsweet', but with improved single gene traits, might have the following genotype (desired genotype shown in parentheses). Seed color is non-dotted black (*DD RR TT WW*), seed size is short (*ll ss*), seed type is non-cracked (*CrCr*), and non-Egusi (*EgEg*). Leaf shape is lobed (*NlNl*), with no seedling leaf variegation (*SlvSlv*), green (not yellow) leaf color (*ylyl*), not delayed green leaf color (*DgDg*), and no defects, for example, not juvenile albino (*JaJa*). The vines are tall (*Dw-1Dw-1 Dw-2Dw-2 Dw-3Dw-3*) and have tendrils (*TlTl*). Flowering habit would be monoecious (*AA*), with yellow flower color (*GfGf*), and plants would be male fertile (*GmsGms Ms-1Ms-1 Ms-2Ms-2*) except for the female parent inbred used for hybrid production.

Fruit of the cultivar are non-bitter (*susu*), elongate shaped (*OO*), have a non-furrowed fruit surface (*FF*), and non-explosive rind (*EE*). The fruit rind pattern is striped ($g^s g^s$), not pencilled (*PP*), not mottled (*MM*), not spotted (*spsp*), and not golden yellow (*GoGo*). Flesh color is red (*YY*), not canary yellow (*cc*), and not white or yellow (*wfwf bb*). Disease resistance is for anthracnose race 1 (*Ar-1Ar-1*), anthracnose race 2 ($Ar\text{-}2^1Ar\text{-}2^1$), *Fusarium* wilt race 1 (*Fo-1Fo-1*), gummy stem blight (*dbdb*), powdery mildew (*PmPm*), and zucchini yellow mosaic (*zymzym*). Insect resistance is for fruit fly (*FwrFwr*) and red pumpkin beetle (*AfAf*). Stress resistance is for cool temperature resistance (*CtrCtr*).

Other watermelon cultivar types include large size fruit with elongate shape and narrow stripes as in 'Jubilee', large size fruit with elongate shape and solid gray rind, as in 'Charleston Gray', medium size fruit with round shape and medium width stripe as in 'Crimson Sweet', and icebox fruit size with round shape and dark solid color as in 'Sugar Baby'.

A cultivar such as 'Jubilee' would have the genotype *OO* (elongate fruit) and $g^s g^s$ (striped rind pattern). 'Charleston Gray' would have the genotype *OO* (elongate fruit) and *mm* (greenish white mottling of the fruit skin). 'Crimson Sweet' would have the genotype $g^s g^s$ (striped rind pattern) and *oo* (round fruit shape). Sugar Baby would have the genotype *GG* (solid dark green rind pattern) and *oo* (round fruit shape).

Elite cultivars could be made available with new flesh colors. The genotype would be *YY* for light red, $y^o y^o$ for orange, *yy* for salmon yellow, *CC* for canary yellow, and *WfWf BB* (or *WfWf bb*) for white flesh. The qualitative genes listed above can also be incorporated into seedless cultivars to improve the quality. Flesh colors other than red (canary yellow and orange) have already been incorporated into seedless cultivars now available commercially.

It would not be sufficient to incorporate just qualitative genes into an elite cultivar. Important quantitative traits would include rapid seed germination at low temperature, early maturity, high fruit yield, crisp fruit flesh, high flesh sugar content, and excellent flavor.

Literature Cited

1. Guner, N. and T. C. Wehner. 2003. The genes of watermelon. Cucurbit Genet. Coop. Rpt. 26: 76-9

Protocol for DNA Extraction from Watermelon Leaves for SSR Marker Studies

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Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Tarek Joobeur and Ralph A. Dean
Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616

Amnon Levi
United States Vegetable Laboratory, USDA-ARS, Charleston, SC 29414

Levi and Thomas developed an improved procedure for the extraction of high-quality DNA from a large sample of watermelon leaves (2). This technique is suitable for isolating high quality DNA used in RFLP and AFLP marker analysis employing restriction enzymes. However, RAPD and SSR marker analysis do not require high quality genomic DNA, so a modified DNA extraction procedure suitable for isolating genomic DNA from a large number of small leaf samples might be sufficient for SSR marker analysis. In this study, we report an alternative DNA extraction procedure from a large number (24 samples per run) of small watermelon leaf samples (≈500 mg) in 1.5 ml micro-centrifuge tubes.

The DNA extracted with our modified protocol is mostly free of other proteins and polysaccharides (Fig. 1, 2), two of the major concerns that originally required the development of a DNA extraction protocol specifically for the watermelon. Furthermore, PCR amplification of the SSR marker URF1 (forward primer: AGC AGC ACC TTG TCT TGT AT; reverse primer: CAC AGA TCC CAC TCA ATC TT) (1) was successful on all the 24 random samples tested (Fig. 3).

Procedure: The following procedure is optimized for the extraction of DNA from 24 samples, using 1.5 ml tubes and a 24 slot microcentrifuge.

Leaf collection and storage: Young (1-4 day old) and tender leaves (quantity 4-6) should be collected. Three procedures were evaluated for storing the leaf samples in -80°C, before DNA extraction:

1. Each sample collected in a polyethylene Easy Zipper Ziploc® Bag resistant to freezing temperatures and stored in a -80°C freezer.
2. Entire leaves harvested, as described by Levi and Thomas (2), ground with three rounds of liquid nitrogen to a fine powder, and stored in 50 ml polyethylene tubes in a -80°C freezer.
3. Entire leaves harvested, 2-4 leaf blade squares (about 5 ∗ 5 mm) cut with scissors or razor blade from the leaves, and stored in a 1.5 ml microcentrifuge tube kept in a -80°C freezer.

We consider the second and third procedures to be the most suitable for long-term storage and sub-sampling of a large number of leaf samples used with the DNA extraction procedure presented herein. These sampling procedures allow DNA extraction from small tissue samples, while avoiding repeated thawing and oxidation of leaf tissue during sub-sampling.

Extraction buffer:

1. In a 50 ml polypropylene tube add:
   - -25 ml of extraction solution (Table 1)
   - -250 mg of Polyvinylpyrrolidone, molecular weight 40,000 (Soluble PVP or PVP-40)

-250 mg Polyvinylpolypyrrolidone (Insoluble PVP or PVPP) (250 mg)

2. Incubate for 10-15 minutes at 60°C and mix vigorously
3. After incubation add 125 µl of 2% β-Mercaptoethanol
4. Incubate at 60°C

DNA extraction procedure:

1. Add 700 µl of extraction buffer to 50-100 mg of leaf tissue (intact or previously ground with liquid nitrogen) in 1.5 ml microcentrifuge tubes; use P1000 pipette tips, cut at 1/3 of their length.
2. Homogenize each sample:
   a. If starting from intact leaf tissue, add quartz or glass sand (the tip of a spatula) and homogenize using a Kontes™ Pellet Pestle™. In this case, it is very important to use tissue from very young leaves (1-4 days).
   b. If starting from leaf tissue previously ground with liquid nitrogen, vortex gently.
3. Incubate for 30 minutes at 60°C and vortex vigorously every 10 minutes.
4. Add to each sample 500 µl of Chloroform:Isoamyl Alcohol (24:1).
5. Vortex vigorously until the mix color turns homogeneous and light-green; release gas and reseal the cap.
6. Centrifuge for 5 minutes at 12,500 rpm.
7. Transfer the supernatant from each sample (≈500 µl) to new 1.5 ml tubes.
8. Add to each sample 500 µl (or 1 volume) of ice-cold Isopropanol.
9. Mix gently by inversion.
10. Incubate for 20 minutes at -20°C.
11. Centrifuge for 15 minutes at 12,500 rpm.
12. Pour supernatant and suspend the pellets in 500 µl 70% ethanol.
13. Centrifuge for 15 minutes at 12,500 rpm.
14. Pour supernatant and dry the pellets at room-temperature then suspend in 100 µl of 0.1X TE.
15. Centrifuge for 5 minutes at 12,500 rpm.
16. Transfer the supernatant from each sample to a new container for final storage (0.5 ml microcentrifuge tubes or 96-well plates).

Literature Cited

1. Guerra-Sanz, J. M. 2002. *Citrullus* simple sequence repeats markers from sequence databases. Molecular Ecology Notes 2(3):223-225.
2. Levi, A., and C. Thomas. 1999. An improved procedure for isolation of high quality DNA from watermelon and melon leaves. Cucurbit Genetics Cooperative Report 22:41-42.

Table 1. Extraction solution for fast DNA extractions from watermelon leaves for SSR marker studies.

Reagent	[Final]	g/l	Notes
Tris-base [a]	0.1 M	12.10	pH=8.5
Sarcosil [b]	0.5 %	5.00	
NaCl	1.4 M	81.82	
EDTA disodium	20.0 mM	5.57	
CTAB [c]	2.5 %	25.00	Pour slowly

[a] Tris hydroxymethyl aminomethane
[b] N-Lauroylsarcosine
[c] Hexadecyltrimethyl-ammonium bromide

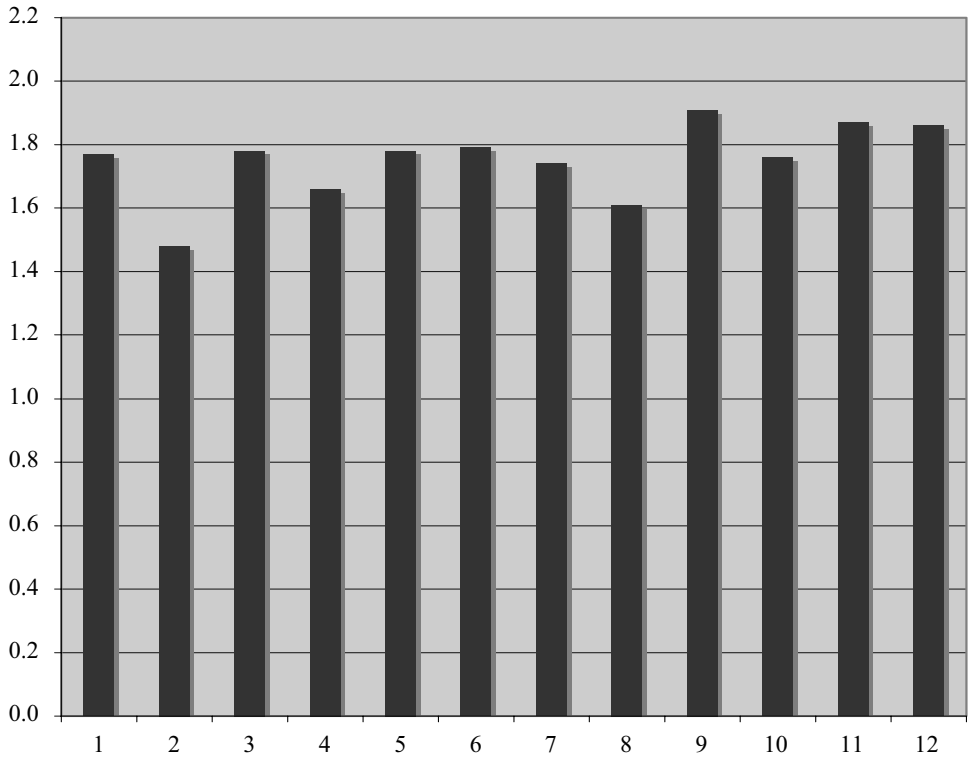

Figure 1. Absorbance ratios at 260/280 (DNA/Proteins) for 12 random samples of DNA extracted from watermelon leaves using the procedure presented herein.

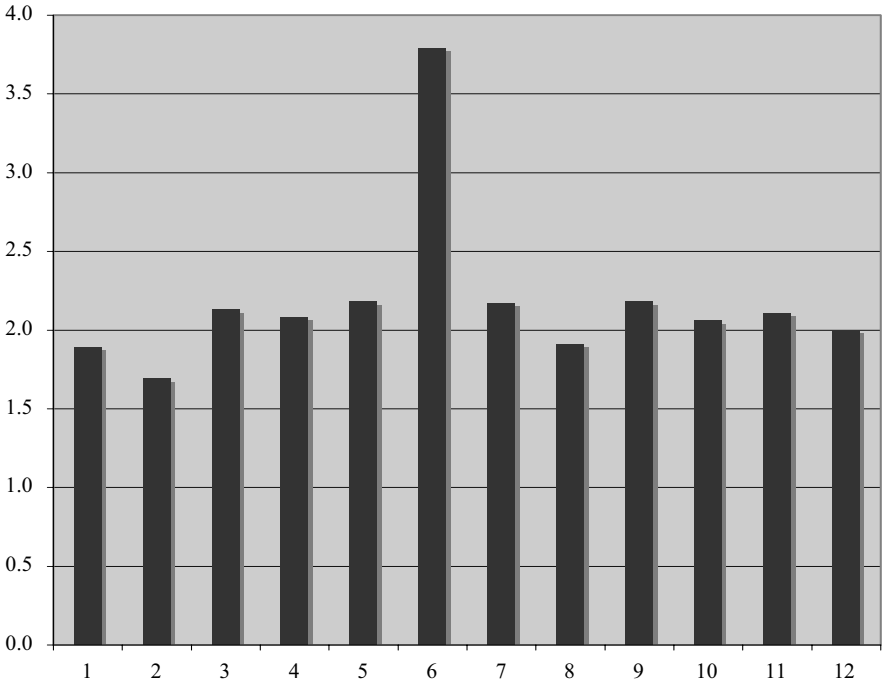

Figure 2. Absorbance ratios at 260/230 (DNA/Polysaccharides) for 12 random samples of DNA extracted from watermelon leaves using the procedure presented herein.

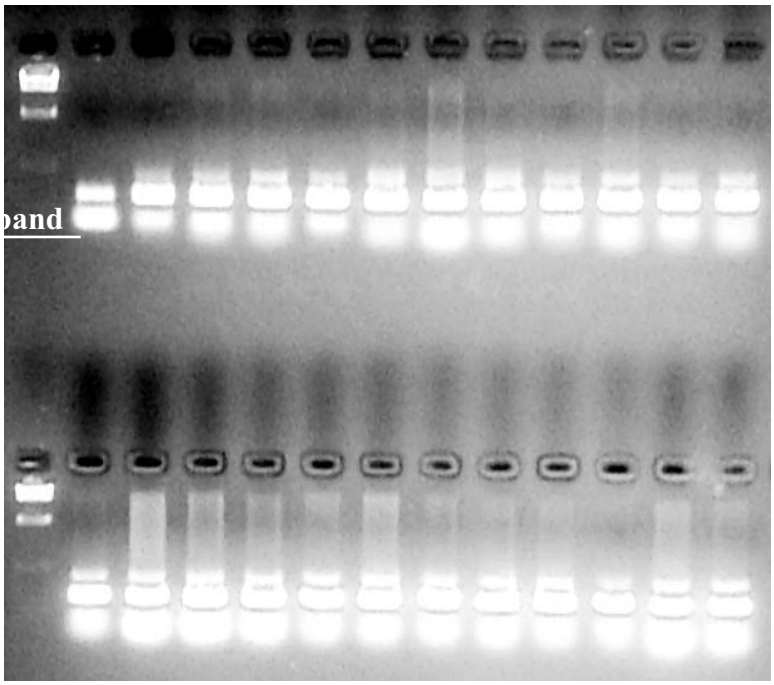

Figure 3. Agarose gel electrophoresis of PCR products from amplification of a non-polymorphic SSR marker from 24 random samples of DNA extracted from watermelon leaves using the procedure presented herein.

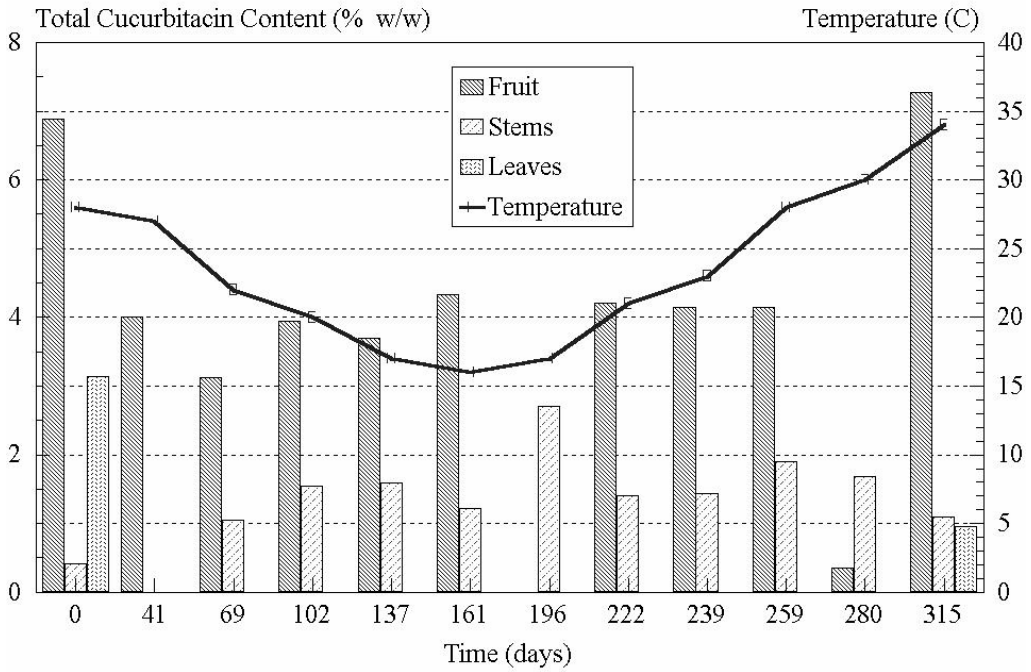

Figure 3. The total cucurbitacin content in elaterium produced from *E. elaterium* fruit, stems and leaves with time and temperature.

