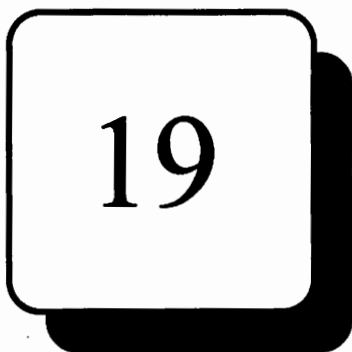# Cucurbit Genetics Cooperative

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19th Annual CGC Business Meeting (1995)

The 19th annual CGC Business Meeting was held on Monday, 31 July 1995, in Montreal in conjunction with the annual meeting of the American Society for Horticultural Science. Eighteen members and other interested individuals were in attendance.

Tim Ng presented summary statistics and cost analyses on the eighteen annual CGC Reports, and provided detailed information on CGC Report No. 18 (1995) as well as an update on the forthcoming Call for Papers for CGC Report No. 19 (1996). With increasing paper, printing and mailing costs, the pro-rated annual fee of $7 per year no longer covers the costs of sending the report and additional correspondence to CGC members, so the pro-rated rates will rise to $8 for the 1997

calendar year. Thus, CGC members renewing for the 1996-97 biennium will pay $15 (exclusive of airmail options) while CGC members renewing for the 1997-98 biennium and thereafter will pay $16. This marks the first rate increase since 1988.

The CGC web site was the next topic of conversation. Tim presented background on the history and rationale for creating a CGC web site and placing it on the U.S. Plant Genome server (see CGC Rept. 19:89-90, 1996). He showed sample screenshots, including an article from CGC Report No. 18 which had been encoded for the web, and spoke of the potential for archiving old reports but also providing links to other cucurbit-related sites on the web. It was decided that digitization of back

issues would begin with the earliest reports, and that eventually all but the most recent five years of CGC reports would be available on the web. Should the time come when all CGC members have access to the Internet, CGC may publish entirely on the web, but in the meantime CGC will still have to publish a printed report every year. However, the most recent gene lists for each of the cucurbit categories will be made available on the web.

Announcements were made of the upcoming Eucarpia meeting on cucurbits, Cucurbitaceae '96, and of plans to update the "other genera" gene list for CGC Report No. 19 (1996). Gary Elmstrom (University of Florida; Pioneer Seeds) recently completed his ten years of service as the CGC Coordinating Committee member for melon (*Cucumis melo*), and David Wolff (Texas A&M, Weslaco) was elected to replace Gary on the Coordinating Committee.

The next annual CGC Business Meeting will be in October at Lexington, Kentucky, in conjunction with the 1996 annual meeting of the American Society for Horticultural Science.

U.S. Cucurbit Crop Germplasm Committee (CCGC) Update

J.D. McCreight, USDA, ARS, Salinas, California

CCGC held its 12th meeting on February 4, 1996 in Greensboro, North Carolina in conjunction with the Southern Association of Agricultural Scientists Annual Meeting. Susan Webb, (University of Florida) replaced Kent Elsey (entomology) and Alex May, (Rogers Seed Co., Gilroy, California) replaced Jon Watterson (plant pathology).

GRIN is now available on the Internet via Gopher (gopher *gopher.ars-grin.gov*) or anonymous

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Comments . . . .

From the CGC Coordinating Committee: The Call for Papers for the 1997 Report (CGC Report No. 20) will be mailed in September 1996. Papers should be submitted to the respective Coordinating Committee members by 31 January 1997, although late submissions may be considered if received prior to our processing deadline. The Report will be published by June/July 1997. As always, we are eager to hear from CGC members regarding our current activities and future direction of CGC.

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

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

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

FTP (ftp *sol.ars-grin.gov* or ftp *192.100.146.3*). For more information contact the GRIN Database Manager, Bldg. 003, Room 407, BARC-West, Beltsville, MD 20705-2350 (301-504-5666 or fax 301-504-5536). GRIN has been updated from GRIN2 to GRIN3 and is greatly improved. A Windows version of pcGRIN is now available. A Macintosh version of pcGRIN has finally been elicited from GRIN, and although not as elegant as the Windows version of pcGRIN, it works on the Macintosh! Contact J.D. McCreight if you're interested in the Macintosh version.

One of two Germplasm Evaluation Proposals submitted to NPGS was funded in 1996: Evaluation of *Cucumis melo* Plant Introductions for Resistance to *Monosporascus cannonballus*, Investigators: David Wolff and Marvin Miller. Two CCGC members (T.C. Wehner and J.D. McCreight) participated in a cucurbit germplasm collection trip jointly funded by USDA, North Carolina State University and Republic of South Africa from April 24-May 8, 1996. They were hosted by Roger Ellis and Mariana Jooste, RSA, Agricultural Research Council, Plant Genetic resources Unit. The expedition collected approximately 113 mostly wild specimens from the northern areas of the Northern, Northwestern and North Cape Provinces.

1996 Watermelon Research Group Meeting

Ray D. Martyn, Dept. Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77845

The 16th Annual Meeting of the Watermelon Research Group was held on February 4, 1996, in Greensboro NC in conjunction with the Southern Region ASHS and the Southern Association of Agricultural Scientists. Approximately 45 people attended. Unfortunately, the severe ice and snow storm kept many people away, but we still had a good discussion session.

Dr. Tom Kucharek, University of Florida, Gainesville, gave a report on watermelon fruit blotch (FB). He indicated that potential sources of inoculum are infected seed, infected transplants, and watermelon crop debris. Fruit blotch has not been observed on the wild citron and it is therefore probably not a potential source of inoculum. Tom also indicated that high humidity in the greenhouse can cause water soaking of the cotyledons similar to that induced by infection and one should be extra careful when rating plants based on water soaking. He also reported that FB increases with increasing temperature. He reported that at 50 F it required 8 days for symptoms to develop and only 5 days at 77 F. At 86 F, disease developed the fastest. In regard to seed contamination, apparently the fermentation that occurs naturally in rotting fruit is adequate in eliminating the FB bacterium from seed. They have also conducted comparison testing of the PCR detection method and the grow out method and they indicate that the tests are in fairly good agreement with each other.

Dr. Rick Latin, Purdue University, reported on studies related to inoculum spread and survival. He reported that the FB bacterium could be recovered from buried watermelon rinds for up to 12 months after burying. Studies on relative humidity and disease indicate that more than just high humidity is required to spread the infection. For example, at 95% RH but no overhead irrigation, almost no disease developed in the greenhouse (5-7%). This is compared to 100% disease at 95% RH with overhead irrigation events. At very low RH (35%) disease spread only about 5 cm from the point source. He also reported on studies on the survival and recovery of the FB bacterium. They examined a number of items common to greenhouses (trays, toothpicks, nuts and bolts). At air temperature, the bacterium could be recovered from used seedling trays up to 14 days after the test, but only up to 7 days in new trays. If the trays were stored at 4 C, however, they could recover the bacterium after 60 days. Rick also showed slides of FB on cantaloupe. Symptoms included sunken pits on the fruit, similar to those caused by anthracnose. On leaves, the symptoms included water soaking and brown, necrotic lesions.

Dr. Don Hopkins, University of Florida, Leesburg, reported on recovery experiments using a double-labeled antibiotic mutant of the bacterium. He reported that PCR of seed washings and grow out tests gave similar results, although there were some false positives with PCR using nested primers (60 cycles). Treatment of seed with either HCl or fermentation completely eliminated the bacterium from seed. Also, most formulations of copper gave adequate control. They also investigated the spread of FB from infested seed in the field by using artificially infested seed at rates of 1 infected seed / 1000 to 100 infected seed / 1000. In the early planted tests (March), almost all of the plants became diseased with the highest infestation levels around 5-6 weeks after planting. In the later planted test, disease began to show up as early as 2 weeks after planting. Rapid spread was correlated with rain events. There was not much difference in total disease between the 1/1000 and 100/1000 infested seed - both were very high. Don suggested that insects such as leaf miners or aphids may be involved in the spread of FB. He also reported that the wild citron could become infected but he had not seen naturally infected citron in the field. Results of screening over 600 PI accessions for tolerance or resistance indicate that three accessions appeared tolerant (less than a disease rating of 5 on a 1-9 scale).

Dr. Billy Rhodes, Clemson University, Clemson, SC, reported that two PIs were resistant in his tests and that dark-colored rinds in Congo were asymptomatic.

Good group discussions occurred throughout the 4 hour meeting. If you have any questions related to the above research reports, please contact the person directly. If you do not know their address, phone, e-mail, etc., contact Ray Martyn and he will be happy to provide it for you.

The refreshments for this year's meeting were kindly provided by Dr. Glenn Price, American SunMelon, Hinton, OK.

Pickling Cucumber Improvement Committee (PCIC)

The Pickling Cucumber Improvement Committee (PCIC) of Pickle Packers International is meeting October 5, 1996, at the Hyatt Regency in Lexington, Kentucky. Titles and abstracts of papers must be submitted by August 1 to Michael Havey (1996 PCIC chair), Department of Horticulture, University of Wisconsin, Madison WI 53706.

Proceedings from Cucurbitaceae '94 and Cucurbitaceae '96

The proceedings from the recent **Cucurbitaceae '94** (USA) and **Cucurbitaceae '96** (Spain) conferences are now available for purchase.

Cucurbitaceae '94, *Evaluations and Enhancement of Cucurbit Germplasm*, was held on 1-4 November 1994 in South Padre Island, Texas, and was organized by Gene Lester (USDA) and James Dunlap (Texas A&M University). The Proceedings for Cucurbitaceae '94 are available as a book for sale through the American Society for Horticultural Sciences (ASHS). The

cost is $20 plus shipping and handling for CGC members ($25 for non-members) plus 4.5% sales tax to Virginia addresses or for sales over the counter. Shipping and handling charges are $5 for domestic orders, $7 for Canadian and Mexican orders, and $10 for all other orders. ASHS can be contacted by phone at (703) 836-4606 (press "0" for operator during business hours). Orders can be faxed, with credit card information, to ASHS at (703) 836-2024, or can be mailed to ASHS at 600 Cameron Street, Alexandria, VA 22314-2562 USA.

Cucurbitaceae '96, the VI Eucarpia Meeting on Cucurbit Genetics and Breeding, was held on 28-30 May 1996 in Málaga, Spain, and was organized by M.L. Gómez-Guillamón, C. Soria, J. Cuartero, J.A. Tores and R. Fernández-Muñoz of the Experimental Station La Mayora, CSIC. The Proceedings are now available as a 350 page book entitled *Cucurbits Towards 2000* (ISBN 605-5163-6), which is divided into three chapters representing the three meeting sessions: *Genetics and Breeding* (18 papers), *Biotechnology and Physiology* (9 papers), and *Plant Diseases and Disease Resistances* (22 papers). The cost of the book is ESP 5,000 (plus mail expenses of ESP 250), and orders can be sent to M.L. Gómez- Guillamón, E.E. La Mayora - CSIC, 29750 Algarrobo Costa, Málaga, Spain (Fax: 34-9-255 26 77, E-mail: *guillamon@mayora.csic.es*).

"Melon" Now Accepted by USDA

It's official! The USDA has now declared that the acceptable name for *Cucumis melo* types is "melon." "Muskmelon" and "cantaloupe" are acceptable synonyms when used to describe muskmelon or cantaloupe type of melons (per USDA). Common sense would dictate that "honeydew" or "winter" or many others are also acceptable, but that the *type* or *kind* name is properly "melon." Our thanks go out to Larry Hollar, who in 1989 instigated the effort to request this change by the USDA.