A Fasciated Mutant in Watermelon

Nihat Guner and Todd C. Wehner
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

A fasciated mutant was observed in colocynth (*Citrullus colocynthis* Schrad.) plant introduction PI 537277. The plant grows normally for several nodes, and then the main stem becomes flatter and wider at 10 to 15cm above the cotyledonary node. Increased numbers of leaves, tendrils, and flowers (staminate and pistillate) are produced on each node of the main stem (Fig. 1). Occasionally, the main stem separates into two flattened stems. The staminate and the pistillate flowers are fertile (Fig. 2).

Fasciated plants have been described in cucumber and melon as well. The trait is controlled by a single recessive gene in both crops (1, 2). In cucumber, the gene has incomplete penetrance (2). It was reported that environmental factors also influence penetrance of the fasciation gene in cucumber. The proportion of fasciated plants of 'Lemon' was increased by irradiation and growth regulator treatments, but these treatments did not induce fasciation in normal monoecious cucumber cultivars (2).

It was also reported that fasciation is associated with opposite leaf arrangement at lower nodes of the main stem (2). We observed an association between opposite leaves and fasciation in PI 537277 as well. A study of fasciation in watermelon is needed to determine the inheritance of the trait.

Literature Cited

1. Gabillard, D. and M. Pitrat. 1988. A fasciated mutant in *Cucumis melo*. Cucurbit Genet. Coop. Rpt. 11: 37.
2. Robinson, R. W. 1978. Fasciation in the cucumber. Cucurbit Genet. Coop. Rpt. 1: 11.

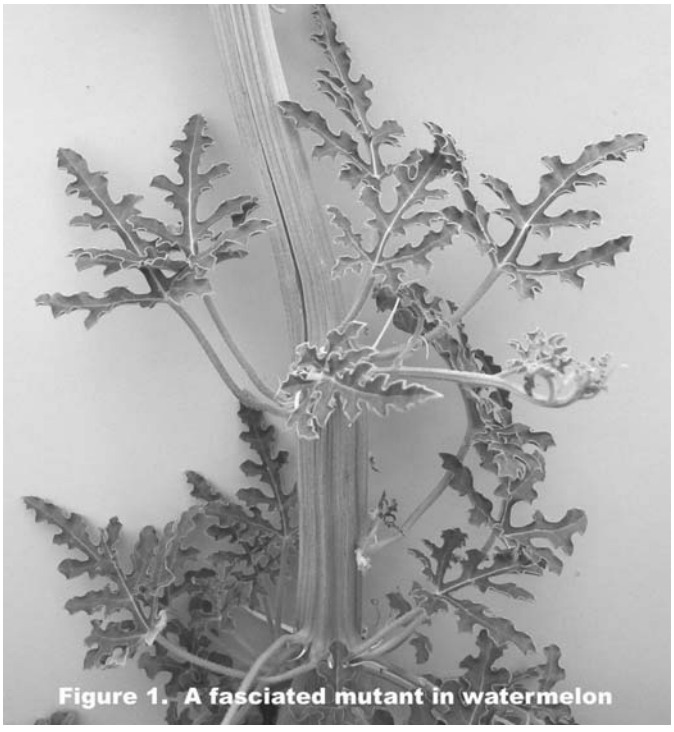

Figure 1. A fasciated mutant in watermelon

Figure 2. Fruit set in faciated mutant

Accessions Having Opposite Leaf Arrangement at the First True Leaf in Watermelon

Nihat Guner and Todd C. Wehner
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Watermelon plants normally have alternate leaves, with a single leaf per node that is positioned 180° from leaves at adjacent nodes. During a screening of the watermelon germplasm collection (totaling 1613 accessions) for disease resistance, we observed an opposite leaf arrangement at the first true leaf of several PI accessions (PI 559995, PI 560008, and PI 560010). These PI accessions were heterogeneous for leaf arrangement, with some plants having only one first true leaf and others having two opposite first true leaves, borne at a 90° angle from the second true leaf (Fig. 1). A very short internode length between first and second true leaves may cause opposite leaf arrangement in watermelon. Opposite leaf arrangement at the first true leaf is unstable. All plants with opposite leaves at the first nodes of their main stem eventually revert to alternate leaf arrangement. Most of the time they reverted at the second node, but occasionally it was at the third node. Opposite leaf arrangement also occurs in cucumber in the cultivar Lemon.

Robinson (1) reported that opposite leaf arrangement in cucumber is controlled by a single recessive gene. In the F_2 generation of reciprocal crosses between alternate- and opposite-leaved cucumber plants, the proportion of seedlings with opposite leaves was significantly less than 25% in each of 26 F_2 populations. The combined segregation ratio was 875 alternate to 86 opposite. Tkachenko (2) concluded that at least three genes are required to produce opposite leaves in cucumber. An alternate explanation is that inheritance is simple, but the single recessive gene has incomplete penetrance. Youngner (3) reported that genes of 'Lemon' for sex expression *(m)* and five fruit locules *(l)* were linked, and were associated with opposite leaves in segregating generations.

Research is needed to determine the inheritance of opposite leaf arrangement at the first true leaf stage in watermelon.

Literature Cited

1. Robinson, R.W. 1987. Inheritance of opposite leaf arrangement in Cucumis sativus L. Cucurbit Genet. Coop. Rpt. 10: 10-11.
2. Tkachenko, N.N. 1935. Preliminary results of a genetic investigation of the cucumber, *Cucumis sativus* L. *Bull. Appl. Pl. Breed. Ser*. 2, 9:311-356.
3. Youngner, V.B. 1952. A study of the inheritance of several characters in the cucumber. Ph.D. Thesis. Univ. Minnesota, St. Paul.

Figure 1. Opposite leaf arrangement at the first true leaf in watermelon.

Lycopene and Total Carotenoid Content as Descriptors for *Citrullus lanatus*: Limitations and Preliminary Trials

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USDA/ARS, South Central Agriculture Research Laboratory, P.O. Box 159, Lane, OK 74555.

Stephen R. King
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Robert L. Jarret
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Amnon Levi
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Ya'akov Tadmor
Newe Ya'ar Research Center, ARO, ISRAEL

Germplasm collections are valuable sources of genetic material for crop improvement. The watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) germplasm collection in Griffin, GA (5) is a good example of a collection that is contributing to the production of improved cultivars. This collection contains approximately 1600 genotypes of the cultivated taxa (*C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*) and is routinely utilized by researchers for the identification of valuable traits such as disease resistance, improved yield, unique flesh characteristics, health-promoting compounds, etc. In order to make the *Citrullus* germplasm collection more user-friendly and valuable to the scientific community genotypes should be characterized to the fullest extent possible. Currently, about 15 characteristics (descriptors) such as flesh color, fruit weight and shape, rind thickness, brix, etc., are used to describe watermelon accessions in the Griffin genebank. However, as new uses are found and trends in watermelon consumption change, it is useful to add or modify descriptors. Two descriptors currently being evaluated are lycopene and total carotenoid content in fruit flesh.

Red fleshed watermelons contain high quantities of lycopene, a carotenoid that imparts the red color. This compound has powerful antioxidant properties and has been shown to lower the risk of myocardial infarction (6) and some cancers. Few red fruits and vegetables contain detectable quantities of lycopene and the USDA-NCC Carotenoid Database officially considers watermelon, on average, to contain higher levels of lycopene than other fresh fruits and vegetables (4). Because of the potential health benefits of lycopene, there is interest in increasing its content in commercial cultivars.

Red and pink fleshed watermelon also contain carotenoids related to lycopene, such as β-carotene and pro-lycopene. The quantities of non-lycopene carotenoids in watermelon have been reported as high as 40% (8) but is more typically between 10-20% of the total carotenoids present in commercial varieties (Perkins-Veazie, unpublished results). Orange watermelons contain predominately pro-lycopene but the other carotenoids present have been reported to comprise as much as 50% of the total carotenoid content (9). Watermelon with white and yellow flesh contain, as of yet, unidentified carotenoids.

The germplasm descriptor list for *Citrullus* does not currently contain a descriptor for fruit pigment content. Addition of descriptors that report fruit lycopene and total carotenoid content will make this information available to the industry in an easy to search format. The following potential descriptors were evaluated: maximum lycopene content, range of lycopene content in red fruit, mean lycopene content of red fruit, percent of red fruit, maximum total carotenoid content, range of total carotenoid content in pigmented fruit, mean total carotenoid content of pigmented fruit, and percent of pigmented fruit. Plant Introduction (PI) lines were evaluated in 2000-2003. One to thirteen pigmented fruits from each of eight PI lines was collected for lycopene and six for total carotenoid analysis.

Results: Lycopene content in fruit flesh ranged from 10 to 81 $\mu g \cdot g^{-1}$ fresh weight (FW). The largest range of lycopene content within a line was 57 $\mu g \cdot g^{-1}$ (PI 385964). PI 288232 had the highest lycopene content detected (81 $\mu g \cdot g^{-1}$) in one fruit and PI 319212 had the highest overall lycopene content with a mean of 64 $\mu g \cdot g^{-1}$ (Table 1). Among the yellow and orange watermelon the total carotenoid content ranged from 3-13 $\mu g \cdot g^{-1}$. The largest range of total carotenoid content within the eight lines was 3-13 $\mu g \cdot g^{-1}$ (PI 629111). This PI line also had the highest total carotenoid content detected (13 $\mu g \cdot g^{-1}$) in one fruit and PI 629111 and NSL 29605 had the highest overall total carotenoid content 8 $\mu g \cdot g^{-1}$ (Table 2).

Using this data, we developed potential descriptors and descriptor codes for the *Citrullus lanatus* PI collection. The four descriptors and their codes are listed in Tables 3 and 4.

Discussion: These preliminary experiments tested the feasibility of using lycopene and total carotenoid content as descriptors for *Citrullus lanatus* PI lines and were used to formulate descriptor codes for documenting various carotenoid contents. Although the proposed descriptors are less informative than the actual data values (Table 5 vs. 1 and 2), the codes are consistent with the GRIN database and are in a format that is easy to use with a search engine. Since PI lines are often heterogeneous in their expression and accumulation of carotenoids, just reporting the average carotenoid content would not be very meaningful. Therefore, we suggest reporting the carotenoid content in eight ways: maximum lycopene content, range of lycopene content in pigmented fruit, mean lycopene content of pigmented fruit, percent of red pigmented fruit, maximum total carotenoid content, range of total carotenoid content in pigmented fruit, mean total carotenoid content of pigmented fruit, and percent of pigmented fruit. These eight descriptors supply information on the number of fruit that are pigmented, how many of the pigmented fruit are red versus yellow or orange, amount of lycopene and or total carotenoids in the fruit, and the range of these compounds present in the PI lines. Non-pigmented fruit are not included because they are typically low in carotenoids (unpublished data) and thus would skew the data making it less informative. In this report, total carotenoid content is documented for yellow and orange fruit only, but it is informative to list this trait for red watermelon as well. Since carotenoid profiles are not well characterized for yellow and orange watermelon total carotenoid content is suggested as a descriptor and not descriptors for individual compounds, such as pro-lycopene.

Obviously, analyzing more fruit will provide more accurate descriptor data. This is especially true since the production environment can affect lycopene levels by 10-20% (7) and PI lines are often grown in the greenhouse which can reduce carotenoid accumulation. For optimal results we recommend testing at least 10 plants from homogeneous lines and more from lines that

demonstrate heterogeneity for fruit color. We are currently analyzing heterogeneous lines to determine an optimal number of fruit for reporting on these descriptors. Only reporting on ripe fruit is imperative, since under-ripe fruit can have 40% less lycopene than ripe fruit of the same variety (Perkins-Veazie, unpublished data).

Methods: *Watermelon Fruit*: *Citrullus lanatus* PI lines from the USDA's *Citrullus lanatus* germplasm collection in Griffin, GA were transplanted in a 0.5 ha field of Bernow fine sandy loam soil at the South Central Agricultural Research Laboratory in Lane, OK. Seedlings were transplanted in April at the three-leaf stage, on black plastic mulch with drip tape. Plants were 0.9 m apart in a single row 9.1 m in length, 3.6 m between reps, and with a 3.1 m alley separating rows. The cultural methods were performed according to Oklahoma State University Extension guidelines (Bulletin No. F-6236). PI 270546 was grown in the greenhouse in winter 2001 until fruit was ripe.

Watermelons were harvested when external appearance suggested ripeness. Ripeness was determined by total soluble solid content, texture, and seed development. Heart tissue from ripe, pigmented watermelons were collected and stored in –80^{o}C until they were processed.

*Sample Preparation***:** All steps from the time watermelons were cut lengthwise were performed in subdued lighting at room temperature. The excised tissue was cut into approximately 2.6 mm^3 cubes or smaller. Samples were pureed either fresh or after storage at -80^{o}C. Tissue (25-500 g) was homogenized in a Waring blender until chunks were less then 4 mm^3 then pureed using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.) with a 20 mm O.D. blade to produce a uniform slurry with particles smaller then 2 mm^3. The samples were not allowed to heat or froth.

*Lycopene Detection Method***:** This method was performed as in Fish et al.(3). Samples were kept on ice unless otherwise stated. Briefly, approximately 0.6 g (determined to the nearest 0.01 g) duplicate samples were weighed from each puree into two 40 ml amber screw-top vials (Fisher, #03-391-8F) that contained 5 ml of 0.05% (w/v) BHT in acetone, 5 ml of 95% ethanol, and 10 ml of hexane. Purees were stirred on a magnetic stirring plate during sampling. To extract lycopene from the samples, they were placed on ice on an orbital shaker at 180 rpm for 15 min. Then, 3 ml of deionized water were added to each vial and the samples were shaken for an additional 5 minutes on ice. The vials were then left at room temperature for 5 min. to allow for phase separation. Absorbance of the upper, hexane layer was measured in a 1 cm path length quartz cuvette at 503 nm after blanking with hexane. The lycopene content of watermelon was then estimated using the absorbance at 503 nm and the sample weight (3, 1).

*Total Carotenoid Detection Method***:** Frozen samples were weighed and homogenized with enough acetone to cover the sample then transferred to a filter paper lined funnel and washed with acetone until all the color was removed. Then 50 ml of hexane was added to the acetone/carotenoid mixture. Water was added to facilitate separation of the layers. The hexane layer was removed, transferred to a 1 cm quartz cuvette and read at 485 nm to estimate total carotenoids (2).

***Acknowledgments*:** Special thanks are given to Dr. Julie Collins, Dr. Penelope Perkins-Veazie, and Dr. Wenhua Lu for their thorough reviews. Thanks to Anthony Dillard, Amy Helms, and Ashly Gammon for technical assistance. This work was partially funded by the Cucurbit Crop Germplasm Committee.

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Table 1: Lycopene content analysis for 8 PI lines.

PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Lycopene Content µg/g[y]	Lycopene Content Range µg/g[x]	Mean Lycopene Content µg/g[x]	% Plants with Pigmented Fruit[w]
270546	1	35	NA	NA	13
288232	4	81	33-81	56	100
319212	2	76	52-76	64	55
385964	13	70	13-70	44	57
525085	2	62	59-62	61	75
525096	2	44	27-44	36	78
560000	2	70	40-70	55	8
482291	5	32	10-32	23	95

[z]Each fruit was harvested from a different plant.
[y]Maximum lycopene content among the fruit analyzed within this PI line.
[x]Range of lycopene content within this PI line.
[w]At least 10 plants were analyzed for this data.

Table 2: Total Carotenoid content analysis for 6 yellow or orange PI lines.

PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Total Carotenoid Content µg/g[y]	Total Carotenoid Content Range µg/g[x]	Mean Total Carotenoid Content µg/g[x]	% Plants with Pigmented Fruit[w]
219887	3	11	5-11	7	100
229749	1	7	NA	NA	NA
601228	3	6	4-6	4	100
629111	2	13	3-13	8	100
NSL29605	3	9	7-9	8	100
NSL68237	3	12	5-12	10	100

[z]Each fruit was harvested from a different plant.
[y]Maximum carotenoid content among the fruit analyzed within this PI line.
[x]Range of carotenoid content within this PI line.
[w]At least 2 plants were analyzed for this data if reported.

Table 3: List of lycopene descriptors and their codes and definitions for red watermelon fruit.

	Descriptors			
	Maximum Lycopene Content	Mean Lycopene Content of Red Fruit	Range of Lycopene Content in Red Fruit	Percent of Red Fruit
Codes	Definitions			
1	≤10 µg/g	≤10 µg/g	≥125 µg/g	0 %
2	11-25 µg/g	11-25 µg/g	100-125 µg/g	1-10 %
3	25-50 µg/g	25-50 µg/g	75-100 µg/g	11-25 %
4	50-75 µg/g	50-75 µg/g	50-75 µg/g	26-50 %
5	75-100 µg/g	75-100 µg/g	25-50 µg/g	51-75 %
6	100-125 µg/g	100-125 µg/g	11-25 µg/g	76-90 %
7	≥125 µg/g	≥125 µg/g	≤10 µg/g	91-100 %

Table 4: List of total carotenoid descriptors and their codes and definitions for watermelon fruit.

	Descriptors			
	Maximum Total Carotenoid Content	Mean Total Carotenoid Content of Pigmented Fruit	Range of Total Carotenoid Content in Pigmented Fruit	Percent of Pigmented Fruit
Codes	Definitions			
1	≤10 µg/g	≤10 µg/g	≥125 µg/g	0 %
2	11-25 µg/g	11-25 µg/g	100-125 µg/g	1-10 %
3	25-50 µg/g	25-50 µg/g	75-100 µg/g	11-25 %
4	50-75 µg/g	50-75 µg/g	50-75 µg/g	26-50 %
5	75-100 µg/g	75-100 µg/g	25-50 µg/g	51-75 %
6	100-125 µg/g	100-125 µg/g	11-25 µg/g	76-90 %
7	≥125 µg/g	≥125 µg/g	≤10 µg/g	91-100 %

Table 5: Data from table 1 and 2 listed in descriptor format.

PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Lycopene Content	Range of Lycopene in Red Fruit	Mean Lycopene Content of Red Fruit	Percent of Red Fruit
270546	1	3	NA	NA	3
288232	4	5	5	4	7
319212	2	5	6	4	5
385964	13	4	4	3	5
525085	2	4	7	4	5
525096	2	3	6	3	6
560000	2	4	5	4	2
482291	5	3	6	2	7
PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Total Carotenoid Content	Range of Total Carotenoid in Pigmented Fruit	Mean Total Carotenoid Content of Pigmented Fruit	Percent of Pigmented Fruit
219887	3	2	7	1	7
229749	1	1	NA	NA	NA
601228	3	1	7	1	7
629111	2	2	7	1	7
NSL29605	3	1	7	1	7
NSL68237	3	2	7	1	7

[z]Each fruit was harvested from a different plant.

Cultivars Suitable for Watermelon Rind Pickles

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Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a major vegetable crop in the U.S., with an average production (1998 to 2003) of about 1.8 million $Mg \cdot year^{-1}$, an average of 75,000 hectares planted (89% harvested), and a yield of 26.4 $Mg \cdot ha^{-1}$. The market value has been stable in the last five years, with an average total value of 280 million dollars (3).

Minor uses of watermelon fruit include edible seeds and watermelon rind pickles. Home gardeners and small industries make rind pickles from the leftover watermelon crop, especially from cultivars having thick and crisp rind. Recently, Simonne et al. (2) compared traditional recipes for watermelon rind pickles for their efficacy, food safety, and quality. We have been getting questions about suitable cultivars for rind pickles from home gardeners, perhaps indicating increased interest.

The watermelon fruit consists of the exocarp, mesocarp, and endocarp. The endocarp is the seed-containing part that is consumed as food, and the mesocarp and exocarp are usually referred to as the rind. The rind is used for making pickles after removing the thin exocarp, leaving the crisp, white mesocarp.

Some old or obsolete cultivars were discontinued from use in the market because of their thick rind, so they would be obvious candidates for use in making watermelon pickles. Some of those old cultivars are still used by home gardeners and heirloom collectors (1), and seeds are available from seed companies. 'Tom Watson', 'Georgia Rattlesnake', and 'Black Diamond' are three heirloom cultivars with good flavor, attractive rind pattern and color, and thick rind. The objective of this study was to determine the rind thickness of old watermelon cultivars, for use in making pickles.

Methods: Cultivars were chosen with thick rind and then classified by cultivar type (size and shape), and flesh color (red and orange). Trials were run in 2001 and 2002 at the Horticultural Research Station in Clinton, NC and at the Field Crops Research Station in Kinston, NC. The trial was a randomized complete block experiment with two years, two locations, and two or four replications per year and location. Eighty obsolete cultivars and adapted checks were included in the trials. Rows were covered with black polyethylene mulch (0.03 mm) at Kinston. No mulch was used at Clinton. Plots were overhead irrigated at Clinton and drip irrigated at Kinston. Individual rows of each plot were 7.3 m long, on 3.0 m centers with 0.6 m between hills, and 2.4 m alleys at each end of the plot.

Results: Cultivars having thick rind included Carolina Cross #183, Cobbs Gem, Florida Favorite, Garrisonian, Malali, Moon & Stars, Navajo Sweet, Smokylee, Stone Mountain, Tendersweet Orange Flesh, Tom Watson, and Weeks NC Giant (Table 1).

In response to requests for favorite recipes for pickles, we have given one here, courtesy of Mrs. R.B. Edwards (Table 2). With the increased use of watermelons to produce ready-to-eat chunks in containers, there will be a large supply of rinds in the cutting area. Those industries may be candidates for increased production of rind pickles in the future.

Table 1. Cultivars for watermelon rind pickles.

Thin Rind (less than 15 mm)
Allsweet, Cream of Saskatchewan, Crimson Sweet, Early Arizona, Early Canada, Golden Midget, Mickylee, New Hampshire Midget, Petite Sweet, Sweetheart, Sugar Baby, Yellow Doll
Medium Rind (15 to 19 mm)
Calhoun Gray, Charleston Gray, Dixielee, Fairfax, Georgia Rattlesnake, Mardi Gras, Mountain Hosier, Regency, Sun Gold, Tendergold, Tastigold
Thick Rind (greater than 19 mm)
Carolina Cross #183, Cobbs Gem, Florida Favorite, Garrisonian, Malali, Moon & Stars, Navajo Sweet, Smokylee, Stone Mountain, Tendersweet Orange Flesh, Tom Watson, Weeks NC Giant

Table 2. Traditional recipe for watermelon rind pickles from the Southeastern U.S. (Mrs. R.B. Edwards, Siler City, NC).

Ingredients:

4 pounds of watermelon rind without green skin and cut in 1/2"∗1/2" cubes
1 gallon of cold water
2 tablespoons of favorite pickling spices (tied in a cheesecloth bag)
3 pints of vinegar
3 pounds of sugar (1 brown : 1 white)
3 tablespoons of alum

Instructions:

1. In the evening, dissolve alum in cold water
2. Add the rind cubes and let stand over night
3. In the morning exchange old for fresh water and let stand for 3 more hours
4. Cook until the rind cubes become tender, then add all other ingredients and boil gently until the rind cubes are clear
5. Remove the spice bag, pack in jars with the hot juice over the rind cubes
6. Seal and sterilize the jars by boiling them

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Heterosis for Yield in a Watermelon Hybrid

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Heterosis in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)), as well as general (GCA) and specific (SCA) combining ability, received much attention in the 1950s and 1960s. Much of the research was done in India (1, 2, 4, 5, 7-10), and was based on diallel or top crosses of elite inbreds, rather than a random set of lines originating from a single population. Overall, these studies indicated the presence of heterosis in watermelon and the importance of GCA in the choice of parents for hybrid production. Two recent studies were run in Brazil on seven intercrossing populations with evaluation of reciprocal crosses (3) and tetraploid females crossed with diploid males for the production of triploid seeds (11). The presence of heterosis in watermelon, and the importance of parents and direction of the crosses was confirmed. The objective of this study was to measure heterotic effects in a hybrid of two important watermelon cultivars. Our data were collected in a replicated trial of the inbred cultivars 'Allsweet' and 'Jubilee' and their F_1 hybrid.

Methods: The experiment was conducted in the summer of 2001 and 2002 at the Horticultural Crops Research Station at Clinton, North Carolina (4 replications/year) and at the Cunningham Research Station at Kinston, North Carolina (2 replications, 2002 only). The experiment at Clinton was a randomized complete block. Rows were direct seeded on raised, shaped beds with rows 3.1 m apart. Plots were 3.7 m long, with 0.6 m between hills, and 2.5 m alleys at each end of the plot. In Kinston, rows were covered with black polyethylene mulch and drip irrigated. The experiment was conducted using recommended practices (6). Plots were harvested twice (26 July and 9 August) in Clinton in 2001, once (25 July) in Clinton in 2002, and twice (23 July and 6 August) in Kinston in 2002 for fruit yield and quality measurements. Data were analyzed using the MEANS, CORR, and GLM procedures and the LSD option of SAS-STAT Statistical Software Package (SAS Institute, Cary, NC). High parent heterosis was calculated as percentage increase of the F_1 hybrid value over the best parent value.

Results: The F_1 hybrid evaluated had 22% more total fruit weight and 32% more total fruit number than 'Jubilee', the best parent (Table 1). There was no difference in percentage marketable fruit in the parents or F_1 hybrid. Average fruit size was similar in 'Jubilee' and the F_1 hybrid, with 'Allsweet' significantly smaller. High parent heterosis was significant for fruit number, but not for fruit weight. It appears that there is high parent heterosis for yield in the 'Allsweet' x 'Jubilee' hybrid.

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Table 1. Yield for two inbred parents and their F_1 hybrid evaluated in trials of two years, two locations, one or two fields, and two replications.

Cultivar or statistic	Total weight [y] (Mg/ha)	Total number [y] (th/ha)	Marketable weight [y] (%)	Fruit size [y] (kg/fruit)
Allsweet	65.9 b	6.8 b	94 a	9.8 b
Jubilee	87.9 ab	7.4 b	93 a	12.9 a
F_1 hybrid	107.1 a	9.8 a	96 a	11.5 a
HP Heterosis [z]	22%	32%	2%	-
Correlation (tot. weight vs. market. weight)		0.98 **		
Correlation (tot. no. fruits vs. market. fruit no.)		0.96 **		
Correlation (% market. weight vs. % market. fruit no.)		0.97 **		

** r-value significant at p-value<0.01

[y] Equal letters correspond to statistically equal means, based upon Fisher's Protected LSD

[z] High Parent Heterosis = $[(F_1\text{-HP})/F_1]*100$; only where HP > F_1, based upon LSD

Estimates of Variance Components and Broad-Sense Heritability for Yield in Watermelon

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Watermelon breeders in the late 1800s were able to release many new cultivars of different types with good quality and early maturity for use in the U.S. By 1900 'Angeleno', 'Chilean', 'Florida Favorite', 'Georgia Rattlesnake', 'Cole Early', 'Kleckley Sweet', and other open pollinated cultivars had been on the market many years (8). In the 20th century, development of high yielding cultivars (total weight per unit area) became a major goal for public and private watermelon breeders (4).

Overall, watermelon yield in the United States has been increasing during the last five years (7), from 24 $Mg \cdot ha^{-1}$ in 1998 up to 29 $Mg \cdot ha^{-1}$ in 2002. Part of the increase in yield might be due to more reliable production practices and to the availability of more efficient pesticides (3), but it is also due to genetic improvement of yield.

Currently, breeders evaluate breeding lines and new cultivars for yield and quality using 1- to 3-row plots, multiple locations, and multiple seasons (planting dates) for trialing. Usually, plots are harvested once by private breeders and several times by public breeders (5). It is important to have genetic variability for yield if progress is to be made in trialing. In addition, it is important to measure the effect of environment (year, location, etc.) on yield.

The objective of this study was to use a diverse set of watermelon cultivars to measure genetic variance for yield and the effect of year and location on broad-sense heritability.

Methods: The experiment was conducted in 2001 and 2002 at the Horticultural Crops Research Station at Clinton, North Carolina and at the Cunningham Research Station at Kinston, North Carolina (2002 only). Rows were direct seeded on raised, shaped beds on 3.1 m centers. Plots were 3.7 m long, with 0.6 m between hills, and 2.5 m alleys at each end of the plot. In Kinston, rows were covered with black polyethylene mulch and drip irrigated. The experiment was conducted using horticultural practices recommended to the growers by the North Carolina Extension Service (6). A total of 80 cultivars were evaluated for fruit yield and quality. There were 72 obsolete cultivars obtained from seed companies, the Seed Savers Exchange, and the National Seed Storage Laboratory (Fort Collins, Colorado). Eight elite hybrid cultivars were included as checks ('Starbrite', 'Stars-N-Stripes', 'Legacy', 'Sangria, 'Fiesta', 'Sultan', 'Regency', and 'Royal Flush'). Plots were harvested twice (26 July and 9 August) in Clinton in 2001, once (25 July) in Clinton in 2002, and twice (23 July and 6 August) in Kinston in 2002 for fruit yield and quality measurements. Individual cull and marketable fruit were weighed to the nearest pound for each plot. Numbers of cull and marketable fruit were also recorded.

Variance components estimates for the experiment were obtained from two different datasets: 1) two years (2001 and 2002), four replications per year, and one location (Clinton), and 2) two locations (Clinton and Kinston), two replications per location, and one year (2002). The regression models used were, respectively:

1) Y=Year+[Replication(Year)]+Cultivar+(Cultivar∗Year)+Error
2) Y=Location+[Replication(Location)]+Cultivar+(Cultivar∗Location)+Error

We analyzed data for the yield traits using variance component analysis, and then calculated broad-sense heritability for each of the two both models as follows (2):

1) $H^2_B=\sigma^2(\text{Cultivar})/[\sigma^2(\text{Error})/(\#\text{rep.}*\#\text{year})]+[\sigma^2(\text{Year})/\#\text{year}]+[\sigma^2(\text{Cultivar})]$
2) $H^2_B=\sigma^2(\text{Cultivar})/[\sigma^2(\text{Error})/(\#\text{rep.}*\#\text{location})]+[\sigma^2(\text{Location})/\#\text{location}]+[\sigma^2(\text{Cultivar})]$

Data were analyzed using the MEANS, GLM, and VARCOMP procedures of SAS-STAT Statistical Software Package (SAS Institute, Cary, NC).

Results: Our estimates of variance components and broad-sense heritability are not definitive, since they are based only on measurements of a diverse set of cultivars of unknown and/or diverse genealogy. Additional research should make use of populations developed from known and divergent parents. Nevertheless, our estimates apply to this dataset, and the diversity of cultivars included in our study allows useful conclusions to be drawn.

The experiments had a large error variance (Table 1). That was probably due to 1) the wide range of fruit-types tested, which might have affected the precision and accuracy of harvest and weighing; 2) the unbalanced number of harvests among locations and years, due to different homogeneity of maturation of the different cultivars in the same field; and 3) the generally high weight of single fruit that, if not accurately measured, might greatly bias the calculation of total yield. The latter might account for the larger error, relative to the other variance components, recorded for total weight per hectare than for the other traits.

Besides error variance, most of the variation in yield traits (total weight and total number) was due to environmental factors (year or location) and to the genotype. A larger interaction variance (genotype x environment) was present in the analysis among locations for total weight. The largest variance component for the percentage of marketable weight was due to the genotype (among locations) or to interaction of genotype and year of testing. The variation of average fruit size and soluble solids content were mostly genotypic in both analyses.

Broad-sense heritability was consistently high in both analyses only for average fruit size. For percentage of marketable weight, broad-sense heritability was very low in both cases, while for the other traits it was medium to high when year was in the model and medium to low when location was in the model. Yield components (total weight and total number) in our experiments had low heritability and, if confirmed in a larger experiment, would explain why yield improvement is so difficult. Watermelon breeders usually develop new cultivars using pedigree and backcross methods and few lines being tested in field trials. The identification of quantitative trait loci (QTL) for yield may improve the efficiency of selection for traits of low heritability relative to phenotypic selection (1).

Our experiments indicate low genotype by environment (year or location) interaction. Thus, trial location may not be a problem for obtaining data that reflects performance in the general region. Therefore, testing on bare ground with overhead irrigation in few locations and one year would be possible for the early stages of selection, where ranking among cultivars matters more than absolute estimates of yield. Furthermore, genetic variability for yield components in our varied set of cultivars was high and should allow progress in selection for increased yield. Nevertheless, more limits to progress might be present in certain fruit classes (fruit size, shape, rind pattern, etc.). The current market appears to be expanding away from certain types (i.e. 'Allsweet' types in the United States or 'Charleston Gray' types in the Middle-East) in favor of new types.

In conclusion, genetic variability for yield components is still present in watermelon and the new fruit types coming into the market might allow breeders to exploit new genetic sources for yield improvement.

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Table 1. Variance components and broad-sense heritability for yield and quality data of the 80 cultivars evaluated in two experiments.

Genetic Parameter	Total Yield		Percentage of marketable weight	Fruit size	Soluble solids
	Weight	Number			
Experiment 1: 2 years, 4 replications of data per year					
σ^2(Year)	188.13	7.16	0.00	0.76	0.00
σ^2(Rep. (Year))	52.37	1.33	11.04	0.15	0.15
σ^2(Cultivar)	229.34	6.38	0.89	5.83	0.57
σ^2(Cultivar ∗ Year)	101.60	1.02	17.14	0.69	0.05
σ^2(Error)	574.55	9.00	89.95	2.55	0.86
H^2_B [a]	0.58	0.57	0.07	0.89	0.84
Experiment 2: 2 locations, 2 replications of data per location					
σ^2(Location)	1170.85	20.17	0.00	0.01	0.33
σ^2(Rep. (Location))	40.54	0.92	6.56	0.12	0.01
σ^2(Cultivar)	192.29	9.17	9.75	6.30	0.51
σ^2(Cultivar ∗ Location)	20.75	0.16	2.63	0.82	0.17
σ^2(Error)	1070.31	12.69	99.73	2.37	0.73
H^2_B [b]	0.18	0.41	0.28	0.91	0.59

[a] $H^2_B=\sigma^2(\text{Cultivar}) / [\sigma^2(\text{Error}) / (\#\text{rep.}*\#\text{year})]+[\sigma^2(\text{Year}) / \#\text{year}]+[\sigma^2(\text{Cultivar})]$

[b] $H^2_B=\sigma^2(\text{Cultivar}) / [\sigma^2(\text{Error}) / (\#\text{rep.}*\#\text{location})]+[\sigma^2(\text{Location}) / \#\text{location}]+[\sigma^2(\text{Cultivar})]$

Some Considerations on Speed of Weighing Watermelon Fruit in Yield Trials

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Watermelon breeders interested in measuring yield of new inbreds and hybrids need an efficient method to get fruit weight. The method should provide data on many cultigens without using excessive time or resources. Often fruit weight in trials is measured by rounding the value either to the nearest integer or to the nearest tenth of the measurement unit (lb or kg). Data from each plot are then changed into standard measures (cwt/A or Mg/ha), and average fruit weight is calculated to characterize the cultivars by size: icebox (<12 lb), small (12-18 lb), medium (18-24 lb), large (24-32 lb), and giant (>32 lb) (1).