Corrigendum

In CGC Report 18 (1995), an error appeared in text of the article "Regeneration Response of a Few Genetic Marker Lines and Commercial Cultivars of *Cucumis melo* L." by Jaagrati Jain and T.A. More (p. 48-49). In the "Results" paragraph, it was stated that M4 cotyledonary leaves explant callus exhibited differentiation in the range of "30.5 6.8 percent" and that shoot buds differentiation was observed in "66.7 7.2 percent" of calli. The correct phrases are "30.5 ± 6.8 percent" and "66.7 ± 7.2 percent" respectively.

An Induced Mutation in Cucumber (*Cucumis sativus* L.): Super Compact

K. Niemirowicz-Szczytt, M. Rucinska and A. Korzeniewska
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A mutant designated as super compact (*scp*) belongs to the series of mutants (1,3-6) which are currently being maintained in a collection originally developed by B. Kubicki. As with all mutants in this collection, the super compact was obtained by ethyleneimine seed treatment of the inbred line 'Borszczagowski' (B). The morphological description of this mutant provided herein was based on 20 plants. The genetic analysis defining its inheritance was based on several hundred individuals.

The super compact mutant can be included in the broad group of growth-type mutants which have drastically reduced main stem length. This mutant can be distinguished in the seedling stage (Fig. 1) by a drastic decrease in shoot length when compared to the standard wild-type. However, it possesses a hypocotyl the standard size as that of the wild-type.

Fully developed plants of the super compact mutant are small [4.7 ± 0.6 cm high (Fig. 2)] when compared to the B line (266.0 ± 6.2 cm). While the length of the internodes of this mutant ranges between 0.2 to 0.5 cm, that of the B line is between 6.1 to 7.3 cm. The mutant plant does not develop lateral branches while the B line possesses lateral branches (24 ± 3). Leaves of this mutant are dark green and a little smaller than the standard wild-type (mutant leaves 13 to 14 cm long, and 15 to 17 cm wide with leaf petiole 9 to 10 cm long, and respective dimensions of standard wild-types are 16 to 17, 20 to 22, 16 to 18 cm). The smaller leaves with shorter petioles typical of the mutant are thicker and slightly wrinkled when compared to the wild-type (Fig. 3). Flower petals of this mutant are wrinkled, and gynoecious flowers develop rarely. The pistils are deformed and never develop into fruit. Staminate flowers of this mutant often contain deformed stamens with fertile (90% stainable) pollen grains which are viable and have been used in backcrosses to heterozygous (phenotypically wild-type) individuals.

The F_1 data presented in Table 1 confirm the recessive character of *spc*. However, deviation from the expected ratios was observed in F_2 and BCP_2 families. Deviations from expected ratios may be due to a lower germination ability of mutant seeds and lower seedling resistances to common cucumber pathogens.

The mutant described herein may be identical to the rosette (*ro*) mutant described by de Ruiter et al. (2). The mutant description given by these researchers is very general, and thus it is difficult to ascertain whether it is genetically identical to the *scp* mutant. The origin of rosette is different, since it was identified as a segregant in Megurk cross progeny. Traits useful in distinguishing the rosette and super compact mutants include rosette's ability to set fruits after self-pollination indicating that female flowers of rosette are anatomically normal. The availability of rosette seeds would allow for allelism tests to be made. Such seeds may be available since the rosette mutant was used relatively recently by Zijlstra in 1987 (7) for linkage studies in *Cucumis sativus*.

Literature Cited

1. Niemirowicz-Szczytt, K., M. Rucinska, A. Korzeniewska and S. Malepszy. 1995. Cucumber (*Cucumis sativus* L.) induced mutations: A female sterile and an independent long hypocotyl mutant. Cucurbit Genet. Coop. Rpt. 18:13-15.

2. Ruiter, A.C. de, B.J. van der Knapp and R.W. Robinson. 1980. Rosette, a spontaneous cucumber mutant arising from cucumber- muskmelon mentor pollen. Cucurbit Genet. Coop. Rpt. 3:4.

3. Rucinska, M., K. Niemirowicz-Szczytt and A. Korzeniewska. 1991. A cucumber (*Cucumis sativus* L.) mutant with yellow stem and leaf petioles. Cucurbit Genet. Coop. Rpt. 14:8-9.

4. Rucinska, M., K. Niemirowicz-Szczytt and A. Korzeniewska. 1992. Cucumber (*Cucumis sativus* L.) induced mutations. II. A second short petiole mutant. Cucurbit Genet. Coop. Rpt. 15:33-34.
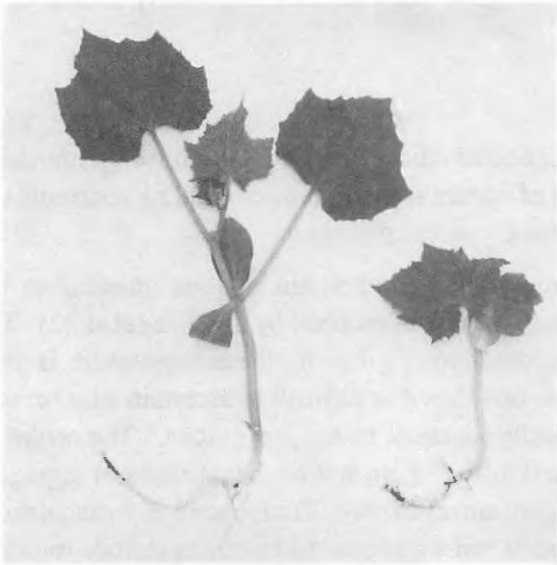
Figure 1. The *super compact* mutant (*spc*; right) compared to wild type seedling (left).

Figure 2. The mature *super compact* mutant plant.
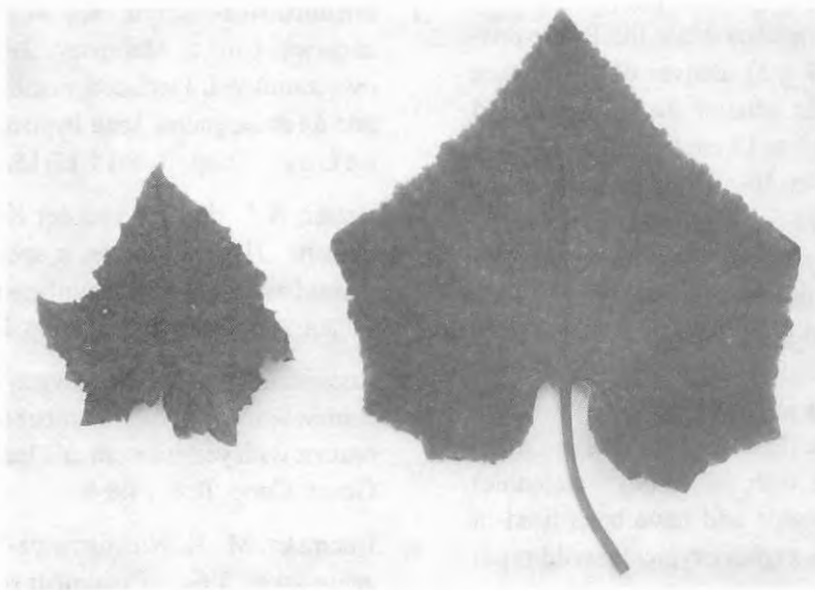
Figure 3. A leave-size comparison of the *super compact* mutant (left) and standard wild type (right).

Table 1. Inheritance of *super compact* (*scp*) gene in cucumber.

Generation	No. observed		No. expected		Ratio tested	x^2	P
	Wild Type	Mutant	Wild Type	Mutant			
P_1 (Normal)	39	0	39	0	1:0	--	
P_2 (Mutant)	0	50	0	50	0:1	--	
F_1	53	0	53	0	1:0	--	
F_2	212	40	189	63	3:1	11.19	0.05
BC P_1	181	0	181	0	1:0	--	
BC P_2	91	87	79	79	1:1	1.00	0.05

5. Rucinska, M., K. Niemirowicz-Szczytt and A. Korzeniewska. 1992. Cucumber induced mutations. III and IV. Divided and gingko leaves. Fifth Eucarpia Cucurbitaceae Symposium, Poland. July 27-31, 1992. pp.66-69.

6. Rucinska, M., K. Niemirowicz-Szczytt and A. Korzeniewska. 1993. Cucumber (*Cucumis sativus* L.) induced mutations: A *Phaseolus* leaf mutant. Cucurbit Genet. Coop. Rpt. 16:14-15.

7. Zijlstra, Z. 1987. Further linkage studies in *Cucumis sativus*. Cucurbit Genet. Coop. Rpt. 10:39.

Cucumber (*Cucumis sativus* L.) Mutants Segregating in M$_2$ Generation After Gamma-Ray Seed and Pollen Irradiation

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S. Iida
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In Japan, the seeds of some hybrid varieties are produced by hand-pollination using monoecious inbred lines. Since technical errors in pollination can cause contamination of self- and/or sib-crossed seeds of female lines used for hybrid production, the purity of individual seed lots must be assessed. Molecular techniques [e.g., restriction fragment length polymorphism (RFLP) of nuclear DNA] are often used in Japan for purity assessments (2). If it were possible to use a recessive morphological marker to assess the purity of female inbred lines, then purity testing could be done more rapidly and economically.

Materials and Methods. Seeds and pollen of inbred line 'T-1' were irradiated with gamma-rays. Line T-1 is monoecious and has been used as a female line to produce a monoecious hybrid. Self- pollinated M$_2$ lines were derived from 100 seed irradiated (100Gy) M$_1$ plants. In addition, 272 M$_2$ lines originating from pollen irradiation were evaluated. These lines were derived from a cross between non-irradiated staminate flowers and the irradiated (20Gy) pistillate flower. The method of pollen irradiation was according to Iida and Amano (1). Forty individuals per one M$_2$ line were examined in order to identify mutant characters.

Results and Discussion. Eleven mutants were observed as a result of seed irradiation and two mutants were observed following pollen irradiation. Characteristics of these mutants are detailed in Table 1. Segregation in the M$_2$ generation suggested that all of these characters were governed by one recessive gene at each locus (Table 2).

Literature Cited

1. Iida, S. and E. Amano. 1990. Pollen irradiation to obtain mutants in monoecious cucumber. Gamma Field Symp. Japan. 29:95- 111.

2. Matsuura, S., A. Saito and Y. Fujita. 1994. An approach for rapid checking of seed purity by RFLP analysis of nuclear DNA in F$_1$ hybrid of cucumber (*Cucumis sativus* L.). J. Japan. Soc. Hort. Sci. 63:379-383.

Table 1. Characteristics of induced mutants.

Mutant	Characteristics
Seed irradiation derived	
Albino-1	White cotyledon. Seedlings die after 6 to 7 days.
Albino-2	White cotyledon. Seedlings die after 10 to 11 days.
Yellow	Yellow cotyledon. Seedlings die before the development of the 2nd true leaf.
Yellow to green	Yellow cotyledon which may revert to normal green. Newly developed true leaves also show some yellow color and may also turn normal green. Seedling growth is as observed in the original inbred line.
Pale green	Pale green stem and fruit. Seedling growth is slower than in the original inbred line, but acquires normal seed fertility.
Round leaf	Round heart shaped leaves. This character is distinguishable after the 5th true leaf stage.
Small leaf	Small leaves. Seedling growth is slightly slower than in the oriiginal inbred line and seed fertility is low.
Mottled leaf	White mottled leaves associated with normal seedling growth. Expression of mottled leaf can be observed after the 10th true leaf stage.
Flat stem	Flat stems. Expression of flat stem can be observed after the 5th true leaf stage. Seedling growth is slower and poor bud development inhibits flowering.
Dwarf-1	Short internode. Seedling growth is slower than in the original inbred line. The leaves, flowers and fruits are normal.
Compact	Reduced size of leaves, flowers, stems, fruits and seeds when compared to the original inbred line. Seed fertility in mutant plants is normal.
Pollen irradiation derived	
Bush	Short internode with thick and vigorous leaves with bush type plant habit.
Dwarf-2	Short internode with small leaves. Seedling growth is slightly slower than that observed in the original inbred line.