Neppl recently surveyed the trialing methods used by watermelon breeders (2) and found that trials are usually conducted using small plots of 1 to 3 rows each, in multiple locations when possible. The vines are not trained to avoid reducing the amount fruit set and the fruit are traced to the proper plot at harvest. Breeders in private companies usually use single harvest trials, public researchers usually use multiple harvest trials. The development of an efficient method for measuring fruit weight would be useful in reducing the cost of yield trials. Methods used by researchers include weighing each fruit for each plot, weighing all the fruit in one load per plot, or weighing one typical fruit per plot and multiplying by the number of fruit in the plot. The first method appears to be most common, while the last method requires that the average fruit be carefully chosen.

Factors affecting efficiency: In our study, yield data were taken by a four person crew, with one person loading the scale, one unloading, one reading the measurement on the scale display, and one person recording the measurement on a portable computer. The crew moved together across the field measuring fruit weights for a series of eight plots using different techniques.

The speed of yield data collection was not affected by whether the data was recorded in tenths or rounded off to the nearest lb or kg. In our case, the limiting factor was the delay from the time the fruit was placed on the scale to the time the weight was displayed. A spring scale may be faster than a digital scale in this case. However, digital scales but not spring scales are available with an extension cord for the display to permit reading while standing away from the scale. That reduces interference with the scale loading crew, permitting the crew to work faster.

There was a large effect on speed due to the coordination and order of the single operations leading to the weighing of each plot. However, this will depend on the number and quality of people available to help do the field work, so was not measured as part of this study.

Precision required on single fruit weights: We were interested in the effect of reduced precision in yield data on the estimate of average fruit weight for cultivars being tested. For this, yield data were obtained from the watermelon trials conducted at the Cunningham Research Station at Kinston, NC (68 accessions) and at the Central Crops Research Station at Clayton, NC (64 accessions) in 2000. Each trial was harvested three times. The plot data were collected as single fruit weights with data taken to the nearest pound or in tenths of a pound. The average fruit weight was the mean of all fruit harvested for each cultivar. Yield was the mean over replications summed over harvests for each cultivar.

Average fruit weight and yield were calculated both from the non-rounded and rounded weights. Correlation analysis was run for the two traits calculated from rounded data vs. the same traits calculated from non-rounded data. The data were analyzed for five fruit-size categories: icebox (<12 lb), small (12-18 lb), medium (18-24 lb), large (24-32 lb), and giant (>32 lb) (1).

Yield estimates measured from values rounded to the nearest pound and not rounded (one decimal position of precision) were completely correlated. Thus, the two measurement methods (rounding vs. non–rounding) have the same accuracy. Furthermore, average fruit weights (rounded vs. non-rounded) were highly correlated, confirming the same conclusions for accuracy. We found the same correlation levels presented above also within the five fruit-size categories. Therefore, there was no effect of measuring weights to the nearest pound or tenths of a pound, regardless of whether working on small-fruited or large-fruited cultivars (Table 1).

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Table 1. Correlation of watermelon weight with and without tenths of the measurement unit (lb).[z]

Trait	Fruit-size [1]	correlation (r)
Yield	Overall	1.00
	Icebox	1.00
	Small	1.00
	Medium	1.00
	Large	1.00
	Giant	1.00
Average fruit-weight	Overall	1.00
	Icebox	0.99 **
	Small	0.99 **
	Medium	0.99 **
	Large	0.99 **
	Giant	0.99 **

** r-value significant at p-value≤0.01

[1] Icebox (<12 lb), small (12-18 lb), medium (18-24 lb), large (24-32 lb), and giant (>32 lb)

[z] Data are single fruit-weights of 2 trials and 4 replications each.

In Search of High Lycopene Watermelon

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The red pigment in red-fleshed watermelon (C*itrullus lanatus* (Thunb.) Matsum. & Nakai) is from the carotenoid lycopene and comprises 70-90% of the total carotenoids in watermelon (Gross, 1987). Watermelon exceeds tomato in average lycopene content (49 µg/g vs 31 µg/g fresh weight) (Holden et al., 1998; USDA National Nutrient Database 2003).

Increasing lycopene in watermelon interested breeders as the plethora of health studies with lycopene ingestion continued to show positive trends for prostate health, cardiovascular improvement, and lowered incidence of certain cancers (Giovannuchi, 1999; Rissanen et al. 2003; Sesso et al., 2004). Currently, there is no set standard for lycopene intake. Although Agarwal and Rao (2000) estimated an average daily intake of lycopene to be 25 mg in Canada, intake is more likely 2.5 mg daily in the U.S., especially if the diet has few tomato products. Vitamins provide 0.03 to 2 mg lycopene and intake from tomatoes, watermelon, red grapefruit juice and tomato products typically provide 2 to 20 mg/serving (USDA National Nutrient Database, 2003).

Synthetic lycopene was approved for use in vitamins in 2003 (IFT, 2003). While lycopene is not produced by animals, it can be isolated from fungi such as the Phycomyces, or obtained from genetically modified bacteria or yeast (Sandmann, 2001). Natural sources of lycopene continue to be of great interest to consumers who wish to eat fruits and vegetables for better health. Although the genetics of lycopene accumulation have not been fully studied, a likely approach to improving lycopene content is to cross high lycopene varieties with other high lycopene varieties. Initial studies underway in our lab demonstrate that 'Dixielee' is the highest lycopene-containing, open pollinated variety. 'Dixielee' was released from the University of Florida breeding program (Crall and Elmstrom, 1979; 1980). In preliminary data, crosses with 'Dixielee' to a low lycopene open pollinated variety ('Black Diamond') resulted in F_1 melons with lycopene levels similar to 'Dixielee', suggesting that lycopene/redness is inherited in a dominant fashion (Table 1). However, the large range of lycopene values of ripe melons indicates that more fruit will need to be assayed before inheritance can be statistically verified.

Watermelons from growers and cultivar trials in Oklahoma and Texas have been analyzed for total lycopene content using spectrophotometric and colorimetric assays (Perkins-Veazie et al., 2001; Davis et al., 2003). Most watermelon fruit of commercial importance, both seeded and seedless, ranged in lycopene content from 50 to 70 µg/g fresh weight (Table 2). Some varieties were found to be extraordinarily high in lycopene, ranging from 80 to 105 µg/g fw lycopene (Table 2). Such variation is well known in tomato, where high pigment lines and processing tomatoes at the red ripe canning stage contain 100 to 150 µg/g (Dumas et al. 2003). Genetically modified tomatoes were reported to contain as much as 300 µg/g (Mehta et al. 2002). Additionally, gac (*Momordica cochinchinensis* (Lour.) Spreng), a cucurbitacea member, is reported to contain 1000-2000 µg/g lycopene in its arils (Ishida et al., 2004). This suggests that watermelon has the potential to contain extraordinarily high amounts of lycopene. The key questions remaining in developing high lycopene watermelons are: how will fruit

quality and flavor be affected (taste, sugars, firmness), how much lycopene can be produced by watermelon (e.g.100, 300, 500 µg/g fw), and will high lycopene fruit be a positive or a negative marketing factor for consumers (eg will high lycopene fruit cause health issues in consumers).

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Table 1. Initial inheritance study of lycopene accumulation using two open pollinated diploid watermelon with low ('Black Diamond') and high ('Dixielee') lycopene content (bc_1=backcross of $F_{1)}$.

Cross	Number of melons	Mean Lycopene content (µg/g)	Standard deviation	Range of lycopene (min/max)
Black Diamond	46	33.06c	6.20	19.90-43.50
Dixielee	31	64.77a	12.84	38.94-93.12
F_1	37	59.25ab	16.41	32.23-107.73
F_2	25	55.05ab	11.32	32.19-81.47
bc_1 x Black Diamond	7	50.12b	18.29	29.53-81.23

Means within column separated by Bonferroni t test, P<0.01. All melons in F1 and F2 crosses were red fleshed and all were used for lycopene analysis.

Table 2. Lycopene content among red-fleshed watermelon varieties and seed company selections sampled from 1999 through 2003.

Variety	Type	Number of melons sampled	Mean Lycopene content (µg/g)	Std dev	Range of lycopene content (min/max)
Sangria	Diploid, Hybrid	475	52.54	8.23	38.90-80.40
Jamboree	Diploid, Hybrid	20	61.06	5.08	52.75-71.39
Imagination	Diploid, Hybrid	17	69.09	10.06	53.86-95.26
Summer Flavor 800	Diploid, Hybrid	123	72.78	8.78	61.96-91.88
Summer Flavor 710	Diploid, Hybrid	11	83.03	7.1	73.57-97.08
Tri-X-313	Triploid	36	60.59	7.44	36.77-76.87
Hazera 6007	Triploid	27	100.00	15.64	67.76-129.28
Hazera 6009	Triploid	20	88.64	12.56	70.70-111.12
Sugar Baby 4N	Tetraploid	14	76.39	15.16	55.13-99.81
Millenium	Triploid	29	74.93	14.73	43.80-96.04
AllSweet	Diploid, Open-pollinated	22	48.39	5.95	38.52-60.67
Calhoun Gray	Diploid, open pollinated	8	37.21	5.7	30.80-45.30

Morphological Traits of Possible Use as Species Markers in *Cucurbita moschata* and *C. argryosperma*

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One aspect of our *Cucurbita* breeding and genetics research at the University of Puerto Rico is the study of the relationship between *C. moschata* and *C. argyrosperma* (1,4,5,6,7), two species often grown in close proximity in Mexico and Central America. Lira-Saade, et al. (2) developed a key to distinguish among the cultivated *Cucurbita* principally based on seed characteristics, followed by peduncle type and pubescence on the leaf and leaf petiole. Wessel-Beaver et al. (7) noted that it is often difficult to distinguish between inter- and intraspecific crosses in the field. The differences can be subtle and variation rather continuous, especially for key taxonomic traits like seed and peduncle type. In addition, these species markers require plants with mature fruits. We became interested in trying to identify species-specific morphological markers that could be observed without the need for fruit and seeds. In her detailed study of *C. argyrosperma* Merrick (3) mentions that the filaments of that species are fused, while the filaments of *C. moschata* are open at the base of the staminate flower. While taking a preliminary look at this trait we also noted that there appeared to be a relationship between filament color and staminate petiole pubescence and *Cucurbita* species. Our objective was to evaluate a large number of *C. moschata* and *C. argyrosperma* accessions in order to determine if these traits could serve as useful species markers in future studies of gene flow between these species.

Materials and Methods: A total of 287 cultivated accessions of *C. moschata* and 64 accessions of *C. argyrosperma* were grown in Isabela, Puerto Rico. The accessions were sampled from the U.S.D.A. Plant Genetic Resource System and from the first author's own collection to represent a wide range of geographic diversity. Geographic diversity of *C. moschata* (grown world-wide) is much greater than *C. argyrosperma* (limited to Mexico and Central America). At least two staminate buds from each of three randomly chosen plants were sampled the day before anthesis. Data was collected directly in the field or buds were held in a cooler and evaluated within 24 hours. Filaments were classified as yellow, intermediate or white in color. The filament base was described as fused or open. The staminate bud petiole and receptacle were classified as glabrous or pubescent. Seven to 27 plants of two inbred lines ('Butternut' and TP411) and two open pollinated cultivars ('Verde Luz' and 'Soler') of *C. moschata* and one S2 line of *C. argyrosperma* (Arg 51-5) were evaluated to access the degree of within-accession variability. Thirty-five F1 plants of the cross *C. argyrosperma* 'Arg 51-5' x *C. moschata* 'PRLongvineSLR' were also evaluated.

Results and Discussion: None of the tested traits serve as species-specific markers (Table 1). *C. argyrosperma* accessions almost always had white, fused filaments inside glabrous staminate flowers. But for all three traits there was a diversity of phenotypes among accessions of *C. moschata*. Many accessions of *C. moschata* were segregating. Contrary to what Merrick (3) observed, the filament base of *C. moschata* can also be fused, although much less often than in *C. argyrosperma*. Many *C. moschata* accessions were classified as glabrous. However, because we included plants with a slight amount of pubescence in this category this may be an over-estimate. We observed that *C. moschata* was usually

very slightly pubescent while *C. argyrosperma* was nearly always completely glabrous.

No formal inheritance study was conducted, but 35 F1 plants of a cross between a white filament *C. argyrosperma* and a yellow filament *C. moschata* had light yellow filaments (data not shown) suggesting that yellow color is partially dominant over white. Among the 351 tested accessions, filament color always fell into three fairly discrete phenotypic classes: white, light yellow and yellow, suggesting simple genetic control by possibly one gene. Filament color can be influenced by environmental effects as evidenced by some phenotypic variation within lines (Table 2). Table 2 suggests that the type of filament base and degree of pubescence were less affected by the environment. However, filaments classified as "open" exhibited a wide range of variation from nearly fused to very open.

While these traits are not completely species-specific, they could be useful in controlled studies of gene flow where one uses parents having known genotypes (assuming that at least some of the traits are monogenic). We plan to conduct more formal inheritance studies in the future.

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Table 1. Comparison of phenotypes found in *C. moschata* versus *C. argyrosperma* for morphological traits associated with the staminate flower bud.

		Number and % of accessions with phenotype			
Trait	Phenotype	*C. moschata* (N=287)		*C. argyrosperma* (N=64)	
Filament color	yellow	146	51%	0	0%
	segregating or lt. yellow	107	37%	10	16%
	white	34	12%	54	84%
Filament base	fused	123	43%	61	95%
	segregating	107	37%	3	5%
	open	57	20%	0	0%
Pubescence on staminate flower	glabrous*	153	53%	62	97%
	segregating	97	34%	2	3%
	pubescent	37	13%	0	0%

*plants with a slight amount of pubescence were also included in this category.

Table 2. Phenotypic variability of three morphological traits within two inbred lines (Butternut and TP411) and two open pollinated cultivars (Verde Luz and Soler) of *Cucurbita moschata,* and within a S2 line of *C. argyrosperma* (Arg 51-5).

	Butternut (N=7)	TP411 (N=25)	Verde Luz (N=24)	Soler (N=27)	Arg 51-5 (N=20)
Filament color:					
Yellow	7	21	24	23	0
Lt. yellow	1	1	0	4	0
White	0	3	0	0	20
Filament base:					
Fused	7	25	13	10	20
Open	0	0	11	17	0
Pubescence:					
Glabrous	7	25	24	25	20
Pubescent	0	0	0	2	0

Enhanced Haploids Regeneration in Anther Culture of Summer Squash (Cucurbita pepo L.)

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Abstract: The best callus induction and proliferation and plant regeneration responses in anther cultures of zucchini type summer squash cv. 'Eskandarani' were obtained when growing the donor plants during summer on 1st of March and during winter on 1st of November. However, the cultures of anthers prepared from donor plants grown during the winter season greatly enhanced callus induction and plant regeneration when compared with those of the summer season. About 60% of the regenerants were haploid (2n = X = 20). We concluded that growing conditions of donor plants are influential factor for optimizing the production of haploids via androgensis in summer squash cv "Eskandrani".

Introduction: Production of double haploid plants has been studied in several vegetable species of the cucurbitaceae family [2, 4, 8]. One of the prominent key points in the induction of the androgenesis is the condition of the donor plants as influenced by the surrounding culture environment especially light and temperature [1, 5, 10]. However, no information is available on this factor as affecting the in vitro androgenesis of zucchini type summer squash. Such information would be important in optimizing tissue culture protocols of androgenesis and enhancing their repeatability and thereby the application towards the improvement of this species. The objective of the present study was, therefore, to investigate the anther culture responses of the summer squash to different planting seasons and dates for the donor plants.

Materials and Methods: Seeds of the summer squash cv ' Eskandrani' were sown during the summer and the winter growing seasons in Malawy Experimental Research Station (27˚ 18′ N latitude), El-Minia Governorate, Egypt. Sowing dates were on the first of March, April, and May during the summer season and on the first of September, October, and November during the winter season. Separate experiments were conducted for these planting dates in randomized complete-blocks with four replicates. Maximum, minimum, average day and average night temperatures along with relative humidity are presented in Table (1). Male flower buds were collected when they were about 9 to 10 mm and exposed to cold pretreatment at 4° C for 4 days. After surface disinfestations of the buds, anthers were excised and placed on MS medium [9] containing 100g/l sucrose and 6 mg/l 2,4-D [8]. The culture was in 100 ml Erlenmeyer flasks containing 30 ml agar-solidified medium (8g/l). The anther cultures were kept in the dark at 35°C for a week followed by 4 weeks at 25°C. The induced callus was subcultured on the same medium for additional 4 weeks. The weight and diameter of the proliferated callus were determined. The proliferated callus was transferred onto MS medium with 0.05 mg/l of both kinetin and α-naphthalene acetic acid (NAA) and incubated under cool white light (16/8, day/night) at 25°C for 4 weeks.

The differentiated callus cultures were subcultured onto the medium without plant growth regulators (PGR) for 4 weeks. The number of the developed plantlets was recorded and expressed as per 100 anther explant cultures. The developed shoots and plantlets were cultured in the medium containing 1 mg indole-3-butyric acid

(IBA). Samples of root tips were used to examine the number of chromosomes in the regenerated plants following the procedure described by Darlington and Lacour [3]. The defined haploid plantlets were immersed in 1% aqueous colchicine solution for 2 h [2]. Shoot-tips from these plantlets were transferred into flasks containing PGR-free medium. Double haploid plantlets, with sufficiently developed roots, were hardened and were then transplanted into pots containing sterilized peat moss. The pots were covered with clear plastic bags for one week. The plants were grown in the soil and observed for the growth and the fruit and seed set. The data were subjected to the combined analysis of variance over years, growing season and the treatments of planting dates. Means were separated with the "Least Significance Difference" (LSD) and additional selected orthogonal comparisons were also tested.