Table 2. Segregation ratios in M_2 generation after gamma-ray seed and pollen irradiations.

Irradiation method	Characters segregated in M_2	No. observed Normal	Mutant	Expected ratio	X^2	P-value
Seed (100Gy)	Albino-1	29	11	3:1	0.133	<0.05
	Albino-2	30	10	3:1	0.000	<0.05
	Yellow	33	7	3:1	1.200	<0.05
	Yellow to green	31	9	3:1	0.133	<0.05
	Pale green	34	6	3:1	2.133	<0.05
	Round leaf	35	5	3:1	3.333	<0.05
	Small leaf	34	6	3:1	2.133	<0.05
	Mottled leaf	35	5	3:1	3.333	<0.05
	Flat stem	35	5	3:1	3.333	<0.05
	Dwarf-1	32	8	3:1	0.533	<0.05
	Compact	33	7	3:1	1.200	<0.05
Pollen (20Gy)	Bush	35	5	3:1	3.333	<0.05
	Dwarf-2	32	8	3:1	0.533	<0.05

Lost Information

J. E. Staub

Vegetable Crops Research, USDA/ARS, Department of Horticulture, University of Wisconsin-Madison, WI 53706 U.S.A.

The Proceedings of the international symposium entitled, "Cucurbitaceae '94: Evaluation and enhancement of cucurbit germplasm" was published in 1995. Information was omitted from a report of the proceedings entitled, "Problems associated with map construction and the use of molecular markers in plant improvement" (1). The information omitted was present in tables which described the results of a survey of vegetable breeders asked to comment on their use of molecular markers for map construction. Because I believe that this survey was a relatively accurate description of the feelings of vegetable breeders at that time, it forms a historical record that should be preserved. Thus, I repeat the text of the article below dealing with this survey and present herein the tables that were omitted from the symposium report.

A survey was conducted in which a group of 25 plant breeders and geneticists (U.S. and Europe) who employ molecular markers for map construction were asked to identify the marker types they use, the level of polymorphism they observe and the intended use of these markers (Table 1). Researchers in this sampling were not currently using AFLPs, SCARs, microsatellites or CAPs for map construction and/or board-based genetic analysis. It was clear from their responses that no one marker type currently under study (i.e., isozyme, RAPD, or RFLP) was preferred, and that the level of polymorphism depended on the species and the marker type utilized. While the level of polymorphisms in apple, *Brassica*

spp., maize, pea, potato, radish and soybean are relatively high (20-~100% bands polymorphic) depending on marker type, polymorphisms in chickpea, cucumber, lentil, onion, pepper, and tomato are considerably lower (0-20%). Researchers intend to use the variation observed at the protein and DNA level to select for economically important qualitative (e.g., disease and insect resistance) and quantitative (e.g., yield and quality components) traits. Several problems such as the low levels of polymorphism (all marker types) observed in some crops species, the reproducibility and repeatability of RAPDs (e.g., *Brassica* spp., pepper, melon), complex banding patterns (e.g., RFLPs in onions), the genetics of the organism (e.g., *Brassica* spp., onions), the lack of true breeding lines (e.g., carrot, garlic), and procedural and technical constraints (e.g., automation for large scale screening purposes) may impede the implementation of marker technologies (Table 2).

Literature Cited

1. Staub, J. 1995. Problems associated with map construction and the use of molecular markers in plant improvement. In: Lester, G.E. and J.R. Dunlap, eds. Proceedings Cucurbitaceae '94: Evaluation and enhancement of cucurbit germplasm. p. 86-91.

Table 1. Relative abundance of molecular marker polymorphisms in an array of crop species.

Crop	Polymorphism (%)^z			Mapped marker (no.)			Intended use for marker assisted selection
	Isozyme	RAPD	RFLP	Isozyme	RAPD	RFLP	
Alfalfa		~60	60-75		80	60	Forage yield & quality, disease resistance
Alfalfa			59-75			100 .	Winter survival, yield
Apple	30-60	~100	30-50	~30	>400	10	Fungal resistance, fruit quality
Barley	5-20	10-30	30-50	~30	~200	~500	Disease resistance, cold tolerance
Bean	10-30	25-40	10-30	~10	~200	2	Photoperiod response, fungal resistance, yield, biomass
Bean	10-50	30-50	40-80	11	20	>700	Disease resistance, quality factors, drought resistance
Carrot	20-40	10-30	30-40				Disease resistance
Cauliflower		60-70			15	250	Cytoplasmic male sterility
Chickpea	0-2	2-10	0-2	10	20	10	Ascochyta blight resistance, drought resistance
Cucumber	0-30	5-10	5-10	21	~100	~70	Disease resistance, plant habit, yield components
B. oleracea			46-88			150	Maturity time, yield
B. rapas			62-87			150	Maturity time, yield
B. napus			46-61			150	Maturity time, yield
Garlic	10-30						Clove quality, disease resistance, fertility restoration
Lentil	5-10	10-20	5-10	20	30	30	Ascochyta blight resistance, winter hardiness
Maize	25-50	40-80	40-80	>40		>1000	Grain yield
Melon	0-5			20			
Onion		5-10	10-20		10		Fertility restoration, bulb color & quality
Pea	20-40	50-75	20-50	~30	>600	~30	Virus and fungal resistance, nitrogen fixation
Pea	10-15	10-20	10-20	25	45	35	Virus resistance, drought tolerance
Pepper		~10			40	900	
Pepper	0-15	0-40			30		
Potato	>80	>80	50-60	15	>60	>100	Dry matter, dormancy, sugar levels, yield components
Radish	40	73		2	63		Yellow's & mosaic resistance, bolting
Soybean	~15	~10	20-50	10	12	500	Disease resistance, iron chlorosis, protein & oil content, photoperiod
Tomato		~10			40	1000	Increased shelflife
Tomato	0-5	0-5	10-30			1000	White fly resistance
Tomato	0-25	0-10			100		

RFLP = restriction fragment length polymorphisms, RAPD = random amplified polymorphic DNA.

Table 2. Common problems encountered in molecular marker development in several crop species.

Crop	Marker Class[z]	Problem	Potential solution
Alfalfa	RFLP	Probes with complex patterns	Screen more probes, low copy sequences
Apple	RFLP	Low level of polymorphism	Alternative marker systems
Bean	RFLP	Low level of polymorphism	Screen different libraries
Bean	RAPD	Clustering of mapped markers	Alternative marker systems
Brassica	RFLP	Duplicate loci	Microsatellites
Brassica	RAPD	Repeatability	Alternative marker systems, SCARs
Carrot	Morph	Lack of true breeding lines	Inbreed
Garlic	Morph	Lack of true breeding lines	Development of a true seed system
Celery	RFLP	Low level of polymorphism	Microsatellites
Chickpea	All classes	Low level of polymorphism	Use wild/unadapted accessions
Cucumber	RFLP	Low level of polymorphism	Alternative marker systems
Cucumber	RAPD	Low level of polymorphism	Alternative marker systems, SCARs
Onion	RFLP	Probes with complex patterns	Screen more probes
Onion	RAPD	Heterozygous populations	
Pea	RFLP	Low level of polymorphism	Alternative marker systems
Pepper	RAPD	Repeatability	Alternative marker systems, SCARs
Melon	RAPD	Repeatability	Alternative marker systems, SCARs
Pepper	RAPD	Low level of polymorphism	Screen more primers
Soybean	RFLP	Low level of polymorphism	Alternative marker systems
Tomato	RFLP	DNA isolation/purification	System automation, robotization
Tomato	RAPD	Low level of polymorphism	Screen more primers

[z]RFLP = restriction fragment length polymorphism, RAPD = random amplified polymorphic DNA, morph = morphological marker.

Environment can Affect the Placement of Discrete Traits on Genetic Maps: The Case of Sex Expression in Cucumber

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Introduction. Several genetic maps have been constructed in cucumber using morphological and biochemical markers (3,4). These maps have incorporated discrete, qualitative loci which have lead to a better understanding of the cucumber genome. Environment can affect the expression of some qualitative loci, and therefore environmental effects should be considered when constructing genetic maps where environment is known to effect traits.

Sex expression studies reported by Shifriss (8), Galun (2) and Kubicki (6) showed that these different sex types are determined by three major loci (F, M, and A). The F locus influences the degree of femaleness ($FF>Ff>ff$), while the M locus determines whether flowers are unisexual ($M\_$) or bisexual (mm). The A locus conditions increased male tendency if a plant is homozygous recessive aa and ff. Interactions between these loci yield the basic sex types found in cucumber.

While this three gene model describes the basic regulation of sex types, a plant's phenotype is also influenced by modifying genes and environmental factors. Environmental factors, such as photoperiod, temperature, and irradiance influence sex expression in cucumber (1). We report here the effects of environment on the placement of the F locus on a genetic map in cucumber.

Materials and Methods. The gynoecious determinate cucumber line G421 possessing normal sized leaves was crossed with the monoecious indeterminate little leaf line H-19. The F_1 was subsequently self-pollinated to produce F_2 progeny and 103 F_3 families.

Frozen tissue from 103 F_2 plants were used for isolation using CTAB buffer (CTAB 2%, NaCl 1.4M, EDTA 20 mM, Tris 0.1M and BME 1%). DNA was extracted using phenol:chloroform:isoamyl alcohol (25:24:1). RNAse was used to remove RNA and the sample was chilled at -20 C for 15 minutes and centrifuged to reduce polysaccharides.

PCR reactions for RAPD analysis were performed in according to Staub et al. (9). PCR products were electrophoresed through 1.6% agarose gels with 0.5 g/ml of ethidium bromide in 1x TAE at 90 V for 3.0 h. A total of 1520 random 10-mer [Operon Technologies, Alameda, Calif. (OPA1 - OPZ20 and OPAA1 - OPAY20) and British Columbia University, Vancouver, BC (BC200 - BC700)] primers were used to screen G421 and H-19 for polymorphisms in the DNA level.

Heritable and repeatable polymorphic loci (80) were used to construct an 83-point map containing three morphological traits (little leaf = ll, determinate = de, and F = gynoecy) . Linkage analysis and the order of markers were estimated with MAPMAKER version 2.0 for the Macintosh (7) using a minimum LOD linkage score of 3.0 for statistical acceptance, and minimum recombinant fraction of 0.4. The Kosambi mapping function was used to convert recombination fractions to map distances (5).

One-hundred three F_3 families, their parents, and the F_1 hybrid were evaluated in two locations in the United States (Georgia and Wisconsin). Treatments were arranged in three replications per location, arranged in a randomized complete block design. The experimental plot consisted of single rows spaced 0.7 m x 1.5 m between plants and rows, respectively.

Sex expression was recorded, and data were interpreted to fit a discrete codominant and dominant genetic model. In the case of codominance, phenotypic classes were designated as staminate, pistillate or staminate/pistillate (heterozygous) flowering. In the case of dominance, the all pistillate and staminate/pistillate phenotypes formed one class and the staminate phenotypes formed the other. The fraction of pistillate nodes in the first 10 flower-bearing nodes was used for recording sex expression as a quantitative trait. Data were collected on the first ten plants of each replication. A node was considered female if it had at least one pistillate flower.

Results and Discussion. Linkage analysis of sex expression resulted in the placement of the F locus on Group B (Figure 1). There was good agreement between the classification of marker class phenotypes for some (e.g., Table 1, families #14, #18, and #19) but not all (e.g.,
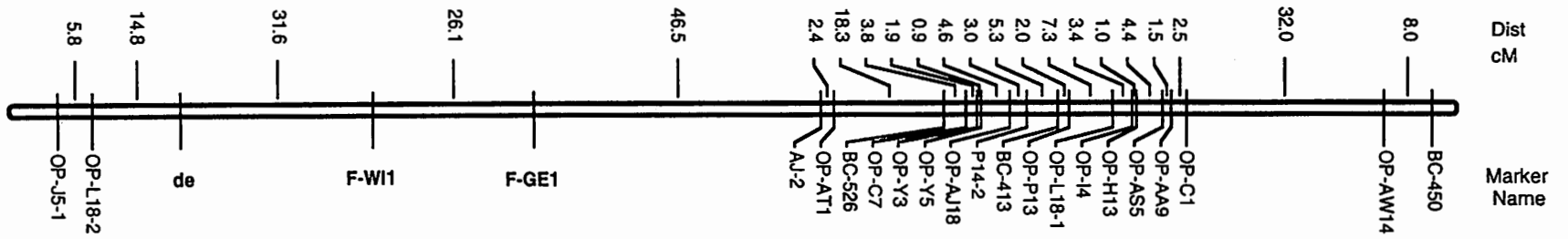
Fig. 1. Cucumber linkage group B constructed from RAPDs in a cross between G421 and H-19.

Table 1. Segregations (dominant and codominant markers) of sex expression in F3 cucumber families derived from an G421 x H-19 mating in two locations used in mapping with MAPMAKER software.

Marker Type	Location	FAMILY						
		13	14	15	16	17	18	19
Codominant	Georgia	B	H	H	H	H	A	A
Codominant	Wisconsin	H	H	B	A	A	A	A
Dominant	Georgia	B	D	D	D	D	D	D
Dominant	Wisconsin	D	D	B	D	D	D	D

B = homozygous for H-19; A = homozygous for G421; H = heterozygous; D = not homozygous for H-19.

Table 1, families #13, #15 - 17) families between locations. That is, families not homozygous for recessive *ff* (*FF* and *Ff*, designated class 'D' in MAPMAKER) for alleles carried by H-19 always corresponded to those families classified as homozygous dominant (*FF* designated 'A' in MAPMAKER) and heterozygous (*Ff* designated 'H' in MAPMAKER) in the codominant model. Likewise, families classified as *ff* (designated as 'B' in MAPMAKER) by the dominant and codominant model always corresponded (Table 1). This tendency is exemplified by families # 13 and # 18. In Georgia these families were classified as H and A for codominant marker, respectively and as D for the dominant marker in the same location.

The relative location of the *F* locus on Group 2 depended upon the segregating plants in the F3 families examined. The classification of sex phenotypes in certain families differed depending upon location. This led to location-dependent placement of the *F* locus on the map. Identification of linkage relationships is dependent upon the proportion of recombinants and parental phenotypes recovered in a segregating population. With a trait like sex expression in cucumber, phenotypes (e.g., gynoecious vs. monoecious) can be classified differently depending upon environmental conditions. Thus, the analysis of a quantitative trait whose expression is recorded by discrete classification of the phenotype and is affected by the environment can lead to bias (i.e., environment specific) during map construction. These biases can be minimized if phenotypes are classified under controlled conditions.

Literature Cited

1. Cantliffe, D.J. 1981. Alteration of sex expression in cucumber due to changes in temperature, light intensity, and photoperiod. J. Amer. Soc. Hort. Sci. 106:133-136.

2. Galun, E. 1961. Study of the inheritance of sex expression in the cucumber. The interaction of a major gene with modifying genetic and non-genetic factors. Genetica 32:134-163.

3. Kennard, W. C., K. Poetter, A. Dijkhuizen, V. Meglic, J.E. Staub, and M.J. Havey. 1994. Linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. Theor. Appl. Genet. 89:42-48.

4. Knerr, L.D. and J.E. Staub. 1992. Inheritance and linkage relationships of isozyme loci in cucumber (*Cucumis sativus* L.). Theor. Appl. Genet. 84:217-224.

5. Kosambi, D.D. 1944. The estimation of map distance from recombination values. Ann. Eugen. 12:172-175.

6. Kubicki, B. 1969. Investigations on sex determination in cucumber (*Cucumis sativus* L.). Genet. Pol. 10:3-143.

7. Lander, E.S. and D. Bostein. 1986. Mapping complex genetic traits in humans: new methods using a complete RFLP linkage map. Cold Spring Harbor Symp. Quant. Biol. 51:49-62.

8. Shifriss, O. 1961. Sex control in cucumbers. J. Hered. 52:5-12.

9. Staub, J. E., J. Bacher and K. Poetter. 1996. Sources of potential errors in the application of random amplified polymorphic DNAs in cucumber. HortScience 31:262-266.

Underestimation of Detection of QTLs When Using Dominant Genetic Markers: The Case of RAPDs in Cucumber

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Introduction. Our laboratory has been interested in using random amplified polymorphic DNAs (RAPDs) as a tool for marker-assisted selection (MAS) in cucumber (*Cucumis sativus* L.). Phenotypic selection based on traits which are conditioned by additive allelic effects can produce economically important changes in breeding populations. MAS provides a potential for increasing selection efficiency by allowing for earlier selection and reducing plant population size used during selection. Selection for multiple loci or quantitative trait loci (QTL) using genetic markers can be effective if a significant association is found between a quantitative trait and markers (1,2,3). Nevertheless, the phenotypic variation that marker loci define is often non-additive, and is a function of genetic linkage, pleiotropy and environment (5). Thus, the efficiency of application of marker loci as predictors of phenotypic variation is dependent upon many factors, and predictions of response to selection (R) are often difficult.

The utility of MAS is further exacerbated when dominant markers are used for characterizing QTLs because of their inability to completely classified all possible phenotypes (i.e., aa, Aa, and AA). In this report, we use sex expression data (see the previous companion article in this issue) and a hypothetical data set in a simulation experiment to provide evidence for the decreased ability of RAPD markers to detect QTLs when compared to codominant markers.

Materials and Methods. There are typically two analytical methods used for the analysis and mapping of QTLs: one-way analysis of variance (ANOVA) and maximum likelihood estimation. While the one-way analysis approach involves least squares estimation and produces an alpha value as a test of significance, maximum likelihood is designed to choose values for variables which maximize a defined function and linkage is tested by the logaritm of the ratio (LOD) of two likelihoods; i.e., the null hypothesis that there is not a QTL at a given site [interval mapping, (4)]. In many analyses a significance level of LOD $\geq$ 3.0 is appropriate as an acceptance level of linkage between two loci. When many loci are considered in QTL mapping, a multilocus analysis involves either maximum likelihood or multi-regression analysis.

Comparison by one-way analysis of variance. Phenotypes in a codominant model are expressed as 1 (AA), 0.5 (Aa), and 0 (aa). In a dominant model phenotypes are expressed as 1 (AA, Aa) and 0 (aa). We have assumed two types of overdominance in our investigations. One type is where the phenotypic value of the heterozygote is higher than either parent and the parents themselves are similar (designated here as type-1), and the other type is where the phenotypic value of one of the parents is higher than the other (designated here as type-2, which may be most realistic) (Table 1).

In the simulations presented here, overdominance models along with the additive and the dominant models are compared to define the efficiency of marker types for their ability to detect QTLs. A one-way ANOVA was performed on a data set which contained hypothetical values for dominant and codominant markers and a trait which was given a range of different types of gene action (Table 1). The mean square error resulting from each ANOVA was used as a sensitivity value for comparisons because it is directly related to the F-test performed in each case. Theoretically, one might expect that while in the case of additive or overdominance, codominant markers would have greater sensitivity in detecting QTLs than dominant markers, the sensitivity of codominant and dominant markers would be similar where the trait was inherited as a dominant.

Comparison by multi-locus analysis. The efficiency of QTL detection was also examined in a multi-locus analysis using backward elimination and forward selection of markers (x_{1-n}). Multi-locus analysis can be used to examine the association between a marker and a QTL, and estimate the contribution which a QTL makes to the expression of a trait's phenotype.

Table 1. Raw data used for simulation to examine the detection of quantitative trait loci (QTL) using dominant and codominant markers by one-way ANOVA, for traits which are conditioned by different types of gene action.

	Trait				Marker	
			Overdominance			
Individual	Additive	Dominance	Type-1	Type-2	Dominant	Codominant
1	0.4	0.4	0.4	0.4	0	0
2	0.5	0.5	0.5	0.5	0	0
3	0.5	0.5	0.5	0.5	0	0
4	0.9	0.9	1.1	1.5	1	0.5
5	1	1	1	1.5	1	0.5
6	1	1	0.9	1.6	1	0.5
7	1	1	1	1.5	1	0.5
8	1	1	1.1	1.5	1	0.5
9	1.1	0.9	0.9	1.6	1	0.5
10	1.5	1	0.5	1	1	1
11	1.5	1	0.5	1	1	1
12	1.6	1	0.6	1	1	1

Table 2. One-way analysis of variance of dominant and codominant markers for traits which are conditioned by different types of gene action.

		Gene Action										
Type of		Additivity			Dominance			Overdominance-1		Overdominance-2		
Marker	df	MS Error	Pr>F	MS Error		Pr>F	MS Error	Pr>F	MS Error		Pr>F
Dominant	10	0.6022	0.0015	0.0222		0.0001	0.4889	0.03	2.0267		0.03
Codominant	9	0.0333	0.0001	0.0200		0.0001	0.0533	0.0001	0.0267		0.0001

Theoretically, the ability of dominant and codominant markers to detect QTLs should differ because of the inability of dominant marker systems to classify all phenotypes. We tested this hypothesis by using a data set containing sex expression phenotypes in F_3 families (see companion article, this issue). Depending upon the grouping of sex phenotypes, the expression of the F locus could be considered codominant (FF, $F\_$, ff) or dominant ($F\_$, ff). We also observed that a random amplified polymorphic DNA (RAPD), OP_AO7, segregated as a codominant marker in this population. Thus, a unique opportunity was provided which allowed for a trait and an independent RAPD marker to be considered as either having codominant or dominant inheritance in any combination in the analysis.

The regression model used attempted to define the predicted dependent variable (y), gynoecious sex expression (conditioned by the F locus) using morphological and marker phenotypes. Two or more x's (e.g., markers) are used to give information about y by means of multiple regression on the x's ($y = Y + b_1x_1 + b_2x_2 + ...b_nx_n$) where b is the regression coefficient (the phenotypic effect associated with each marker) of a given x and Y represents the mean phenotypic distribution of the population). In the case of the analysis we modeled y as $y = Y + b_1x_1 + b_2x_2$ (where x_1 = the morphological marker F, and x_2 = the RAPD marker OP_AO7). This regression model was used in backward elimination and forward selection as follows:

Case 1 $y = x_1$ and x_2 (both codominant)

Case 2 $y = x_1$ and x_2 (both dominant)

Case 3 $y = x_1$ and x_2 (dominant and codominant)

Results and Discussion. It was determined that both through simulation and by the analysis of a data set composed of sex and associated RAPD marker phenotypes that QTL effects are underestimated by dominant molecular markers. This observation was made by comparing the sensitivity of dominant and codominant marker systems.