Results and Discussion: Greater weight and larger sized callus were obtained when the anther explants were prepared from plants sown earlier in the summer season as on March than those that were sown later especially on May (Table 2A). The squash plants sown late in the winter season (November) gave anthers producing enhanced callus weight and diameter comparing with the early planting in October and September (Table 2B). The number of the regenerated plants per 100 anthers exhibited similar response to the planting dates of the donor plants in the summer and the winter seasons. Therefore, significant positively correlated existed between the number of the regenerated plants per 100 anthers and the both of the callus weight (r = 0.973 **) and the callus diameter (r = 0.978 **). The percentage of the abnormal regenerated plants did not differ within each of the summer (Table 2A) and winter (Table 2B) planting dates.

Comparison of the average overall the winter vs. the summer revealed a significant difference towards enhanced callus weight, diameter and the number of the regenerated plants per 100 anthers in the winter season. The percentage of the abnormal regenerated plants significantly reduced in the cultures of anthers prepared from plants grown in the winter season. The number of regenerated plants from anther-derived callus of the squash sown in November was more than twice as those planted in March. Heberle-Bors [5] indicated that short-day (8 h) and relatively low temperatures (15-18° C) were preferable for growing the anther donor *Nicotiana tabacum* plants. In a study conducted by Yong et al. [10], the explants from cauliflower plants grown in the winter and the spring were more responsive than those grown in the summer or early autumn. Furthermore, Matsubara et al. [7] reported that the best differentiation of the anther-derived embryoids of pepper was from plants grown during September and October (15 to 25° C). The enhanced responses of the tissue culture towards plant differentiation could be attributed to the slow maturity and synchronized development of the microspores and thus prolonging their responsive stage [1]. Also, surrounding environment of the donor plants could affect the indogenous hormonal level [6] and thereby the conditioning of the microspores towards stimulating their responses in tissue cultures. Our cytological observation indicated that about 60% of the regenerated plants were haploids (2n = X = 20) while 23% were diploid (2n = 2X = 40) and 17% were aneuploid (2n = X = 21-39). Spontaneous doubling was also observed by Dryanovska [4] occurring for the regenerants in anther culture of cucurbitaceae members. Dihaploid plants obtained by colchicine treatment developed normal fruit and seed set.

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Table 1. Maximum, minimum and average day and night temperatures along with the relative humidity recorded during the months of the summer (A) and the winter (B) growing season of the anther-explant donor summer squash (cv Eskandrani), Malawy Metreological Station, Egypt.[z]

Months	Temperature (C)				Relative Humidity
	Maximum	Minimum	Average. Day	Average Night	
			(A)		
March	26.4	8.4	22.4	15.4	75.4
April	36.2	15.6	30.3	20.2	68.3
May	36.6	18.4	30.2	20.5	68.6
June	35.2	20.3	32.3	23.4	65.3
			(B)		
September	35.4	20.5	25.4	28.5	70.5
October	30.5	17.6	30.2	20.3	75.4
November	28.3	10.3	23.5	15.2	78.3
December	21.6	8.4	18.2	10.4	84.1

[z] Average of 2 seasons, 1998 and 1999.

Table 2. Average diameter and weight of the proliferated callus from anther culture of summer squash cv 'Eskandrani' and the number of the regenerated plants and the percentage of the abnormal plants in those anther-derived callus[z].

Treatments	Callus proliferation		Plant regeneration	
	Diameter (cm)	Weight (g)	Plants/100 anthers (no.)	Abnormal plants (%)
			(A)	
A- Summer season				
March	2.5	2.9	118	24.6
April	2.4	2.6	39	24.6
May	2.1	2.2	13	24.5
LSD $_{0.05}$	0.1	0.1	6	ns [y]
			(B)	
B- winter season				
September	2.6	2.9	126	17.4
October	3.0	3.3	211	17.4
November	3.3	3.4	260	17.4
LSD $_{0.05}$	0.2	0.1	28	ns
	* [x]	*	*	*
Summer vs winter				

[z] Data were pooled from 2 seasons, 1998 and 1999.
[y,x] Nonsignificant and significant at 0.05 probability level, respectively.

Development and Characterization of Microsatellite Markers (SSR) in *Cucurbita* Species

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Introduction: At present, microsatellite (SSR) markers are the most useful tools for relationship studies as well as mapping, due to their easy handling, co-dominant inheritance, and multiallelic and highly polymorphic nature. They provide stable (anchor) points of physical orientation in the plant genome. Their major drawbacks are the high amount of labour and high costs required for their development. So far, no published SSR marker exists for *Cucurbita* species. Conventional methods of SSR isolation consist of screening partial genomic libraries with appropriate probes (4) followed by large amounts of sequencing work. Meanwhile, to reduce time requirement and costs, several alternative isolation strategies have been introduced for SSR marker development (8). We adapted a method, which relies on the digestion-ligation reaction of the amplified fragment length polymorphism procedure (AFLP, 7). The DNA-fragments are then fished by Streptavidin coated Dynabeads (5, and personal communication of T.C. Glenn). In this communication we describe the first results of applying this technique for SSR isolation in *C. pepo*.

Material and Methods: *Plant material:* For SSR development genomic DNA was extracted from the Austrian oil-pumpkin variety "Gleisdorfer Ölkürbis". For the relationship study using the newly developed SSR markers, 48 genotypes were selected. This included representatives of the species *C. pepo*, *C. maxima*, *C. moschata* and *C. ecuadorensis*. Within the *C. pepo* group, beyond representatives of all cultivar types a described by Paris (3), emphasis was put on hull-less pumpkin genotypes (numbers 18 to 36 in Fig. 1).

DNA isolation: Genomic DNA was extracted from young leaves of oil-pumpkin plants according to the procedure of the Wizard® Genomic DNA Purification Kit, supplied by Promega Corp., WI, USA (www.promega.com).

Microsatellite enrichment, screening and sequencing: SSR isolation was done following a slightly modified procedure of Schable et al. (5). After digestion with *RsaI*, fragments were ligated to SuperSNX24 linkers and hybridized to biotinylated microsatellite oligonucleotides. These were captured with streptavidin coated paramagnetic beads (Dynal). Uncaptured DNA was washed away and the remaining DNA was amplified using the SuperSNX24 primer. The product was ligated into AccepTor Vector (Novagen), inserted into NovaBlue Singles Competent Cells and screened for inserts using the β-galactosidase gene. Positive clones were amplified using T7 and SP6 primers and screened for their size. Sequencing of fragments with a size above 500 bp was done by an outside company (IBL, Vienna). For automated microsatellite sequences the search sequences were exported to SSRIT (http://www.gramene.org/gramene/searches/ssrtool). All sequences were checked against each other using the FASTA program (http://www.ebi.ac.uk/fasta33/nucleotide.html). PCR primers were designed using Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), in the 18-25 bp range. They were synthesised by MWG Biotech AG (D-85560 Ebersberg). PCR

amplifications for primer testing were performed in 15µl volume using an Eppendorf Mastercycler Gradient thermal cycler. The final PCR concentration was 40 ng of genomic DNA, 2.25 µM of each primer, 1x reaction buffer containing 1.5 mM $MgCl_2$, 3.75 mM dNTP and 0.5 U *Taq* polymerase. Cycling parameters were 94°C for 60 s, followed by 32 cycles of 94°C for 25 s, an annealing step between 48°C and 58°C for 25 s and elongation at 72°C for 25 s, finally 72°C for 5 min. PCR products were scored on an 12% acrylamide gel stained with silvernitrate (6) (Fig.1). The annealing temperature, which gave the best result, was chosen for follow up experiments.

Primer evaluation and relationship study: Amplification using 22 primers was performed in 10µl volume (26 ng of genomic DNA, 1.49 µM of each primer, 1x reaction buffer containing 1.5 mM $MgCl_2$, 2.5 mM dNTP and 0.35 U *Taq* polymerase) with a MWG Primus 96 plus Thermocycler. Fragment separation and staining was done according to Stift et al. (6), allele scoring and sizing by manual screening.

Statistical analysis: The SPSS software package was used for data processing. Distance between clusters was calculated as the average distance between all pairs of genotypes (UPGMA). The relationship between genotypes was measured by squared Euclidean distance.

Results and Discussion: The library enrichment in microsatellite repeats resulted in 1704 positive clones. 621 had a size above 500 bp and had been sequenced. 334 (54%) contained a repeat. Duplicates or repeats too close to the cloning site were eliminated. Primer pairs were designed for 81 (13%) sequences. 56 (9%) primer pairs amplified a product of the expected size, 25 did not give any amplification product. The 56 primer pairs were tested for polymorphism and quality on a set of 12 selected *Cucurbita* genotypes. 29 of the primers gave only monomorphic bands. From the remaining 27, the best 22 primer pairs were used for the relationship study (Fig. 2). The number of alleles per locus ranged from 2 to 6 with an average of 4.4. The relationship study of the selected genotypes using 22 *Cucurbita* specific SSR markers is in full agreement with previous results of Decker (1) and Katzir et al. (2), by grouping the *C. pepo* genotypes into the two subspecies *ovifera* and *pepo* (1). The Cocozelle genotype "Striato d'Italia" (Z6) is far away from the Zucchini genotypes, as was also found by Katzir et al. (2). Within the *ssp. ovifera* in this sample of genotypes, no correspondence to the cultivar groups as established by Paris (3), can be seen. The analysis clearly differentiated the species *C. maxima* and *C. moschata,* from each other and from the *C. pepo* group. As expected, most of the oil-pumpkins bred by the company "Gleisdorf" are clustered together. Other oil-pumpkins in the vicinity might be derivatives of Styrian material.

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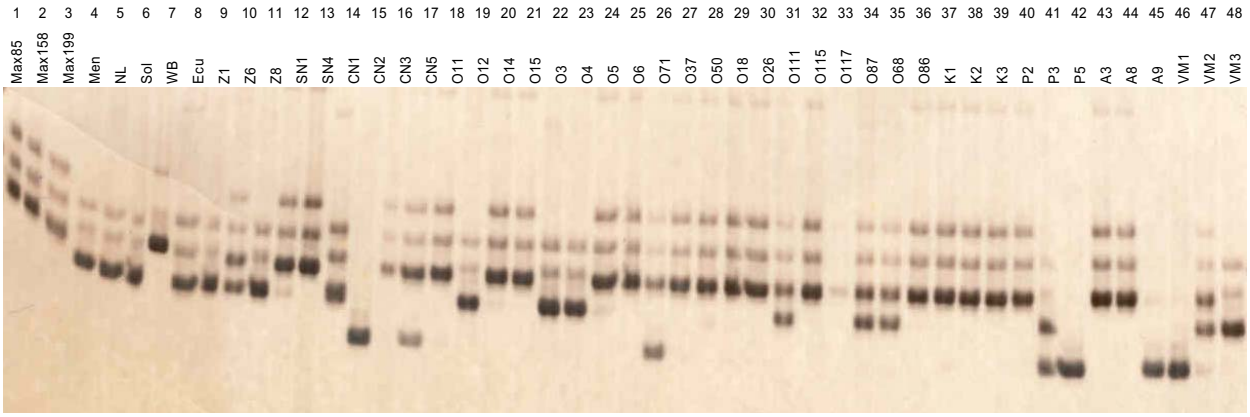

Fig. 1: Characterization of 48 *Cucurbita* genotypes for SSR marker SGA3 separated in 12% polyacrylamide, stained with silvernitrate. The marker detected one amplification site per genotype (smallest fragment) with 4 alleles. Eight of the genotypes are heterozygous, e.g., 10, 17, 26 etc.

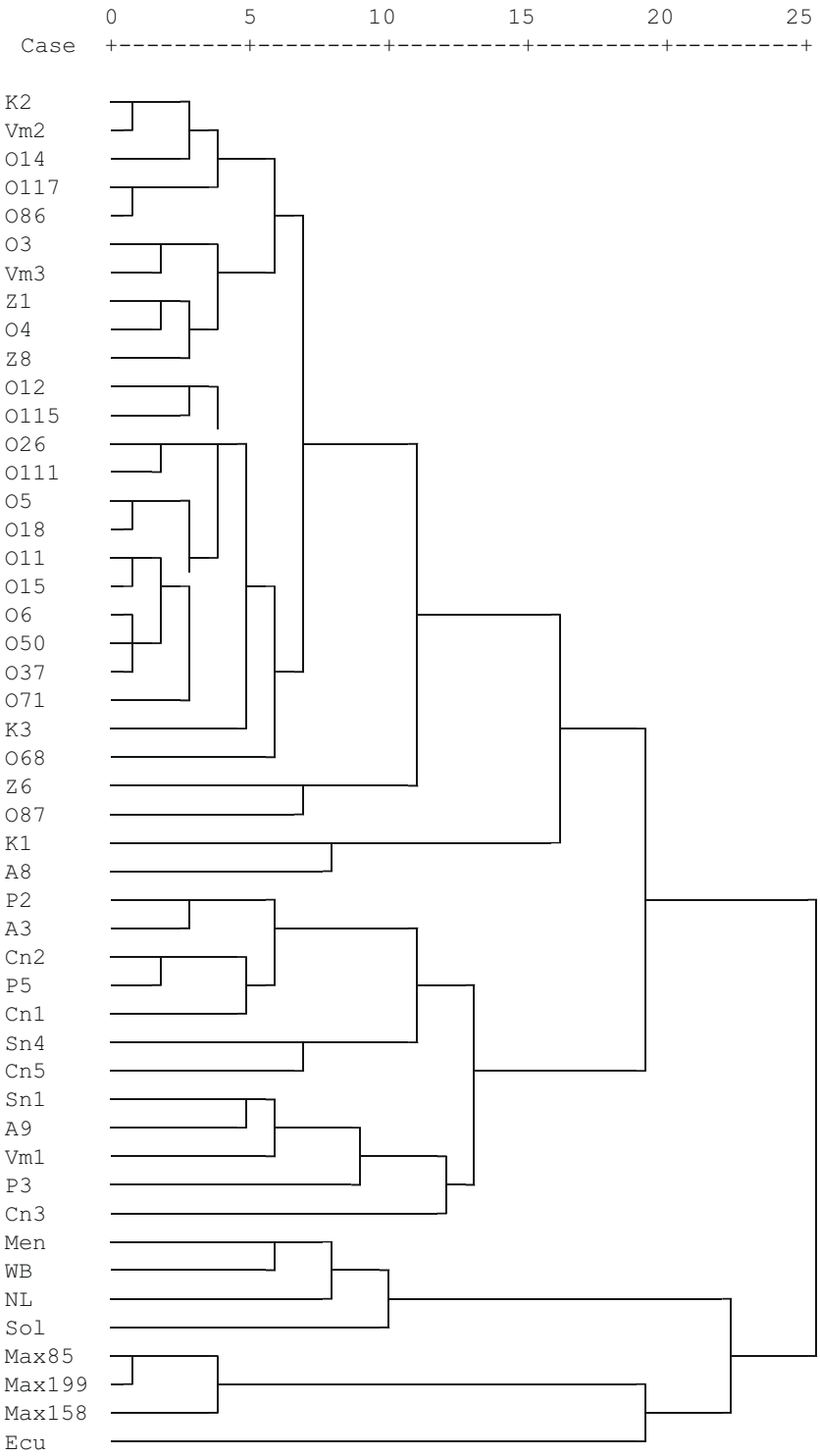

Fig. 2: Dendrogramm based on *C. pepo* SSR markers scoring 48 genotypes of *C. pepo*, *C. moschata*, *C. maxima* and *C. ecuadorensis*. The abbreviations are as in Table 1.

Max85	*C. maxima*	Max85
Max158		Max158
Max199		Max199
Men	*C. moschata*	Menina
NL		Nig. Local
Sol		Soler
WB		Waltham Buttern.
Ecu	*C. ecuadorensis*	Grif 9446 01 SD
	C. pepo	
Z1	**Zucchini**	True French
Z6		Striato d' Italia (Cocozelle)
Z8		Erken
SN1	**Straightneck**	General Patton
SN4		Sunray
CN1	**Crookneck**	Bianco Friulano
CN2		Courge Cou Tours
CN3		ohne Namen
CN5		Sundance
K1	**Pumpkin**	Pomme d'Or
K2		Tondo di Padana
K3		Chinese Miniatur
O11		Retzer Gold
O12		Gleisdorfer Ölk.
O14		Sepp
O15		Markant
O3		Chinesischer
O4		S-Afrika
O5		Lady Godiva
O6		Estancia Bugar
O71		Georgica
O37		Miranda
O50		Slovenska Golica
O18		Kakai
O26		Lu's Ölkürbis
O111		Szentesi Oliva
O115		09H4 CZ
O117		PM 18
O87		Pulawska
O68		Anton Berger
O86		PI 285611
P2	**Scallop**	unknown
P3		Early White Bush
P5		Galeux
A3	**Acorn**	Tay Belle
A8		Thelma Sanders Sweet Potato
A9		Yugoslavian finger
VM1	**Veg. Marrow**	unknown
VM2		Bulgarian Summer
VM3		Alba

Table 1: List of *Cucurbita* genotypes used for the realtionship study with *Cucurbita* specific SSRs.
(O = Oil-pumpkin)

Variability Analysis of Underutilized Nutritive Vegetable Kartoli: Indian collection

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Abstract: Experiment was conducted to screen the *Momordica diacia* Roxb (kartoli) germplasm lines for high yield and fruit characters. Exploration and collection of kartoli germplasm was done from main growing areas of country. Total of twenty four diverse germplasm of vegetable was grown in randomized block design with three replications during 2002. The widest range was recorded for fruit length, while narrowest range was observed for days to female flower anthesis. The characters like days to female flower anthesis, fruit set to edible maturity, plant height, fruit diameter, fruit length, number of fruits/plant and yield/plant expressed maximum variability at phenotypic and genotypic level. Estimates of genetic advance were high for yield/plant and number of branches/plant. The germplasm DR/05-08-02 with maximum yield was considered as a promising line, whereas DR/NKV-02-06-01 for smaller fruit size.

Horticultural crops are gaining importance throughout the world not only as food or economic crops but also for their importance as raw material for value-added products and industrial uses. Minor cucurbitaceous vegetables are very nutritious with high vitamins and minerals matter. The specific medicinal and curative properties are very well appreciated. Kartoli is one of the important members of the family cucurbitacae(1). This priced vegetable is available in Northeastern states, UP, Bihar, Maharashtra, MP, Kerala, WB and Andman Island of the country (3). The delicious young tender fruits are used as vegetables.
Spine gourd (*Momordica dioica* Roxb) is protein rich vegetable. The pots of female and male plants in a ratio of 9:1 provide 8-100 fruits (2). The cost of fruits varies from Rs 10 to 20 per kg for culinary purpose. Nutritional value of Kartoli per 100 gm edible part : moisture 84.1 %, protein 3.1 gm, fat 1.0 gm, fibre 3.0 gm, carbohydrate 7.7 gm, calcium 33 mg, phosphorus 42 mg, iron 4-6 mg, carotein 162 mg, thiomin 0.05 mg, riboflavin 0.18 mg and niacin 0.06 mg etc. Fruits are used in ulcers, piles, sores and obstection of liver and spleen. It posses several medicinal properties and is said to be good for those suffering from cough, bile and other digestive problems. The unripe fruits work as appetizer astringent in barrels. The seeds are used for chest problems and simulate urinary discharge.

Material and Methods: Spine gourd shows a wide range of genetic diversity in shape and size of the leaf, fruit shape and colour. However, a good spine gourd should have medium sized fruits (10-15 g), dark green in colour, tough and false spines, and quick edible maturity 30-35 days after flowering, resistant to epilachana beetle, fruit borer and good yield(4). The present investigation was carried out to measure the extent of variability, expected genetic advance, etc among 24 diverse genotypes of Kartoli in randomized block design with three replications at research farm of IIVR Varanasi. Observations were recorded on reproductive and vegetative viz number of days to female flower anthesis, flowerer to fruit set fruit set to maturity, flower to seed maturity, number of branches per plant, plant height, fruit diameter, individual fruit weight, number of seeds/fruit, number of fruits/plant, fruit length , fruit colour, skin surface of fruit etc. Except the fruit colour and fruit skin surface, the mean data were analysed statistically.

Results and Discussion: The widest range was recorded for fruit length, while narrowest range was observed for days to female flower anthesis. The characters like days to female flower anthesis, fruit set to edible maturity, plant height, fruit diameter, fruit length, number of fruits/plant and yield/plant expressed maximum variability at phenotypic and genotypic level. Estimates of genetic advance were high for yield/plant and number of branches/plant. The medium gain was found for plant height, number of fruits/plant. The remaining characters were of low advance.

Kartoli are under utilized vegetable with high nutritional, medicinal and economic values. Its immature tender green fruits are consumed as vegetable, young leaves, flowers and seeds are also edible. The genetic improvement for higher yield could be achieved through clear understanding of the type and amount of variability present in genetic stocks. Knowledge of genetic diversity allows the breeder to select the suitable genetic base and use them in breeding programme.

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Table 1: Variability in characters for evaluated genotypes

	Days to female flower anthesis	Fruit set to edible maturity	Flowers to seed maturity	Fruit Diameter (cm)	Fruit length (cm)	No of fruit per plant	Individual fruit weight(gm)
Average	34.86	19.2	29.9	3.00	5.72	61.62	13.64
Phenotypic variability	8.46	10.08	6.74	12.43	17.19	22.73	7.26
Genotypic variability	7.18	6.15	3.81	4.25	1.86	22.1	4.38
Genetic advance	4.37	1.49	1.29	0.09	0.02	27.29	0.74
Genetic advance as % of mean	12.54	7.76	4.31	3.00	0.35	44.29	5.43

Table 2: Variability in evaluated Genotypes of spine gourd

Character	Range	
Early plant vigour	Poor	Good
Plant growth	Short viny	Long viny
Flower colour	Light yellow	Yellow
Fruit shape	Spindle	Tapering
Fruit surface	Light tubercle	Deep tubercle
Blossom end fruit shape	Acute	Blunt
Fruit skin colour	Light green	Green

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 $p \leq 0.05$ 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 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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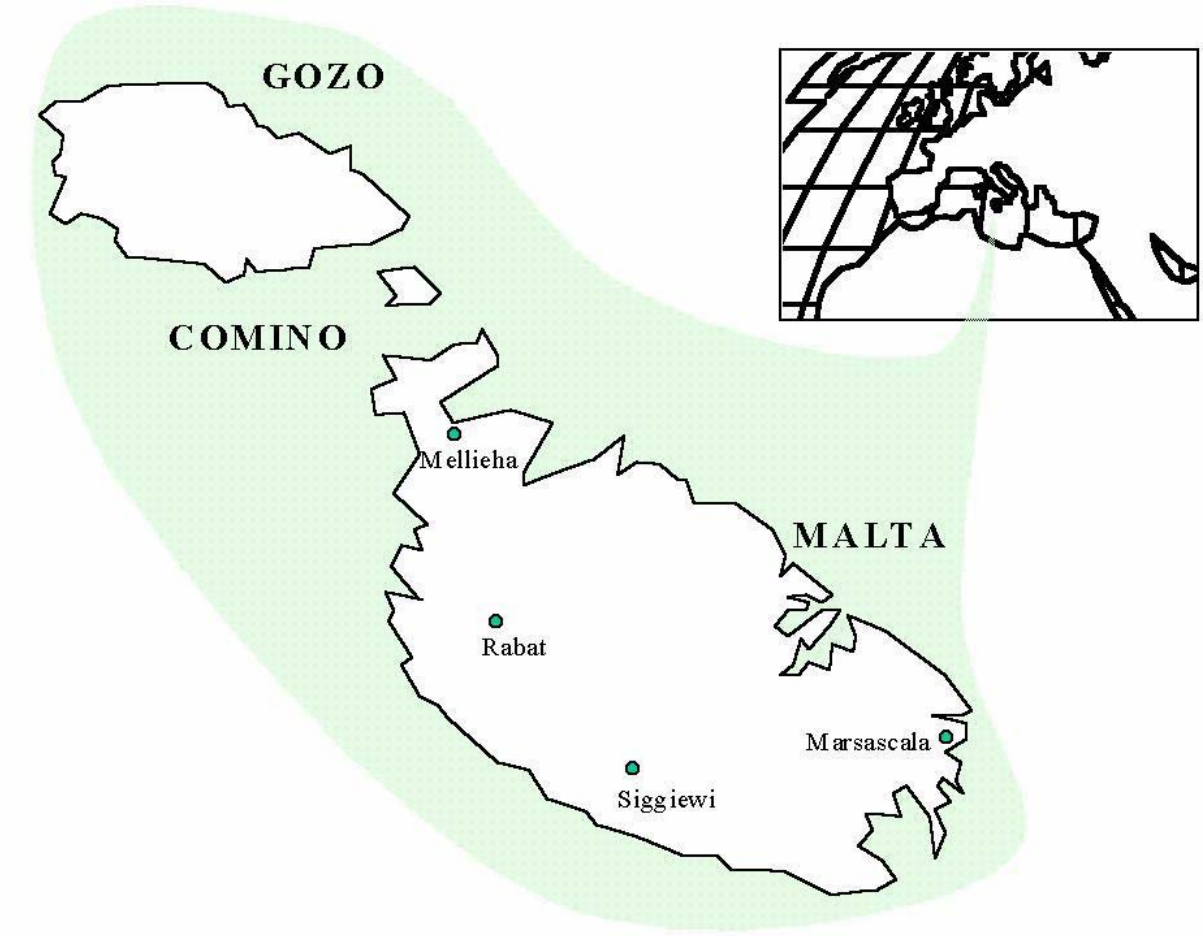

Figure 1. The localities chosen for soil sample collection

Table 1. The moisture, gravel and soil contents of the different soils on which *E. elaterium* grows.[Z]

Localities	Soil Description	% Moisture Content	% Gravel Content	% Soil Content
M'Scala	Terra soil	18.6600 ± 1.7106	46.8467 ± 0.9192	53.1533 ± 0.9192
	(*Terra rossa* type)	(2.9629)	(1.5921)	(1.5921)
Siggiewi	Rendzina soil	17.9300 ± 2.7900	29.9750 ± 0.1150	70.0250 ± 0.1150
	(Xerorendzina type)	(3.9457)	(0.1626)	(0.1626)
Mellieha	Rendzina soil	10.5000 ± 1.0800	36.1600 ± 0.5200	63.8400 ± 0.5200
	(Brown rendzina type)	(1.5274)	(0.7354)	(0.7354)
Rabat	Carbonate raw soil	17.7900 ± 2.2303	6.5033 ± 1.6734	93.4967 ± 1.6734
	(Fiddien series)	(3.8629)	(2.8985)	(2.8985)
ANOVA		p>0.1232	*p<0.0001*	*p<0.0001*

[Z]Values represent means ± 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 *E. elaterium* grows. [Z]

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

[Z]Values represent means ± S.E.M of 4 - 6 determinations. Standard deviation values are indicated in brackets. ANOVA results aer also tabulated (v=19).

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 24ºC and a relative humidity of about 95 ± 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 temperature-dependent. 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 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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Figure 1. The percentage germination of *E. elaterium* seeds with time

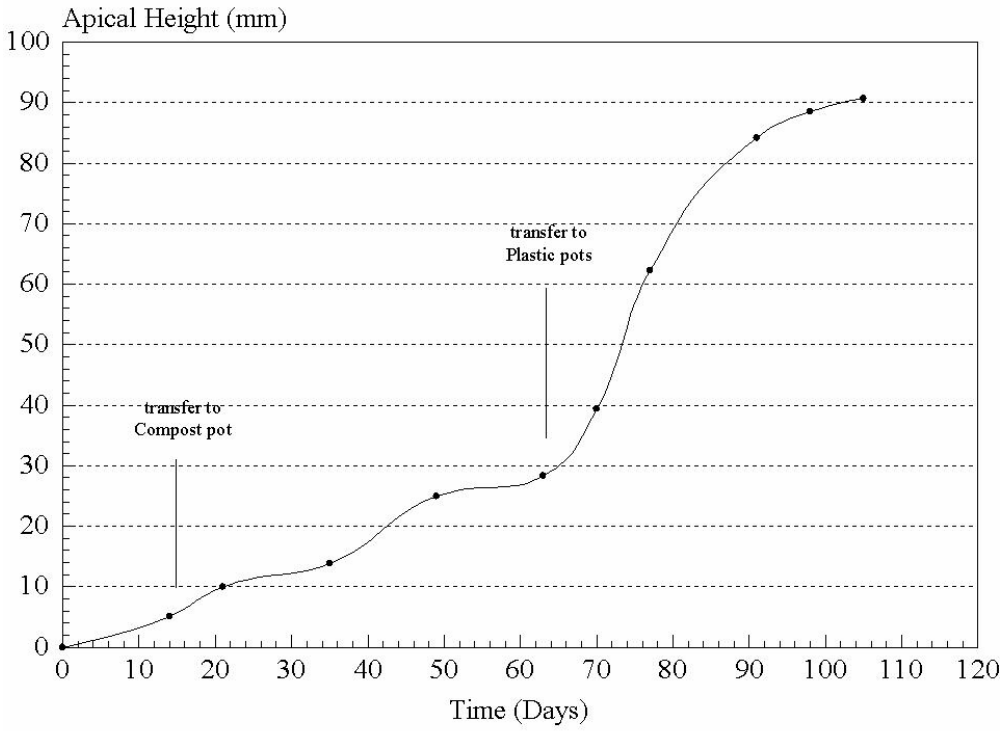

Figure 2. The apical height of *E. elaterium* seedlings with time

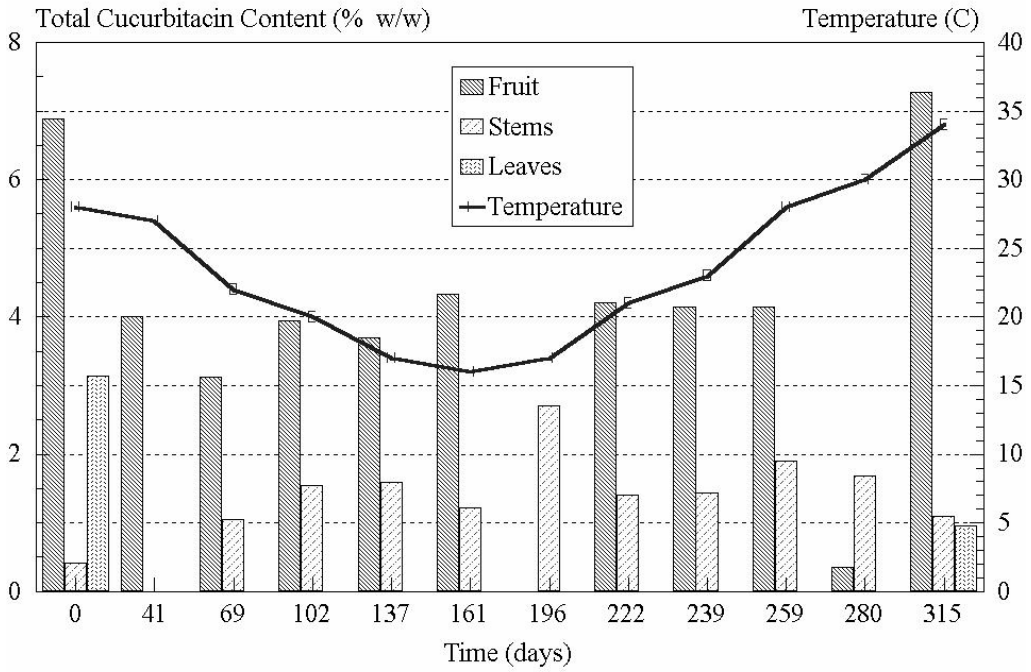

Figure 3. The total cucurbitacin content in elaterium produced from *E. elaterium* fruit, stems and leaves with time and temperature.

Gene List for *Cucurbita* species, 2004

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A complete list of genes for *Cucurbita* species was last published 12 years ago (33). Since then, only updates have been published (72, 73).

The genus *Cucurbita* L. contains 12 or 13 species (50). As far as is known, all have a complement of 20 pairs of chromosomes (2n = 40).

This new gene list for *Cucurbita* contains much more detail concerning the sources of information, being modeled after the one for cucumber presented by Wehner and Staub (96) and its update by Xie and Wehner (100). In order to more easily allow confirmation of previous work and as a basis for further work, information has been added concerning the genetic background of the parents that had been used for crossing. Thus, in addition to the species involved, the cultivar-group (for *C. pepo*), market type (for *C. maxima*, *C. moschata*), and/or cultivar name are included in the description wherever possible.

Genes affecting phenotypic/morphological traits are listed in **Table 1**. The data upon which are based identifications and concomitant assignment of gene symbols vary considerably in their content. No attempt is made here to assess the certainty of identifications, but gene symbols have been accepted or assigned only for cases in which at least some data are presented. The genes that are protein/isozyme variants are listed in **Table 2**. It can be seen from Tables 1 and 2 that a large number of genes, 65, have been identified for *C. pepo* L. For *C. moschata* Duchesne and *C. maxima* Duchesne, 21 and 19 genes have been identified, respectively, and for the interspecific cross of *C. maxima* × *C. ecuadorensis* Cutler & Whitaker, 29, of which 25 are isozyme variants. One or two genes have also been identified in four of the wild species (*C. okeechobeensis* Bailey, *C. lundelliana* Bailey, *C. foetidissima* HBK and *C. ecuadorensis*) and in several other interspecific crosses. Notably, no genes have been identified for the other two cultivated species, *C. argyrosperma* Huber and *C. ficifolia* Bouché.

Some genes are listed as occurring in more than one species. This does not necessarily indicate that these genes reside at identical locations in the genome of different species.

New additions to the list of *Cucurbita* genes include a number of omissions as well as a number of new genes published after the last update. Those that had been omitted are: *Bn, pm-1, pm-2*, and *s-2*, and Wmv^{ecu}. Those that have been published since the last update are: *Cmv, grl*, $l\text{-}1^{BSt}$, $l\text{-}1^{iSt}$, $L\text{-}2^{w}$, $m\text{-}zym^{mos}$, *prv, qi*, *sl*, *wmv,* zym^{mos}, *Zym-2*, and *Zym-3*. In addition, there are many additions to the list of isozyme variants.

Symbols of genes that have been published in previous lists but have been modified for this list are *Pm* (to be used solely for powdery mildew resistance in *C. lundelliana*, with the separate designation *Pm-0* for resistance in and derived from *C. okeechobeensis*), and *Zym* (with separate designations for different sources of resistance, viz. zym^{ecu} from *C. ecuadorensis*, *Zym-0* from *C. moschata* 'Nigerian Local', and *Zym-1* from *C. moschata* 'Menina', and zym^{mos} from *C. moschata* 'Soler').

Before choosing a gene name and symbol, researchers are urged to consult this Gene List as well as the rules of Gene Nomenclature for the Cucurbitaceae that appears near the end of this Cucurbit Genetics Cooperative Report in order to avoid confusion arising from duplication of gene names and symbols. Please contact us if you find omissions or errors in this Gene List.