Comparison by one-way analysis of variance. The mean square error (MSE) was larger in each case of gene action examined for a dominant marker when compared to a codominant marker (Table 2). The increase in MSE in the case of dominant marker systems is due to the inability to distinguish the AA and Aa genotypic classes.

This increase in sensitivity (lower MSEs) in codominant marker systems results in the potential detection of a higher frequency of QTLs when compared to dominant marker systems. Thus, dominant marker systems tend provide a conservative estimate of the number of QTLs that affect a trait's expression. Because of this fact, the misidentification of spurious associations between dominant markers and QTLs is decreased. Moreover, the QTLs detected when individual dominant marker probability values ($P < 0.01$) are combined in the analyses ($P < 0.05$) result in accurate, but conservative, reflections of reality.

Comparison by multi-locus analysis. The results of multi-locus regression analysis was as follows ($x_1 = F$ and $x_2 = $ OP_AO7):

Forward selection	Marker	R2	P > F
Case 1 Both codominant	1	0.621	0.0001
	2	0.015	0.0509
Case 2 Both dominant	1	0.289	0.0001
	2	0.027	0.0591
Case 3 Codominant & dominant	1	0.621	0.0001
Backward elimination			
Case 1 Both codominant	1&2	0.637*	0.0001
			0.0509
Case 2 Both dominant	1&2	0.637	0.0001
			0.0509
Case 3 Codominant & dominant	1	Kept codominant marker	
	2	Eliminated dominant marker	

* combined R^2 for both equations.

From these analyses, two conclusions may be drawn: 1) forward selection underestimates the contribution of dominant markers (case 2 above) to the phenotypic variance; and 2) in a combination codominant-dominant marker (case 3 above), the dominant marker tends to be eliminated from the model (both in forward selection and backward elimination). Markers with small effects tend to be eliminated from the model, thus supporting the contention of the conservative nature of dominant markers for the detection of QTLs.

Literature Cited

1. Edwards, M and L. Johnson. 1994. RFLPs for rapid recurrent selection. Proc. Symposium on Analysis of Molecular Marker Data, p. 33-40. August, 1994. Corvallis, Oregon.

2. Edwards, M.D. and N.J. Page. 1994. Evaluation of marker- assisted selection through computer simulation. Theor. Appl. Genet. 88:376-382.

3. Lande, R. and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743-756.

4. Lander, E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

5. Lark, K.G., K. Chase, F. Adler, L.M. Mansur, and J.H. Orf. 1995. Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele. Proc. Natl. Acad. Sci. 92:4656-4660.

Once-over Harvest Yield of Cucumber Hybrids Made with a Determinate Parent

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Cucumber (*Cucumis sativus* L.) plant architecture offers new ways of improving cultivar performance in once-over harvest. Besides the indeterminate (normal) type, two plant types that have been used successfully in cucumber breeding are the determinate (*dede* genotype) and the small leaf, multiple branched type originating from LJ 90430, an accession from India belonging to *C. sativus* var. *hardwickii* (R.) Alef. Although *C. s.* var. *hardwickii* has small, bitter fruits with little known horticultural value, the multiple branching, multiple fruiting habit is interesting to plant breeders (Horst and Lower, 1978). In previous plant breeding work, our experience has been that a determinate male parent can improve the yield of the hybrid significantly. Since the determinate trait is recessive, the positive effect on the hybrid may be due to other genes that just happened to be in the determinate inbreds being used, or to additive effects of the *de* allele (the *Dede* heterozygote having some of the useful traits of the *dede* homozygote). The research of Lower and Den Nijs (1979) on lines isogenic for determinate (*DeDe* and *dede*) and compact (*CpCp* and *cpcp*) offers some insight to the heterotic effects of determinate type in hybrids heterozygous at the *de* locus.

The determinate trait is controlled by a single, recessive gene named *de* (Denna, 1971; George, 1970; Hutchins, 1940; Odland and Groff, 1963; Robinson et al., 1976). There are modifier genes involved with the determinate type, and early flowering causes greater expression of the dwarf, determinate character than late flowering. Plants homozygous recessive for *de* have short vines because the apical meristem changes into floral buds (Hutchins, 1940). Originally, breeders used the determinate plant type hoping that the smaller vines would improve yield by providing a higher optimum planting density than for the normal type. However, in a two year, multiple harvest trial with 3 planting densities (24, 36, and 72 thousand plants/ha (TPH)), both determinate 'Spacemaster' and indeterminate 'Pacer' had an optimum density above 72 TPH for yield (Munger et al., 1982). Optimum density of pickling cucumbers tested at 4 planting densities

(65, 129, 258, and 516) for yield ($/ha) in multiple-harvest trials was around 129 TPH for the two determinate cultivars, and 258 TPH for the two indeterminate cultivars (Wehner and Miller, 1987). Therefore, the optimum density for the determinate plant type was not higher than for the indeterminate plant type.

A major advantage of *C. s.* var. *hardwickii* has been its high combining ability for fruit number in crosses with indeterminate *C. sativus* inbreds (Kupper and Staub, 1988). Unfortunately, determinate inbreds crossed to LJ 90430 produce an unexpected F_2 progeny, with the determinate, multiple-branched combination missing or deficient in number (Delaney and Lower, 1984, 1985). Thus, it may be difficult to develop inbreds with both the determinate and the multiple-branched habit.

The objective of this study was to compare cucumber hybrids involving a determinate male parent crossed with indeterminate, determinate, or *C. s.* var. *hardwickii* female parents for yield in once-over harvest. Additionally, we were interested in measuring the effect of planting density, and whether the hybrids had different optimum densities for maximum yield.

Methods. The experiment was conducted at the Agricultural Research Station near Hancock, Wisconsin. Recommended cultural practices were used, including raised beds and overhead irrigation. The experiment was a randomized complete block design with 3 replications. Treatments were 3 hybrids (indeterminate, determinate, and *C. s.* var. *hardwickii* - all crossed with a common determinate) and 4 densities (26, 52, 108, and 215 TPH).

Plots were 3 rows wide and 3.1 m long, with 1.5 m alleys separating their ends. Rows were 0.77 m apart center to center. The center row was harvested once-over when 'Calypso' check plots had 10 % oversized (50 mm diameter) fruits. There were adequate staminate flowers and bees available for pollination. No pollination problems (excessive crooked or nubbin fruits) were observed.

Plots were seeded on 12 June 1986 with 2 seeds per hill. Plots were thinned to the correct stand two weeks later, and all plots (center row only) harvested on 5 August. Data were collected on number of plants, total fruits, cull fruits, and oversized fruits per plot. From those data, we calculated total yield (total fruits per hectare), number of fruits per plant (total fruits per plot / total plants per plot), marketable yield (total minus cull fruits/ha), early yield (oversized fruits/ha), and percentage culls (culls out of total).

Analysis of variance was performed on plot data and regression was used to determine optimum density for maximum yield.

Results. We were most interested in marketable yield, although the conclusions would have been the same for total yield, since % culls was not a significant effect over density or plant type. Total and marketable yield were highly correlated, and were maximized at the highest density tested (215 TPH) for the hybrids made with the determinate or *C. s.* var. *hardwickii* female parent (Table 1). For the hybrid made with the indeterminate female parent, total and marketable yield were maximized at 108 TPH.

Early yield was maximized at 108 TPH for the hybrids made with indeterminate and determinate female parents, and at 52 TPH for the hybrid made with the *C. s.* var. *hardwickii* female parent. The hybrid made with *C. s.* var. *hardwickii* was later than the other two hybrids, reflecting the late flowering habit of the *C. s.* var. *hardwickii* female parent. Growers willing to wait one or two weeks longer for harvest may be able to obtain significantly higher yields, especially with *C. s.* var. *hardwickii* plant types.

As expected, fruits per plant decreased as plant density increased (Table 1). However, the drop in number of fruits per plant (2.4 to 3.4 at 26 TPH to 0.9 to 1.2 at 215 TPH) was more than compensated by the increase in plants per hectare.

The determinate x determinate hybrid had a high total and marketable yield, but the *C. s.* var. *hardwickii* x determinate hybrid was slightly higher. Although the differences among plant types were not significant, this test does demonstrate the value of determinate and *C. s.* var. *hardwickii* inbreds for producing elite hybrids for use by the processing industry in once-over harvest.

Conclusions. Marketable yield was maximized at about 215 TPH for the determinate x determinate and *C. s.* var. *hardwickii* hybrids, and at 108 TPH for the determinate x indeterminate hybrid. Maximum yield occurred at the highest or second highest plant density despite the fact that the number of fruits per plant dropped to their lowest values. The *C. s.* var. *hardwickii* plant type had significantly lower early yield than the other two plant types, but total yield was the highest of the three types. Since *C. s.* var. *hardwickii* does require more time to reach maturity, growers must be willing to wait for the fruits to reach proper size.

This experiment involved only one hybrid of each plant type tested at a single location and year, so caution must be used in interpreting these results. However, determinate and *C. s.* var. *hardwickii* plant types appear to offer promise over the indeterminate type as parents for hybrid production using high yielding determinate male parents.

Literature Cited

1. Delaney, D.E. and R.L. Lower. 1984. Effects of the determinate locus on number of lateral branches in crosses between four cucumber lines and *Cucumis sativus* var. *hardwickii*. Cucurbit Genet. Coop. Rpt. 7:3-5.

2. Delaney, D.E. and R.L. Lower. 1985. Segregation of the determinate (*de*) allele in crosses between *Cucumis sativus* L. and *C. sativus* var. *hardwickii* R. (Alef.). Cucurbit Genet. Coop. Rpt. 8:2-3.

3. Denna, D.W. 1971. Expression of determinate habit in cucumbers (*Cucumis sativus* L.). J. Amer. Soc. Hort. Sci. 96:277-279.

4. George, W.L. 1970. Genetic and environmental modification of determinate plant habit in cucumbers. J. Amer. Hort. Sci. 95:583-586.

5. Horst, E.K. and R.L. Lower. 1978. *Cucumis hardwickii*: a source of germplasm for the cucumber breeder. Cucurbit Genet. Coop. Rpt. 1:5.

6. Hutchins, A.E. 1940. Inheritance in the cucumber. J. Agric. Res. 60:117-128.

7. Kupper, R.S. and J.E. Staub. 1988. Combining ability between lines of *Cucumis sativus* L. and *Cucumis sativus* var. *hardwickii* (R.) Alef. Euphytica 38:197-210.

Table 1. Fruit production (total yield, total fruits per plant, marketable yield, early yield, and percentage culls) from hybrids made with an indeterminate, determinate, or *C. s.* var. *hardwickii* female parent crossed with a determinate male parent.[z]

Female parent Plant type	Plant density	Total yield	Fruits /plant	Mark. yield	Early yield	% culls
Indeterminate	26	87.5	3.4	84.6	7.2	3.3
	52	149.2	2.9	134.9	11.5	10.2
	108	202.3	1.9	180.8	12.9	11.1
	215	193.7	0.9	173.6	11.5	11.3
Determinate	26	83.2	3.2	78.9	4.3	4.6
	52	116.2	2.3	110.5	11.5	4.3
	108	202.3	1.9	183.6	15.8	9.4
	215	246.7	1.1	225.2	7.2	9.1
Hardwickii	26	61.7	2.4	58.8	0.0	5.3
	52	91.8	1.8	86.1	5.7	6.5
	108	203.7	1.9	189.4	1.4	6.9
	215	262.5	1.2	249.6	0.0	4.7
Mean		158.4	2.1	146.3	7.4	7.2
CV (%)		27	26	28	78	70
F ratio: Type		NS	NS	NS	**	NS
F ratio: Density		**	**	**	NS	NS
F ratio: TxD		NS	NS	NS	NS	NS

Correlation (total vs. fruits/plant) r = -0.41*
Correlation (total vs. marketable) r = 0.99**
Correlation (total vs. early) r = 0.15[NS]
Correlation (total vs. % culls) r = 0.1[NS]

[z] Data are means of 3 replications of once-over harvest from the center row of a 3-row plot.
   Density, total, marketable, and early yield are in thousands per hectare.
*,**,[NS] Indicates significant at the 5% or 1% level, or not significant, respectively.