Several cases of genetic linkage have been reported: *D – mo-2* (56) and *M – Wt* (*C. pepo*) (66) and *Bi – Lo-2* (*C. ecuadorensis* × *C. maxima*) (30). Some of the isozyme variants observed by Weeden & Robinson (95) were also found to be linked to one another. RAPD markers have been categorized and organized into linkage groups and are not listed here but can be found in Brown and Myers (4) and Zraidi and Lelley (101). These two maps cannot be easily compared, as they were constructed using different mapping populations; RAPD markers are population-specific. Neither map gives complete coverage of the *Cucurbita* genome. Both maps contain morphological traits, either as single genes or as quantitative trait loci (QTLs). These traits are listed in **Table 3** along with the most tightly linked RAPD markers.

In many species, knowledge of the genome has moved beyond mapping markers linked to phenotypic traits to isolating and sequencing the genes that control the traits. Sequenced genes can be valuable to breeders and geneticists, as the differences in the gene sequences that result in the phenotypes of interest can be used as markers in marker-assisted selection. Unlike random markers, these gene-specific, allele-specific markers are completely linked to the genes of interest. Genes can be isolated through widespread sequencing of genomic or cDNA libraries, through map-based cloning, or by functional homology with sequenced genes from other species. In addition, genes which code for a known protein such as an enzyme can be isolated by working backwards from the protein. Many of the genes sequenced in *Cucurbita* at present have been sequenced this way. Map-based cloning is the most effective way to identify the DNA sequence of genes for phenotypic and morphological traits. This requires maps of much higher resolution than those presently available for *Cucurbita*. Most of the genes sequenced in *Cucurbita* have been isolated by researchers doing comparative studies of specific genes across plant families; usually only a single allele is available. Nonetheless, we have included a list of the sequenced genes in **Table 4** as the sequences could be useful as a starting point for breeders interested in isolating the genes from lines of differing phenotype. In addition to the genes listed here, there exists a collection of partial sequences from mRNA for genes differentially expressed during seed development in *C. pepo*. These expressed sequence tags were identified in a study of the naked seed trait. The Gene Accession numbers for these sequences are CD726806 through CD726832.

Table 1. Phenotypic/Morphological Characteristics

Gene Symbol: Preferred	Synonym	Character	Species	Reference(s)
a		*androecious*. Found in 'Greckie'; produces only male flowers, recessive to *A*.	*pepo*	39
B		*Bicolor*. Precocious yellow fruit pigmentation; pleiotropic, affecting fruit and foliage, modified by *Ep-1*, *Ep-2* and *Ses-B*. Originally from 'Vaughn's Pear Shaped' ornamental gourd. *B* in *C. moschata* 'Precocious PI 165561' derived from *C. pepo* through backcrossing. Complementary to *L-2* for intense orange, instead of light yellow, fruit-flesh color.	*pepo, moschata*	52, 68, 78, 85, 87
B^{max}	*B-2*	*Bicolor*. Precocious yellow fruit pigmentation, from subsp. *andreana* PI 165558	*maxima*	86, 89
Bi		*Bitter* fruit. High cucurbitacin content in fruit. *Bi* from *C. maxima* subsp. *andreana* and *C. ecuadorensis*; *bi* from *C. maxima* subsp. *maxima*, including 'Queensland Blue'. Linked to *Lo-2*. In *C. pepo*, *Bi* from wild Texan gourd; *bi* from zucchini squash.	*maxima, maxima* × *ecuadorensis, pepo*	11, 28, 30
bl		*blue* fruit color. Incompletely recessive to *Bl* for green fruit color, in hubbard squash.	*maxima*	31
*Bn**		*Butternut fruit shape*, from 'New Hampshire Butternut', dominant to *bn* for crookneck fruit shape, as in 'Canada Crookneck'.	*moschata*	48
Bu		*Bush* habit. Short internodes; dominant to vine habit, *bu*, in young plant stage. In *C. pepo*, *Bu* in 'Giant Yellow Straightneck' and near-isogenic line of 'Table Queen', *bu* in 'Table Queen' acorn. In *C. maxima*, *Bu* from inbred line, *bu* from 'Delicious'.	*pepo, maxima*	17, 29, 84
Cmv		*Cucumber mosaic virus resistance*, from Nigerian Local. Dominant to *cmv* for susceptiblity, from 'Waltham Butternut'.	*moschata*	3
cr		*cream* corolla. Cream to nearly white petals, *cr* from *C. okeechobeensis*; *Cr* from *C. moschata* 'Butternut' incompletely dominant (yellow petals for *Cr/cr*, and orange for *Cr/Cr*)	*moschata* × *okeechobeensis*	75
cu		*cucurbitacin*-B reduced; *cu* for reduced cucurbitacin-B content of cotyledons of 'Early Golden Bush Scallop'; *Cu* for high cucurbitacin content of cotyledons of 'Black Zucchini'.	*pepo*	83
D		*Dark* stem. Series of three alleles observed in *C. pepo*: *D* for dark stem and dark intermediate-age	*pepo, maxima*	25, 42, 55, 56, 59, 67, 80

		fruit, D^s for dark stem but fruit not affected, and *d* for light stem and fruit not affected, with dominance $D > D^s > d$. *D* from 'Fordhook Zucchini', D^s from 'Early Prolific Straightneck'; *d* from 'Vegetable Spaghetti'. Epistatic to genes *l-1* and *l-2* when either is homozygous recessive; linked to *mo-2*. In *C. maxima*, only the fruit was observed: *D* for dark intermediate-age fruit from the zapallito 'La Germinadora'; *d* for light intermediate-age fruit from a variant zapallito breeding stock.		
de		*determinate* plant habit; stem lacking tendrils and terminating with female flowers. Recessive to *De* for indeterminate plant habit. *De* from 'Jeju' and 'Sokuk', *de* from inbred designated "Det".	*moschata*	40
Di		*Disc* fruit shape. From scallop squash, dominant to spherical or pyriform.	*pepo*	91, 97
Ep-1		*Extender of pigmentation-1*; modifier of *B*. *Ep-1* incompletely dominant to *ep-1* and additive with *Ep-2*. *Ep-1* from 'Small Sugar 7 × 7' pumpkin; *ep-1* from 'Table King' acorn.	*pepo*	90
Ep-2		*Extender of pigmentation-2*; modifier of *B*. *Ep-2* incompletely dominant to *ep-2* and additive with *Ep-1*. *Ep-2* from 'Table King' acorn; *ep-2* from 'Small Sugar 7 × 7' pumpkin.	*pepo*	90
Fr		*Fruit fly* (*Dacus cucurbitae*) resistance. *Fr* from 'Arka Suryamukhi', dominant to *fr* for susceptibility.	*maxima*	49
fv		*fused vein.* Fusion of primary leaf veins, subvital male gametophyte; found in hull-less-seeded pumpkin breeding line.	*pepo*	7, 8
G	*a, m*	*Gynoecious* sex expression; dominant to *g* for monoecious sex expression.	*foetidissima*	18, 23
Gb		*Green band* on inner side of base of petal, from a scallop squash; dominant to *gb*, for no band, from a straightneck squash.	*pepo*	19
gc		*green corolla*. Green, leaf-like petals, sterile; in unspecified F2 population.	*pepo*	92
gl		*glabrous*, lacking trichomes	*maxima*	37
Gr	*G*	*Green rind.* Dominant to buff skin of mature fruit. *Gr* from 'Long Neapolitan', *gr* from 'Butternut'.	*moschata*	71
grl		*gray leaf*. Recessive to green leaf. Recessive *grl* derived from cross of zapallito-type line of *C. maxima* and a butternut-type line of *C. moschata*. Dominant *Grl* from zapallito-type *C. maxima*.	*maxima* × *moschata*	41
Hi		*Hard rind inhibitor*. *Hi*, for hard-rind inhibition, from *C. maxima* 'Queensland Blue'; *hi*, for no hard-rind inhibition, from *C. ecuadorensis*.	*maxima* × *ecuadorensis*	30

Hr		*Hard rind. Hr* for hard (lignified) rind in ornamental gourd, straightneck squash, and zucchini; *hr* for soft (non-lignified) rind in 'Small Sugar' pumpkin and 'Sweet Potato' ('Delicata'). Complementary to *Wt* for *Warty* fruit.	*pepo*	44, 79
i		*intensifier* of the *cr* gene for cream flowers. *Cr/— I/—* for intense orange or yellow flowers, *Cr/— i/i* for light orange or yellow flowers, *cr/cr I/—* for cream flowers, *cr/cr i/i* for white flowers. *I* from *C. moschata* 'Butternut', *i* from *C. okeechobeensis*.	*moschata* × *okeechobeensis*	75
I-mc	I_{mc}	*Inhibitor* of *mature* fruit color; dominant to *i-mc* for no inhibition. *I-mc* in a scallop squash.	*pepo*	9
I-T		*Inhibitor* of the *T* gene for trifluralin resistance. *I-T* from 'La Primera'; *i-t* from 'Ponca' and 'Waltham Butternut'.	*moschata*	1
l-1	*c, St*	*light fruit coloration-1*. Light intensity of fruit coloration. Series of five alleles observed in *C. pepo* which, in complementary interaction with the dominant *L-2* allele, give the following results: *L-1* for uniformly intense/dark fruit coloration, from 'Fordhook Zucchini'; $l\text{-}1^{BSt}$ for broad, contiguous intense/dark stripes, from 'Cocozelle'; $l\text{-}1^{St}$ for narrow, broken intense/dark stripes, from 'Caserta'; $l\text{-}1^{iSt}$ for irregular intense/dark stripes, from 'Beirut' vegetable marrow; *l-1* for light coloration, from 'Vegetable Spaghetti', with dominance of $L\text{-}1 > (l\text{-}1^{BSt} > l\text{-}1^{St}) \geq l\text{-}1^{iSt} > l\text{-}1$. In *C. maxima*, *L-1* from the zapallito 'La Germinadora'; *l-1* from a variant zapallito breeding stock.	*pepo, maxima*	25, 42, 57, 62, 63, 67, 76, 85
l-2	*r*	*light fruit coloration-2*. Light intensity of fruit coloration. Series of three alleles observed in *C. pepo*, which, in complementary interaction with dominant alleles at the *l-1* locus, give the following results: *L-2* for intense/dark fruit coloration, with *L-1* from 'Fordhook Zucchini' and intense/dark fruit stripes, with $l\text{-}1^{BSt}$ from 'Cocozelle'; allele $L\text{-}2^{w}$ has delayed and weaker effect than *L-2*, from *C. pepo* subsp. *fraterna*; *l-2* for light coloration, from 'Vegetable Spaghetti', with dominance of $L\text{-}2 > L\text{-}2^{w} > l\text{-}2$. Dominant *L-2* is also complementary with *B* for intense orange, instead of light yellow, fruit-flesh color and with recessive *qi* for intense exterior color of young fruit. In *C. maxima*, *L-2* from the zapallito 'La Germinadora'; *l-2* from a variant zapallito breeding stock.	*pepo, maxima*	25, 42, 52, 58, 60, 67
lo-1	*l*	*lobed leaves-1*; recessive to *Lo-1* for non-lobed leaves	*maxima*	20
Lo-2		*Lobed leaves-2*. *Lo-2* for lobed leaves in *C. ecuadorensis* dominant to *lo-2* for unlobed leaves in	*ecuadorensis* × *maxima*	30

		C. maxima. Linked to *Bi*.		
lt		*leafy tendril*. Tendrils with laminae; *lt* found in ornamental gourd.	*pepo*	77
ly		*light yellow corolla*. Recessive to orange yellow; *ly* found in ornamental gourd.	*pepo*	77
M		*Mottled* leaves. *M* for silver-gray areas in axils of leaf veins, dominant to *m* for absence of silver-gray. For *C. maxima*, *M* in 'Zuni' and *m* in 'Buttercup' and 'Golden Hubbard'. For *C. pepo*, *M* in 'Caserta' and inbred of 'Striato d'Italia' cocozelle; *m* in 'Early Prolific Straightneck' and 'Early Yellow Crookneck'. For *C. moschata*, *M* in 'Hercules' and 'Golden Cushaw', *m* in butternut type. Weakly linked to *Wt*.	*pepo, maxima, moschata*	13, 66, 76, 81
Mldg		*Mottled light* and *dark green* immature fruit color; germplasm unspecified. Dominant to *mldg* for non-mottled.	*moschata*	5
mo-1		*mature orange-1*; complementary recessive gene for loss of green fruit color prior to maturity. *Mo-1* from 'Table Queen' acorn; *mo-1* from 'Vegetable Spaghetti'.	*pepo*	56
mo-2		*mature orange-2*; complementary recessive gene for loss of green fruit color prior to maturity. *Mo-2* from 'Table Queen' acorn; *mo-2* from 'Vegetable Spaghetti'. Linked to *D*.	*pepo*	56
ms-1	ms_1	*male sterile-1.* Male flowers abort before anthesis, derived from a cross involving 'Golden Hubbard', recessive to *Ms-1* for male fertile.	*maxima*	82
ms-2	ms_2	*male sterile-2.* Male flowers abort, sterility expressed as androecium shrivelling and turning brown; *ms-2* from 'Eskandarany' (PI 228241).	*pepo*	22
ms-3	*ms-2*	*male sterile-3*.	*maxima*	37
*m-zym^{mos}**		*modifier* of dominance of *zucchini yellow mosaic* virus resistance; confers resistance to otherwise susceptible *Zym^{mos}/zym^{mos}* heterozygotes. *M-zym^{mos}* in 'Soler', *m-zym^{mos}* in 'Waltham Butternut' and 'Nigerian Local'.	*moschata*	51
n	*h*	*naked* seeds. Lacking a lignified seed coat, *n* from oil-seed pumpkin.	*pepo, moschata*	27, 80, 87, 99, 102
pl		*plain light* fruit color, *pl* from 'Beirut' vegetable marrow and 'Fordhook Zucchini'; *Pl* in 'Vegetable Spaghetti'.	*pepo*	53
*Pm, Pm-0**		*Powdery mildew* resistance. Resistance to *Podosphaera xanthii*; *Pm* from *C. lundelliana*; *Pm-0* from *C. okeechobeensis* and in *C. pepo*	*lundelliana, okeechobeensis, pepo*	10, 12, 35, 70

pm-1		*powdery mildew* resistance in *C. moschata*. Series of three alleles: $pm\text{-}1^P$ for susceptibility from 'Ponca' dominant to $pm\text{-}1^L$ for resistance from 'La Primera', which is dominant to $pm\text{-}1^W$ for susceptibility in 'Waltham Butternut'.	*moschata*	2
pm-2		*powdery mildew* resistance in *C. moschata* 'Seminole', recessive to *Pm-2* for susceptibility	*moschata*	2
prv		*papaya ringspot virus resistance*, in Nigerian Local, recessive to *Prv* for susceptibility, in 'Waltham Butternut'.	*moschata*	3
qi		*quiescent intense*. Recessive to *Qi* for not intense and complementary to *L-2* for intense young fruit color; little or no effect on mature fruit. *Qi* from 'Vegetable Spaghetti'; *qi* from 'Jack O'Lantern' pumpkin and 'Verte non-coureuse d'Italie' cocozelle.	*pepo*	58, 61
Rd		*Red* skin. Red external fruit color; dominant to green, white, yellow and gray. *Rd* from 'Turk's Cap'; *rd* from 'Warted Hubbard'.	*maxima*	43
ro		*rosette* leaf. Lower lobes of leaves slightly spiraled, *ro* derived from an ornamental gourd.	*pepo*	44
s-1	*s*	*sterile.* Male flowers small, without pollen; female flower sterile. Derived from crossing 'Greengold' with 'Banana'.	*maxima*	32
s-2		*sterile.* Male flowers small, without pollen and female flower sterile; mutant in powdery mildew resistant, straightneck squash breeding line.	*pepo*	6
Ses-B		*Selective suppression* of gene *B*. Suppression in foliage of precocious yellowing conferred by *B*. *Ses-B* in straightneck breeding line dominant to *ses-B* in 'Jersey Golden Acorn'.	*pepo*	88
sl		*silverleaf* resistance. Recessive to *Sl* for susceptibility. *Sl* from 'Soler'; *sl* from PI 162889 and butternut types.	*moschata*	26
Slc		*Squash leaf curl* virus resistance; derived from *C. moschata*.	*pepo*	46
sp		*spaghetti* flesh, breaking into strands after cooking	*pepo*	45
T		*Trifluralin* resistance. Dominant to susceptibility to the herbicide; modified by *I-T*. *T* in 'La Primera'; *t* in 'Ponca' and 'Waltham Butternut'.	*moschata*	1
uml		*umbrella-like*; leaves shaped like partially opened umbrella. Recessive *uml* derived from a cross of *C. maxima* 'Warzywna' and a *C. pepo* inbred; dominant *Uml* from 'Warzywna'.	*maxima* × *pepo*	69
v		*virescent.* Yellow-green young leaves, *v* found in 'Golden Delicious'.	*maxima*	21