8. Lower, R.L. and T.P.M. den Nijs. 1979. Effect of plant type genes on growth and sex-expression of pickling cucumber. HortScience 14:435.

9. Munger, H.M., R. Washek and R.W. Riker. 1982. Responses to spacing of Spacemaster cucumber. Veg. Improv. Newsl. 24:3-4.

10. Odland, M.L. and D.W. Groff. 1963. Linkage of vine type and geotropic response with sex forms in cucumbers *Cucumis sativus* L. Proc. Amer. Soc. Hort. Sci. 82:358-369.

11. Robinson, R.W., H.M. Munger, T.W. Whitaker and G.W. Bohn. 1976. Genes of the Cucurbitaceae. HortScience 11:554-568.

12. Wehner, T.C. and C.H. Miller. 1987. Optimum plant density for multiple-harvest yield of determinate and indeterminate cucumbers. Cucurbit Genet. Coop. Rpt. 10:29-30.

Correlation between Leaf Size and Horticultural Characteristics in Cucumber

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The little-leaf character was discovered in an inbred selection in Arkansas (1), and it was determined that this characteristic was controlled by single recessive gene at locus *ll* (3). Little-leaf (*ll ll*) is an interesting character which has been observed in cucumber. There are no Japanese greenhouse cultivars with high fruiting ability and small leaves (2). Little-leaf genotypes are horticulturally interesting (especially in greenhouse cultivation) and we have decided to introduce the little-leaf character into our breeding materials. Prior to introgression of this trait, horticultural characteristics of the little-leaf genotype were investigated. It was determined that leaf size is correlated with high number of fruits under greenhouse cultivation in Japan (2). Therefore, we felt it important to clarify the genetic and/or physiologic relationships between leaf size and other horticultural characteristics; mainly fruiting ability.

Materials and Methods. 'Sakata' cucumber, the local pickling variety grown in northern Japan, was used as the donor of the little-leaf character. Although 'Sakata' is the smallest cucumber genotype in our genetic stocks, we were not sure that 'Sakata' contained the *ll* gene. 'Sakata' was crossed to line 'PA-1' and 'NB-1', independently, and F_1 plants were self- pollinated to produce F_2 progeny for segregation analyses. 'PA- 1' is gynoecious and possesses large leaves, and gynoecious 'NB-1' is an intermediate-size leaf genotype. Some morphological characteristics of the three parental lines and two derived F_1 progeny are listed in Table 1. One hundred and forty individuals per population were tested in the greenhouse at Utsunomiya, Japan in the summer of 1995. Leaf lengths and areas of the hypocotyl, cotyledon, true leaf, petiole and internodes of the main and lateral stems were measured. Since plants were segregated for sex type the number of pistillate flowers used was artificially controlled. Pistillate nodes were removed by pinching. The main stem was terminated at the 17th node by pinching. The first three lateral branches which appeared at the first three nodes of the main stem were removed. Fourteen primary lateral branches which appeared in the 4th to 17th nodes of the main stem were removed at the second node. Secondary branches were removed as they appeared. All pistillate flowers which formed on the main stem and the second node of the primary lateral branches were removed before flowering. The number of fruits on 14 nodes of the first node of the first lateral branch were counted as a measure of fruiting potential. Since there were no insects as pollinators in this greenhouse, fruits were parthenocarpic.

Results and Discussion. In segregating populations derived from 'PA-1' x 'Sakata', the true leaf area was significantly correlated with the petiole length (0.422^{**}), the internodes of main stem (0.463^{**}), and lateral branches (0.397^{**}). On the other hand, leaf area was correlated with nursery stage characteristics and fruiting potential (Table 2). In segregating populations derived from 'NB-1' x 'Sakata' true leaf area was significantly correlated with the petiole length (0.603^{**}), but not correlated with the other length and fruiting potential characteristics examined (Table 3).

These data suggested that leaf size is correlated with some length characteristics, but not correlated with fruiting potential under the Japanese greenhouse cultivation used in this experiment. Some little-leaf segregants with normal internode length and high fruiting ability were observed and selected. These segregants may be useful germplasm as a new plant type in Japanese breeding programs.

Literature Cited

1. Goode, M.J., J.L. Bowers and A. Bassi, Jr. 1980. Little-leaf, a new kind of pickling cucumber plant. Arkansas Farm Res. 29:4.

2. Matsuura, S. and Y. Fujita. 1995. Correlation between agronomical characters of cucumber cultivars grown in Japan. J. Soc. Hort. Sci. 64:305-313.

3. Wehner, T.C., J.E. Staub and C.E. Peterson. 1987. Inheritance of little-leaf and multi-branched plant type in cucumber. Cucurbit Genet. Coop. Rpt. 10:33-34.

Table 1. Morphological characteristics of three parental cucumber inbreds and two derived F_1 hybrids.

Name	Hypocotyl length (cm)	Leaf area(cm^2)		Petiole length (cm)	Internode length (cm)		Number of fruit
		Cotyledon	True leaf		Main stem	Lateral stem	
PA-1	5.8	27.2	766.9	28.5	7.3	21.4	12.3
NB-1	4.0	19.6	548.1	25.9	7.2	8.1	10.5
Sakata	5.7	17.2	363.7	20.3	8.2	15.9	0.0
F_1(PA-1 x Sakata)	6.0	29.4	711.1	25.5	9.1	17.5	2.8
F_1(NB-1 x Sakata)	5.9	20.7	565.5	27.9	9.0	13.8	2.7

Table 2. Correlations among six morphological characteristics and number of fruit in F_2 population derived from 'PA-1' x 'Sakata'.

Characters	1)	2)	3)	4)	5)	6)	7)
1) Hypocotyl length	1						
2) Leaf area (cotyledon)	.185*	1					
3) Leaf area(true leaf)	.123	.194*	1				
4) Petiole length	.109	.147	.422**	1			
5) Internode length (main stem)	.163*	.077	.463**	.181*	1		
6) Internode length(lateral stem)	.084	.157	.397**	.084	.469**	1	
7) No. of fruit	.162*	.229**	.222*	-.129	.300**	.293*	1

*, **significant at 5.0, 1.0% level, respectively.

Table 3. Correlation among six morphological characteristics and number of fruit in F_2 population derived from 'NB-1' x 'Sakata'.

Characters	1)	2)	3)	4)	5)	6)	7)
1) Hypocotyl length	1						
2) Leaf area (cotyledon)	.124	1					
3) Leaf area(true leaf)	.025	.258**	1				
4) Petiole length	-.012	.205*	.603**	1			
5) Internode length (main stem)	.301**	.237**	.237**	-.143	1		
6) Internode length(lateral stem)	.225**	.121	.023	-.124	.421**	1	
7) No. of fruit	-.098	-.084	.049	.024	.062	.117	1

*, **significant at 5.0, 1.0% level, respectively.

Effects of Calcium on Cucumber (*Cucumis sativus* L.) Seed Germination, Seed Storage and Seedling Growth

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Introduction. Calcium is not only an macroelement, but one of the major regulators of plant metabolism (2,3). It has been reported that soaking peanut seeds with 30 mmol/l of Ca^{2+} increases the germination percentage and vigor of maize seeds (1). The optimum Ca^{2+} concentration in these experiments was 10 mmol/l. We report here the effects of Ca^{2+} on cucumber seed germination, storage and seedling growth.

Material and Methods. Four concentrations of $CaCl_2$, (5 to 40 mmol/l), were used to coat cucumber seeds of 'Jing 4-3-1'. Seed receiving no such treatment were used as the control. The germination percentage, rate and index of coated seeds and artificially aged coated seeds were evaluated in a temperature cabinet at 25 C. Artificial aging of seed was accomplished by seed treatment in a closed container held at 42 C and a relative humidity of near 100%. Measurements were taken from seedlings grown in vermiculite medium and ambient light, temperature and humidity.

Results and Discussion. A Ca^{2+} concentration of 10mmol/l was optimal for seed germination. Higher (40mmol/l) and lower (5mmol/l) Ca^{2+} concentrations did not promote germination. The germination percentage, germination rate and germination index of seeds coated with 10mmol/l Ca^{2+} were 9.4%, 10.9% and 6.6% higher, respectively, than control. The difference in germination percentage between treatment and control was statistically significant. A germination index of 5mmol/l Ca^{2+} was significantly lower than that of control (Table 1). All coated seed treatments (5-40 mmol/l) Ca^{2+} were more tolerant to storage than that of control

(Table 2). The germination percentage, germination rate, and germination index of artificially aged coated seeds was 43.9-55.3%, 41.7-57.5% and 24.8-44.1% higher, respectively, than control. Significant differences between the treatments and control were recorded for germination percentage and germination rate. Although treatments were not significantly different, germination decreased as Ca^{2+} concentration was increased. Significant differences among treatments and between any treatment and the control were not detected for seedling height, leaf area and seedling index. The stem width of seedlings derived from seeds coated with 5 and 10 mmol/l Ca2+ were 16.7% and 3.3% larger, respectively, than control. The fresh weight of seedlings derived from seeds treated with these Ca^{2+} concentrations were 11.3% and 9.6% lower than control (Table 3).

In summary, Ca^{2+} seedcoat treatment improved seed storage. The optimal Ca^{2+} concentration for cucumber seedcoat treatment is between 5 to 20 mmol/l.

Literature Cited

1. Donglin, L. 1995. Application and effect analysis of soybean seedcoat. Modern Agriculture 6:23.

2. Xizhang, S. and Changming, L. 1990. A new type of seedcoat. Seed World 9:31.

3. Songquan, S. 1991. Regulating effects of calcium on seed germination. Seed 5:34.

Table 1. Influences of Ca^{2+} seedcoat treatment of cucumber seed on germination.

Ca^{2+} concentration (mmol/L)	Germination percentage (%)	Germination power (%)	Germination index
0 (Control)	78.0 bAB	73.3a	17.38
5	77.3 bB	69.3 a	14.41cB
10	85.3 aA	81.3 a	18.53 aA
20	82.7 abAB	78.0 a	16.38 bAB
40	81.3 abAB	71.3 a	16.18 bAB

Table 2. Germination of artificially aged Ca^{2+} coated seeds of cucumber.

Ca^{2+} concentration (mmol/L)	Germination percentage (%)	Germination power (%)	Germination index
0 (Control)	37.8	36.7 bB	8.12 a
5	58.9 aA	57.8 aA	11.70 a
10	58.7 aA	55.6 aA	10.52 a
20	54.4 aA	52.0 aA	10.13 a
40	56.7 aA	54.4 aA	10.42 a

Table 3. Effects of Ca^{2+} seedcoating of cucumber seed on seedling growth.

Ca^{2+} conc. (mmol/L)	Height (cm)	Stem width (cm)	Fresh weight (g)	Leaf area (cm^2)	Seedling index
CK	5.4 a	0.300 bB	2.287 aA	23.2 a	1.327 a
5	5.9 a	0.3465 aA	2.030 bA	24.6 a	1.087 a
10	5.7 a	0.310 bB	2.070 bA	23.2 a	1.077 a
20	5.7 a	0.300 bB	2.270 aA	23.0 a	1.337 a
0	5.2 a	0.300 bB	2.133 abA	23.3 a	1.237 a

Variation in Free Proline Content of Cucumber *(Cucumis sets* L.) Seedlings under Low Temperature Stress

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Introduction. Recently a greenhouse industry has rapidly emerged in some areas of China. Grafting cucumber seedling onto the rootstock of *Cucurbita ficifolia* is an effective way of obtaining high yields from greenhouse cucumber varieties during the winter and early spring. Studies relating to the characterization of chilling tolerance in cucumber *(Cucumis sativus* L.) are important. Elucidation of the biochemical mechanism of graft facilitated chilling tolerance in cucumber might lead to improved environmental management technologies.

Materials and Methods. The seedlings of 'Shangdong Thom' (CS) cucumber were grafted onto *Cucurbita ficifolia* (GS) and were then evaluated under artificially-induced low temperature stress (0 ± 1 C, 3 ± 1 C and 6 ± 1 C). The content of free proline (CFP) in the leaves of seedlings under temperature stress were examined at 12h, 24h, 48h and 72h after stress initiation. Experimental treatments were arranged in a randomized complete block design with 3 replications.

Result and Analysis. During the low temperature stress, the CFP in CS and GS was similar under different low temperature stress conditions (Figure 1). The CFP increased during the exposure period. This increase in CFP varied among treatment temperatures. After 72h at 0 ± 1 C, CFP of CS plants attained 1209.2 µg/g DW, rising from an initial value of 25.3 µg/g DW (i.e., a 46.7 fold increase), while the CFP of GS plants attained 830.7 µg/g DW from a base CFP of 25.3 µg/g DW (i.e., a 31.8 fold increase). During the same exposure period, the CFP of CS plants held at 3 ± 1 C increased by 14.8 fold, and plants at 6 ± 1 C increased 4.5 fold. The CFP of GS plants held at 3 ± 1 C increased 9.1 fold and plants held at 6 ± 1 C increased 5.9 fold. Plants held at 0 ± 1 C experienced an increase in the range of CFP at 12h and 24h regardless of genotype. Similar results occurred at 12h for CS and GS when plants were held at 3 ± 1 C. However, a second increase in CFP occurred after 48h in CS plants, and CFP increased slightly after 24h in GS plants. There was a slight increase in the CFP of CS and GS plants held at 6 ± 1 C.

The expression of chilling damage occurred later in GS than in CS plants. The degree of damage observed in GS plants was slightly less than that in CS plants under different temperature stress regimes. After assessing the relationship between CFP content and chilling damage, it might be hypothesized that the observed increase in CFP during increasing stress exposure is an indication of cucumber's adaptation to the low temperature. In most instances higher CFP values in cucumber resulted in higher tolerance to low temperatures. The second rapid increase period of CFP is associated with the expression index of chilling damage in cucumber.

Literature Cited

1. Tang, Z.C. 1984. Accumulation of proline in stressed plants and its possible significance. Plant Physiology Communications 1:15- 21.

2. Handa, S. et al. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant. Physiol. 80:938- 945.

3. Carpita, N.C. 1986. Incorporation of proline and aromatic amino acids into cell walls of maize coleoptiles. Plant. Physiol. 80:660-666.

4. Stewart, C.R. et al. 1986. The effects of benzyladenine, cycloheximide, and cordycepin on wilting-induced abscisic acid and proline accumulations and abscisic acid- and salt-induced proline accumulation in barley. Plant. Physiol. 82:703-707.

5. Paleg, L.G. et al. 1984. Proline and glycine betaine influence protein solvation. Plant. Physiol. 75:974-978.

Content of free
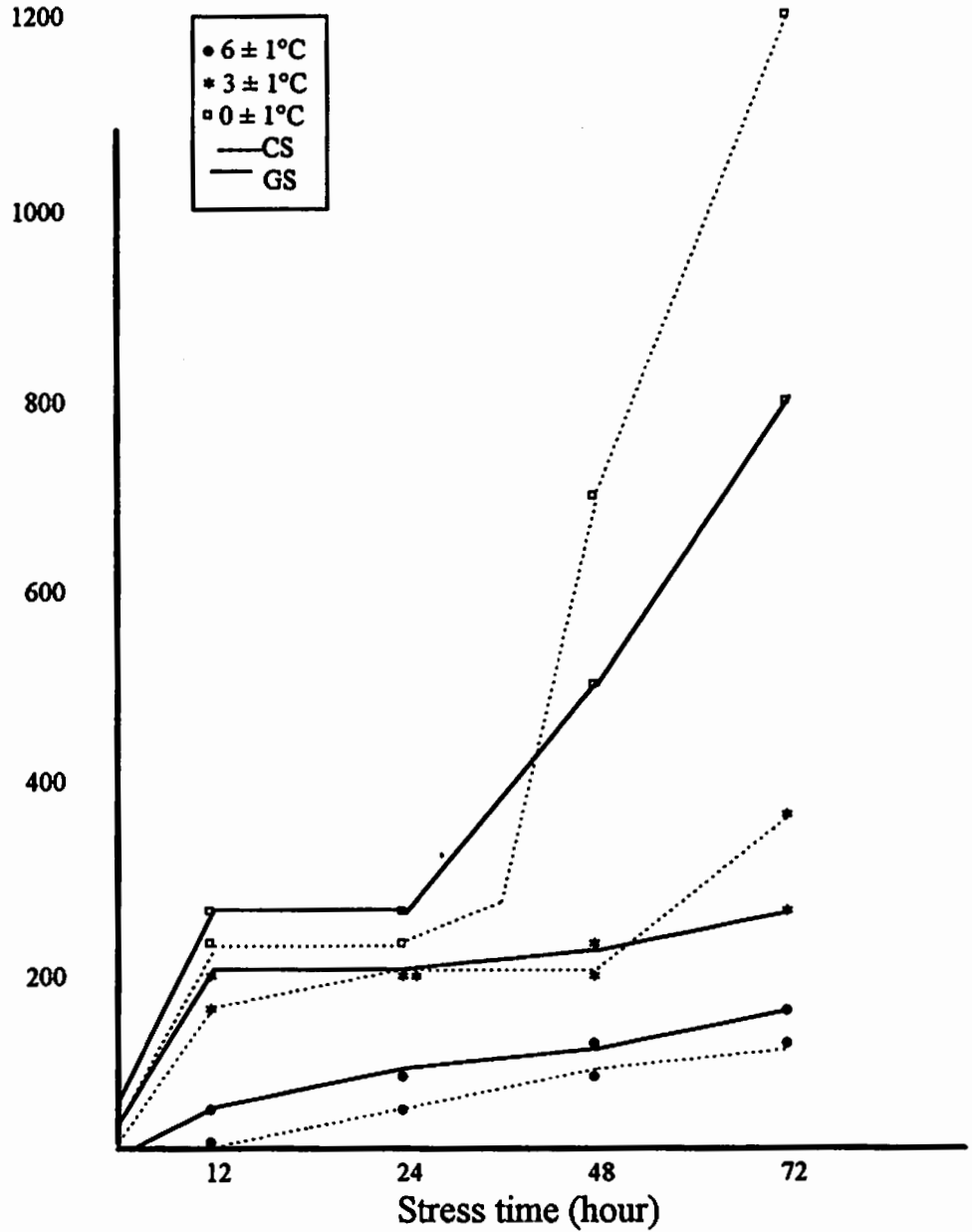proline
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Fig. 1. Variation in free proline content of *Cucumis sativus*
L. seedling leaves subjected to differing temperatures and
exposure times.

The Evaluation of Cold Tolerance in Chinese Cucumber Varieties

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Introduction. The northern part of China is in the temperate growing zone. The cold monsoons of the Northwest are responsible for a longer cold season in China than in other countries of similar latitude. As a result, vegetable production is unfavorable during this season. However, microclimates can be established within greenhouses constructed of plastic, which allow for favorable vegetable production at this time of year. This important horticultural practice is used for the breeding and production of cucumber. This paper examines heat sources used in such greenhouses and their efficiency of operation.

Materials and Methods. Seven cucumber varieties (BG3, BG5, ED6, ED10, FC15, FC17 and ME18) from the breeding program at Northwestern Agricultural University, P.R. China, were evaluated. DH23 cucumber was used as a check variety in the field plot experiments described herein. Experiments were designed such that varieties were arranged in a randomized complete block design with 3 replications, and plant-two-rows in plastic greenhouses from February to June, 1995. Thirty plants were examined in each replication on $4.4\,m^2$ centers. Air temperature was sampled at a height of $1.5m$ at the center of the greenhouse and at a given point $1.5m$ height outside the greenhouse. The air temperature sampling times were at 8:00, 14:00, and 20:00 HRS., Peking time. The variables (X_N) recorded were: $X = \{X_1, X_2, X_3\}$; where X = sum for all cucumber varieties examined, X_1 = total fruit yield, X_2 = cumulative temperature 10 C from February to June 1995 inside plastic greenhouse, and X_3 = disease resistance of the cucumber varieties (% of undamaged control). The matrix (A) of coefficients of the variables assessed was: $A = [0.5, 0.3, 0.2]$; where A = matrix of coefficients of the variables assessed; 0.5 = coefficient of X_1, 0.3 = coefficient of X_2, and 0.2 coefficient of X_3. Fruit grades (Y_N) of varieties were designated as: $Y = \{Y_1, Y_2, Y_3\}$; Y = sum for all cucumber grades assessed, Y_1 = grades of unimproved (acceptable) varieties examined, Y_2 = grades of improved varieties examined, and Y_3 = grades of inferior varieties examined.

The operational equation used for X_1 and X_2 was:

$$u(X_1, X_2) = \begin{cases} 1 & x_{1,2} \geq a_1 \\ \dfrac{X_i - a_2}{a_1 - a_2} & a_1 \geq x_{1,2} \geq a_2 \\ 0 & a_2 \geq x_{1,2} \end{cases}$$

where $u(x_1, x_2)$ = the functional value of the x_1 and x_2, a_1 = the lower limit of the fruit grades of acceptable varieties, and a_2 = the upper limit of the fruit grades of inferior varieties. The operational equation used for disease resistance of the cucumber varieties was:

$$u(x_3) = \frac{X_i - X_{min}}{X_{max} - X_{min}}$$

where $u(X_3)$ = the functional value of X_3, X_i = observation date, X_{max} = the maximal value of the observation date, and X_{min} = minimal value of the observation date.

Results and Discussion. *Air Temperature.* The daily mean air temperature and the cumulative temperature of 10 C were higher inside than outside the plastic greenhouse. The monthly mean air temperature in March was 6.9 C higher inside the greenhouse than outside. The monthly mean air temperature in April was 5.5 C higher inside than outside the greenhouse. The cumulative temperature 10 C in March was 18.6 C higher inside the greenhouse than outside the greenhouse in April (Table 1).

Resistance to Cold Stress. A table of differential criterion was constructed from the observational data and experiences of horticultural experts (Table 2).

Grade Evaluation. Values obtained from equations 1 and 2 allowed for the computation of functional values for total yield of cucumber in each experiment, cumulative temperature 10C, disease resistance ratings, and the functional values of grade matrixes. The results are as follows:

Table 1. A comparison of air temperature and cumulative temperature of ≥10C inside and outside plastic greenhouse.