W	*Weak* fruit coloration. Dominant to *w* for intense-pigmented mature fruit; *W* from scallop squash. Complementary to *Wf* for white external fruit color.	*pepo*	54, 91, 97
wc	*white corolla*. Derived from 'Ispanskaya' × 'Emerald'. Recessive to *Wc* for normal orange-yellow corolla	*maxima*	38
Wf	*White flesh*. Dominant to *wf* for colored flesh. *Wf* in a scallop squash, *wf* in a straightneck squash. Complementary to *W* for white external fruit color.	*pepo*	19, 54, 91
Wmv	*Watermelon mosaic virus resistance*. From "Menina" and "Nigerian Local", dominant to *wmv* for susceptibility in 'Musquée de Provence' and 'Waltham Butternut'. May be linked with or identical to *Zym-1*.	*moschata*	3, 24
Wmv^{ecu}*	*Watermelon mosaic virus* resistance. From *C. ecuadorensis*, in a cross with an unspecified *C. maxima*.	*maxima* × *ecuadorensis*	95
Wt	*Warty* fruit. Dominant to non-warted, *wt*, and complementary to *Hr*, with fruit wartiness being expressed only in the presence of the dominant *Hr* allele. *Wt* in straightneck, crookneck, and 'Delicata'; *wt* in zucchini, cocozelle, and 'Small Sugar' pumpkin. Weakly linked to *M*.	*pepo*	66, 79, 91
wyc	*white-yellow corolla*; isolated in 'Riesen-Melonen'. Recessive to *Wyc* for normal orange-yellow corolla.	*maxima*	38
Y	*Yellow* fruit color. *Y* for yellow fruit color of intermediate-age fruits, from straightneck and crookneck squash, dominant to *y* for green intermediate-age fruit color, from vegetable marrow, ornamental gourd, and cocozelle.	*pepo*	66, 76, 84, 85, 91
yg	*yellow-green* leaves and stems	*maxima*	37
Ygp	*Yellow-green placenta*. Dominant to yellow placental color. *Ygp* in a scallop squash, *ygp* in a straightneck squash.	*pepo*	19
ys	*yellow seedling*. Lacking chlorophyll; lethal	*pepo*	44
zym^{ecu}	*zucchini yellow mosaic* virus resistance, recessive to susceptibility; zym^{ecu} from *C. ecuadorensis*, Zym^{ecu} from *C. maxima* 'Buttercup'.	*ecuadorensis*	74
zym^{mos}*	*zucchini yellow mosaic* virus resistance, recessive to susceptibility; zym^{mos} from 'Soler', Zym^{mos} from 'Waltham Butternut'.	*moschata*	51
*Zym-0**	*Zucchini yellow mosaic* virus resistance. *Zym-0* from *C. moschata* 'Nigerian Local' dominant to *zym-0* for susceptibility from 'Waltham Butternut'. Perhaps one of two separate genes for resistance in 'Nigerian Local'.	*moschata*	3, 47, 51

Zym-1	*Zucchini yellow mosaic* virus resistance. *Zym-1* from *C. moschata* 'Menina' dominant to *zym-1* for susceptibility from *C. moschata* 'Waltham Butternut'. *Zym-1* transferred via backcrossing to *C. pepo* 'True French' zucchini, in which it confers resistance through complementary interaction with *Zym-2* and *Zym-3*. *Zym-1* is either linked with *Wmv* or also confers resistance to watermelon mosaic virus.	*moschata, pepo*	24, 51, 64, 65
Zym-2	*Zucchini yellow mosaic* virus resistance-*2*. Dominant to susceptibility and complementary to *Zym-1*. *Zym-2* from *C. moschata* 'Menina'. *Zym-2* in *C. pepo* derived from *C. moschata*, in near-isogenic resistant line of 'True French' zucchini; *zym-2* from *C. pepo* 'True French'.	*moschata, pepo*	64
Zym-3	*Zucchini yellow mosaic* virus resistance-*3*. Dominant to susceptibility and complementary to *Zym-1*. *Zym-3* from *C. moschata* 'Menina'. *Zym-3* in *C. pepo* derived from *C. moschata*, in near-isogenic resistant line of 'True French' zucchini; *zym-3* from *C. pepo* 'True French'.	*moschata, pepo*	64

*Proposed new gene symbol.

Table 2. Isozyme Variants

Gene Symbol					
Preferred	Synonym	**No. alleles observed**	**Character**	**Species**	**Reference(s)**
Aat-1	*Aat*	8	*Aspartate aminotransferase-1*. Variant among accessions.	*pepo*	16, 34
Aat-3		2	*Aspartate aminotransferase-3*. Variant among wild populations.	*pepo*	16
Aat-4		3	*Aspartate aminotransferase-4*. Variant among wild populations.	*pepo*	16
Aat-mb		2	*Aspartate aminotransferase – microbody*	*maxima* × *ecuadorensis*	95
Aat-m1		2	*Aspartate aminotransferase mitochondria-1*	*maxima* × *ecuadorensis*	95
Aat-m2		2	*Aspartate aminotransferase mitochondria-2*	*maxima* × *ecuadorensis*	95
Aat-p2		2	*Aspartate aminotransferase plastid-2*	*maxima* × *ecuadorensis*	95
Acp-1		2	*Acid phosphatase-1*	*maxima* ×	95

				ecuadorensis	
Acp-2		2	*Acid phosphatase-2*	*maxima* × *ecuadorensis*	95
Aldo-p		2	*Aldolase – plastid*	*maxima* × *ecuadorensis*	94
Est-1	*Est*	2	*Esterase*	*maxima* × *ecuadorensis*	93, 95
Gal-1		2	*β-galactosidase-1*	*maxima* × *ecuadorensis*	95
Gal-2		2	*β-galactosidase-2*	*maxima* × *ecuadorensis*	95
G2d-1		3	*Glycerate dehydrogenase-1*. Variant among wild populations.	*pepo*	16
G2d-2		2	*Glycerate dehydrogenase-2*. Variant among wild populations.	*pepo*	16
Got-1		5	*Glutamine oxaloacetate-1*. Variant among accessions, wild populations, and among *Cucurbita* species.	*pepo*	14, 15, 36, 98
Got-2		3	*Glutamine oxaloacetate-2*. Variant among species.	*maxima* × *ecuadorensis*	98
Gpi		2	*Glucosephosphate isomerase*. Variant among accessions.	*pepo*	34
Gpi-3		2	*Glucosephosphate isomerase-3*. Variant among wild populations.	*pepo*	16
Gpi-c1		2	*Glucosephosphate isomerase cytosolic-1*	*maxima* × *ecuadorensis*	95
Gpi-c2		2	*Glucosephosphate isomerase cytosolic-2*	*maxima* × *ecuadorensis*	95
Idh-1		4	*Isocitrate dehydrogenase-1*. Variant among accessions, wild populations, and *Cucurbita* species.	*pepo*	14, 15, 16, 36, 98
Idh-2		2	*Isocitrate dehydrogenase-2*. Variant among accessions, wild populations, and *Cucurbita* species.	*pepo*	14, 15, 16, 36, 98
Idh-3		2	*Isocitrate dehydrogenase-3*. Variant among accessions and populations.	*pepo*	14, 15, 16, 36
Lap-1	*Lap*	4	*Leucine aminopeptidase*. Variant among *C. pepo* accessions.	*maxima* × *ecuadorensis*; *pepo*	16, 34 , 93, 95

Mdh-1	*Mdh*	7	*Malate dehydrogenase*. Variant among accessions.	*pepo*	34
Mdh-2		3	*Malate dehydrogenase-2*. Variant among accessions, wild populations, and *Cucurbita* species.	*pepo*	14, 15, 16, 36, 98
Mdh-3		3	*Malate dehydrogenase-3*. Variant among accessions, wild populations, and *Cucurbita* species.	*pepo*	14, 15, 16, 36, 98
Mdh-m1		2	*Malate dehydrogenase mitochondria-1*	*maxima* × *ecuadorensis*	95
Mdh-m2		2	*Malate dehydrogenase mitochondria-2*	*maxima* × *ecuadorensis*	95
Mdh-c2		2	*Malate dehydrogenase cytosolic-2*	*maxima* × *ecuadorensis*	95
Per-1		2	*Peroxidase-1*	*maxima* × *ecuadorensis*	95
Per-2		3	*Peroxidase-2*. Variant among accessions and wild populations.	*pepo*	14, 15, 36
Per-3		2	*Peroxidase-3*	*maxima* × *ecuadorensis*	95
Pgi-1		2	*Phosphoglucase isomerase-1*	*pepo*	14
Pgi-2		2	*Phosphoglucase isomerase-2*. Variant among *Cucurbita* species.	*pepo*	14, 36, 98
Pgi-3		4	*Phosphoglucase isomerase-3*. Variant among accessions, wild populations, and *Cucurbita* species.	*pepo*	14, 15, 36, 98
Pgm-1	*Pgm*	2	*Phosphoglucomutase*. Variant among accessions.	*pepo*	34
Pgm-2		4	*Phosphoglucomutase-2*. Variant among accessions, wild populations, and *Cucurbita* species.	*pepo*	14, 15, 36, 98
Pgm-5		2	*Phosphoglucomutase-5*. Variant among wild populations.	*pepo*	16
Pgm-6		2	*Phosphoglucomutase-6*. Variant among wild populations.	*pepo*	16
Pgm-c2		2	*Phosphoglucomutase cytosolic-2*	*maxima* × *ecuadorensis*	95
Pgm-p		2	*Phosphoglucomutase plastid*	*maxima* × *ecuadorensis*	95
Skd-1		6	*Shikimate dehydrogenase*. Variant	*pepo*	16

		among wild populations.		
Skdh	5	*Shikimate dehydrogenase*. Variant among *C. pepo* accessions.	*maxima* × *ecuadorensis*; *pepo*	34, 95
Sod-1	2	*Superoxide dismutase-1*	*maxima* × *ecuadorensis*	95
Tpi-c2	2	*Triosephosphatase isomerase cytosolic-2*	*maxima* × *ecuadorensis*	95
Tpi-p2	2	*Triosephosphatase isomerase plastid-2*	*maxima* × *ecuadorensis*	95

Table 3. Mapped Phenotypic/Morphological Characteristics

Trait	**Symbol**	**Linked Marker(s)**	**Recombination Distance (cM)**	**Reference(s)**
Seed Coat	*n*	AK11_340	4.4	101
Fruit Length	(QTL)	AE07_165, AC10_490, AJ20_420, P13_750, J01_600, AO20_1200, T08_460, AB08_540, AE09_1600		101
Fruit Width	(QTL)	AE07_165, AJ20_420, AM10_950, AG08_440		101
Fruit Length/width Ratio	(QTL)	AE07_165, AC10_490, AJ20_420, P13_750, J01_600		101
No. of Fruit Chambers	(QTL)	P13_950, AE08_470		101
Precocious yellow fruit	*B*	I10_1700	27.1	4
Leaf Indentation	(QTL)	F10_400, K11_950, G2_400		4
Leaf Mottle	*M*	H14_600 U489_1200	13.0 16.3	4
Mature Fruit Color	[none given]	G17_700	9.7	4
Fruit Shape	(QTL)	F8_1050, B8_900, H19_500		4

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Table 4. Genes with known DNA sequence

Gene Symbol*	**Gene Accession**	**(Putative) Function**	**Source**	**Ref.**
AIG-2	AY666083	aspartic protease inhibitor	*C. maxima*	**
PRB1	AY326308	phloem RNA-binding protein	*C. maxima* 'Big Max'	**
GAIP	AY32630, AY326307	gibberellic acid insensitive phloem protein (two very similar genes)	*C. maxima* 'Big Max'	**
FAD2	AY525163	omega-6 fatty acid desaturase	*C. pepo* zucchini	**
NIP1	AJ544830	Nod26-like protein	*C. pepo* zucchini	23
PP2	AY312402	phloem protein 2 lectin (includes promoter region)	*C. moschata* crookneck	**
PP2	AF150627	phloem protein 2 lectin	*C. moschata* crookneck	**
PP2	Z22647	phloem protein 2 lectin	*C. pepo* 'Autumn Gold'	42
PP2	Z17331	phloem protein 2 lectin	*C. maxima* 'Big Max'	2
PP2	L31550, L31551, L31552	phloem protein 2 (three alleles)	*C. maxima*	**
GA2OX, GA20OX, GA3OX	AJ315663, AJ302041, AJ308480, AJ302040	gibberellin oxidases (two sequences for GA2OX)	*C. maxima* 'Riesenmelone'	**

	U61385	gibberellin 20-oxidase	*C. maxima* 'Riesenmelone'	25
	U63650	gibberellin 2 beta,3 beta hydroxylase	*C. maxima* 'Riesenmelone'	26
	AJ006453	gibberellin 3 beta hydroxylase	*C. maxima* 'Riesenmelone'	**
	U61386	gibberellin dioxygenase	*C. maxima* 'Riesenmelone'	24
Moschatin 1 through 5	AF462349, AF504011, AY25646, AY27921, AY279217	ribosome-inactivating protein	*C. moschata* crookneck	**
CPS1	AB109763	copalyl diphosphate synthase; gibberellin biosynthesis	*C. maxima*	**
CPS	AF049905, AF049906	copalyl diphosphate synthase; gibberellin biosynthesis (2 genes)	*C. maxima*	37
Hsc70	AF527794, AF527795, AF527796	cell-autonomous heat shock protein; chaperonin 70 (multiple sequences)	*C. maxima*	1
	AB061204	thioredoxin h	*C. maxima*	**
Puga, Pugb, Pugc	AB055116, AB055117, AB055118	glutathione S-transferase	*C. maxima*	**
CYP88A	AF212990, AF212991	cytochrome P450; ent-kaurenoic acid oxidase (multiple alleles)	*C. maxima* 'Queensland Blue'	13
PP2	AF520583	phloem protein 2	*C. digitata* PI 240879	**
PP2	AF520582	phloem lectin	*C. argyrosperma* subsp. *sororia*	**
	L32700, L32701	phloem lectin	*C. argyrosperma*	2
	X56948	malate synthase	*Cucurbita* sp.*** 'Kurokawa Amakuri Nankin'	29
pMCPN60	X70867, X70868	chaperonin 60	'Kurokawa Amakuri Nankin'	40
PCPK	AY07280, AY072802	phloem calmodulin-like protein kinases	*C. maxima* 'Big Max'	47
	X55779	ascorbate oxidase	*C. maxima* 'Ebisu Nankin'	7
AAO	D55677	ascorbate oxidase	*C. maxima*	21
chitP1	AB015655	chitinase	*C. maxima* 'Ebisu Nankin'	**
PLC	AF082284	chitinase	*C. moschata* crookneck	20

PV72	AB006809	vacuolar sorting receptor	'Kurokawa Amakuri Nankin'	36
	D88420	stromal ascorbate peroxidase	'Kurokawa Amakuri Nankin'	28
	D78256	isocitrate lyase	'Kurokawa Amakuri Nankin'	27
	D70895	3-ketoacyl-CoA thiolase	'Kurokawa Amakuri Nankin'	19
	D83656	thylakoid ascorbate peroxidase	'Kurokawa Amakuri Nankin'	45
	D49433	hydroxypyruvate reductase	'Kurokawa Amakuri Nankin'	12
MP28	D45078	membrane protein	'Kurokawa Amakuri Nankin'	16
	D38132	glyoxysomal citrate synthase	'Kurokawa Amakuri Nankin'	18
	D29629	aconitase	'Kurokawa Amakuri Nankin'	10
	D16560	prepro2S albumin	'Kurokawa Amakuri Nankin'	8
	D14044	glycolate oxidase	'Kurokawa Amakuri Nankin'	39
	AF002016	acyl CoA oxidase	'Kurokawa Amakuri Nankin'	9
PP36	AF274589	cytochrome b5 reductase	*C. maxima* 'Big Max'	**
pAPX	AB070626	peroxisomal ascorbate peroxidase	'Kurokawa Amakuri Nankin'	33
CM-ACS3	AB038559	ACC synthase	*C. maxima*	43
CmATS	AB049135	acyl-(acyl-carrier protein); acyltransferase	*C. moschata* 'Shirogikuza'	**
	Y00771	glycerol-3-phosphate acyltransferase transit peptide	*C. moschata* 'Shirakikuza'	17
	AB002695	aspartic endopeptidase	*C. pepo*	14
PS-1	AF284038	phloem serpin	*C. maxima*	46
SLW	AF170086, AF170087	silverleaf whitefly-induced protein (multiple genes)	*C. pepo* zucchini 'Chefini'	41
aprX	Y17192	anionic peroxidase	*C. pepo* zucchini 'Black Beauty'	3
cpCPK1	U90262	calcium-dependent calmodulin-independent protein kinase	*C. pepo* zucchini	6
PP16	AF079170, AF079171	mRNA movement protein; phloem transport (multiple alleles)	*C. maxima* 'Big Max'	44
AOBP	D45066	transcription factor binding to ascorbate oxidase	*C. maxima*	22
accW	D01032	auxin-induced 1-aminocyclopropane-1-carboxylate synthase	*C. maxima* 'Ebisu'	32
	U37774	auxin-induced 1-aminocyclopropane-1-carboxylic acid synthase	*C. maxima*	31
ACC1	M58323	1-aminocyclopropane-1-carboxylate synthase	*C. pepo*	34

ACC1A, ACC1B	M61195	1-aminocyclopropane-1-carboxylate synthase (2 genes, tightly linked)	*C. pepo* zucchini	15
PHP-1	D86306	proton-translocating inorganic pyrophosphatase	*C. moschata* crookneck	**
PP1	U66277	phloem filament protein	*C. maxima* 'Big Max'	4
pfiAF4	X81647	trypsin inhibitor	*C. maxima* 'Supermarket Hybrid'	30
pfiBM7	X81447	chymotrypsin inhibitor	*C. maxima* 'Supermarket Hybrid'	30
	M15265	phytochrome	*C. pepo* zucchini 'Black Beauty'	35
NADH	M33154	nitrate reductase	*C. maxima*	5
	M36407	11S globulin beta-subunit	'Kurokawa Amakuri Nankin'	11
	AF206895	18S ribosomal RNA	*C. pepo*	**
	AF479108	26S ribosomal RNA	*C. pepo*	38

* Gene symbols were assigned by the researchers isolating the gene; they have no correspondence to the official *Cucurbita* gene symbols.
**Unpublished: Genes can be submitted directly to Genbank, wthout being published in a journal.
*** 'Kurokawa Amakuri Nankin' was identified only as "*Cucurbita* sp."

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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.

References

1. CGC Gene List Committee. 1982. Update of cucurbit gene list and nomenclature rules. CGC 5:62-66.
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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.

2004 CGC Membership Directory

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