Month	Mean air temperature		Cumulative temperature of ≥10C	
	Inside	Outside	Inside	Outside
March	14.6	7.7	426.6	0
April	19.1	13.6	574.3	408.0
May 1-7	21.5	16.5	150.3	115.1

Table 2. Differential criterion used for classification of cucumber varieties.

Factors	Acceptable varieties (Y_1)	Improved varieties (Y_2)	Inferior varieties (Y_3)
Total output (X_1)	≥0.90	0.70-0.89	<0.70
≥10C (X_2)[1]	≥0.90	0.75-0.89	<0.75
Disease resistance	≥0.95	0.80-0.94	<0.80

[1]Cumulative temperature.

Table 3. Matrixes used in the calculation of fruit grade evaluation of the cucumber varieties examined.

Code name of cucumber varieties	Matrixes	Fruit Grade Components			Results of Evaluation
		Y^1	Y^2	Y^3	
BG3	$B_3 =$	0.6250	0.3750	0	good seed
BG5	$B_5 =$	0.2640	0.4412	0.2941	intermediate seed
ED6	$B_6 =$	0.3809	0.5714	0.477	intermediate seed
ED10	$B_{10} =$	0.2000	0.3000	0.5000	bad seed
FC15	$B_{15} =$	0.6250	0.3750	0	good seed
FC17	$B_{17} =$	0.2124	0.2567	0.5310	bad seed
ME18	$B_{18} =$	0.29140	0.4412	0.2647	intermediate seed
DH23(CK)	$B_{23} =$	0.4414	0.2627	0.2941	good seed

$$BG3 \ R_3 = \begin{Bmatrix} Y_1 & Y_2 & Y_3 \\ 1 & 0 & 0 \\ 0.6667 & 0.3333 & 0 \\ 1 & 0 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$ED10 \ R_{10} = \begin{Bmatrix} y_1 & y_2 & y_3 \\ 0 & 0 & 1 \\ 0 & 0.3333 & 0.6667 \\ 0.3333 & 0.6667 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$BG5 \ R_5 = \begin{Bmatrix} 0 & 0.6667 & 0.3333 \\ 0 & 0.3333 & 0.6667 \\ 1 & 0 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$FC15 \ R_{15} = \begin{Bmatrix} 1 & 0 & 0 \\ 0.6667 & 0.3333 & 0 \\ 1 & 0 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$ED6 \ R_6 = \begin{Bmatrix} 0.3333 & 0.6667 & 0 \\ 0.3083 & 0.6500 & 0.0417 \\ 0.8333 & 0.1667 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$FC17 \ R_{17} = \begin{Bmatrix} 0 & 0 & 1 \\ 0.1333 & 0.2417 & 0.06250 \\ 0.3333 & 0.6667 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$ME18 \ R_{18} = \begin{Bmatrix} 0.3333 & 0.6667 & 0 \\ 0.2000 & 0.2500 & 0.5500 \\ 1 & 0 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

$$DH23 \ R_{23} = \begin{Bmatrix} 0.6667 & 0 & 0.3333 \\ 0.3333 & 0.6667 & 0 \\ 1 & 0 & 0 \end{Bmatrix} \begin{matrix} X_1 \\ X_2 \\ X_3 \end{matrix}$$

Composite operations (B) for matrix A and matrix R are: B = AOR. Data resulted in the establishment of matrix B which is a comprehensive evaluation for the cucumber varieties. According to U-max, the data resulted in the definition of classes for the cucumber varieties examined (Table 3).

The cold winter monsoon in northern China produces lower winter temperatures than in other countries of identical latitude. For example, latitude is greater in Cologne, Germany (50°56'N) than in Harbin, China (45°45'N) but the mean monthly temperatures in December to February are 18.8 - 21.8 C more in Cologne than in Harbin, China. While the mean air temperature in January is -1.0 C in Xi'an, China (34'15'N) and 3.7 C in Tokyo, Japan (35°41'N), the mean air temperature in February is 2.1 C in Xi'an and 4.3 C in Tokyo. Moreover, there are lower air temperatures in Xi'an than in Tokyo. The winter climate of China allows plastic greenhouses to accumulate solar energy which increase inside ambient temperatures. This microclimate is beneficial for vegetable growth.

The Relationship between Papilla Formation and Resistance of Cucumber to *Sphaerotheca fuliginea* (Schlecht.) Poll.

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Introduction. Papillae are the wall oppositions occurring between the cell wall and plasmalemma when fungal infection pegs penetrate a plant host. The function of the papillae with respect to the expression of powdery mildew resistance have been investigated in barley and wheat in some detail (1-4). However the results of these studies are not entirely consistent. The mechanism of powdery mildew resistance in cucumber has received limited study. Therefore, a preliminary study was designed to elucidate the mechanism of resistance to powdery mildew in cucumber using germplasm possessing varying disease resistance.

Materials and Methods. *Plant and pathogen*. Six cultivars [Jinza No. 2 and 3511 (resistant), Jinyan No. 6 (moderately resistant), Heidan No. 1 and Nongcheng No. 3 (moderately susceptible), and Changchun Thorn (susceptible)] were grown in a greenhouse. An isolate of *Sphaerotheca fuliginea* (Schlecht) Poll. obtained from field-infected cucumber plants was maintained on a growth chamber-grown susceptible cultivar, Changchun Thorn.

Inoculation, sampling, staining and microscopy observation. The day before inoculation, powdery mildew spores on the leaves were knocked off to ensure establishment of fresh inoculum. The second leaf of plants in the 4-leaf stage were inoculated, and then cultured in a greenhouse at 18-25 C. Leaf samples were taken at 8, 10, 12, 14, 16, 24 and 48h after inoculation, made transparent with saturated trichloroacetaldehyde monohydrate, and stained with lactophenol-cotton blue solution. Observations were made using Olympus light microscopy.

Results and Discussion. The papillae observed were spherical or semi-spherical in shape. Papilla formation was not observed in any cultivar 8h after inoculation. The papillae appeared within the host epidermic cells in the middle of appressoria 10h after inoculation in resistant cultivars, and 2h later in susceptible cultivars. The papillae were observed in all the cultivars (79.1 to 87%) 48h after inoculation. Thus, there was no functional difference in the reaction of the cultivars examined (Table 1). The frequency of papilla formation was not associated with the degree of host resistance to powdery mildew (Table 1). This result is consistent with those of Wright, et al. (5). The formation of haustoria marks successful penetration. Our observations also indicate that some appressoria produce papillae but fail to form haustoria, and that the number of papillae not forming haustoria increases as the resistant level rises in cucumber varieties. This means that the papillae function to resist penetration (Note: Table 2, 48h after inoculation).

Approximately 6.7% of the papillae in susceptible cultivars did not form haustoria. In contrast, over 20% of the papillae formed haustoria in resistant cultivars. This observation may be related to the time of papilla formation. The papillae appear early in resistant cultivars, thus hindering the formation of haustoria. Conversely, haustoria formation occurs later in susceptible cultivars. In susceptible cultivars the pathogen penetrates the host and immediately forms haustoria. Thus the pathogen escapes the potential beneficial impact of papillae and successfully establishes a parasitic relationship.

These results demonstrate that the papillae are important structures contributing to a host's resistance to powdery mildew. Papillae act to augment plant barriers to penetration of the pathogen in resistant plants. This function increases as the resistance level of the host rises. This type of resistance depends mainly on the interaction of host resistant genes and the susceptible genes of the pathogen. The papillae appear early on the leaves of resistant cucumbers and the pathogen has greater difficulty in penetrating the papillae and developing haustoria. The papillae appear late in leaves of susceptible

Table 1. Papilla formation among different cucumber cultivars.

Cultivar	Resistant level	No. matured appressoria	No. papillae	Papilla formation rate (%)
Jinza No. 2	Resistant	100	87	87.0
3511		122	102	83.6
Jinyan No. 6	Moderately resistant	129	112	86.8
Heidan No. 1	Moderately susceptible	115	91	79.1
Nongcheng No.3		104	86	82.7
Changchun Thom	Susceptible	101	83	82.2

Table 2. Papilla ability against penetration among different cucumber cultivars.

Cultivar	Resistant level	Total No. papillae	No. papillae without haustoria	Anti-penetration rate (%)
Jinza No. 2	Resistant	94	223	24.5
3511		102	21	20.6
Jinyan No. 6	Moderately resistant	106	15	14.2
Heidan No. 1	Moderately susceptible	111	9	8.1
Nongcheng No.3		95	8	8.4
Changchun Thom	Susceptible	104	7	6.7

plants and the pathogen can penetrate host epidermal cells and establish a parasitic relationship before the papillae are fully developed.

The formation and function(s) of the papillae are not completely clear. For example, not all of the appressoria produce papillae and some appressoria fail to penetrate the host. Furthermore, papillae function against a pathogen's penetration in susceptible cultivars. Likewise, some papillae do not function against pathogen penetration in resistant cultivars. Thus, papillae formation and the genetics of papillae production are attractive areas for further research.

Literature Cited

1. Aist, J.R. 1976. Papillae and related wound plugs of plant cells. Ann. Rev. Phytopathol. 14:145-163.

2. Aist, J.R and H.W. Israel. 1967. Papilla formation: Timing and significance during penetration of barley coleoptiles by *Ersiphe graminis hordei*. Phytopathology 67:455-461.

3. Stolzenburg, M.C., J.R. Aist, and H.W. Israel. 1984. The role of papillae in resistance to powdery mildew conditioned by the *ml-o* gene in barley. I. Correlative evidence. Physiol. Plant Pathol. 25:337-346.

4. Stolzenburg, M.C., J.R. Aist, and H.W. Israel. 1984. The role of papillae in resistance to powdery mildew conditioned by the *ml-o* gene in barley. II. Experimental evidence. Physiol. Plant Pathol. 25:347-361.

5. Wright, A.J. and J.B. Heale. 1988. Host responses to fungal penetration in *Erysiphe graminis* f. sp. *hordei* infections in barley. Plant. Pathol. 37:131-140.

CMV Resistance in Three Sources of Cucumber

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Introduction. Resistance in cucumber (*Cucumis sativus* L.) to Cucumber Mosaic Virus (CMV) traces back to an accession of 'Tokyo Long Green' and its inheritance has been characterized by numerous researchers (1,2,4). CMV resistance shows a dominance type expression, but is affected by background genotype, genetic modifiers, and environment. Provvidenti (3) discovered that the line 'TMG-1' is resistant to CMV, but did not report its inheritance. Herein, I report the initial characterization of the CMV resistance in TMG-1 and compare it to other known sources of resistance.

Materials and Methods. An isolate of CMV (522) was the gift of Dr. R. Provvidenti (Geneva, NY) and was maintained by monthly transfers on 'Zucchini Select' squash. Approximately 5 cm^2 of freshly harvested expanding leaf tissue was ground in a mortar in 5 ml of phosphate buffer (0.05 M K2HPO4, pH 8.8) until tissue was completely homogenized. Fully expanded cotyledons were dusted with carborundum and the virus suspension inoculated by gentle rubbing with the pestle. Plants were maintained in insect-proof cages. Sources of CMV resistance were 'Wisconsin SMR18', 'Marketmore 76' (MM76), and TMG-1. Single plants from SMR18, MM76, and TMG-1 were grown, propagated by cuttings, and the root stock inoculated with CMV as described above. 'Straight 8' plants (susceptible to CMV) were similarly propagated and the root stocks inoculated with CMV to identify a single susceptible plant (ST8-5). After determining the virus phenotype, root stocks were discarded. Propagules of single CMV-resistant SMR18, MM76, and TMG-1 plants were crossed as males to propagules of ST8-5. Single F_1 plants were self-pollinated to generate the F_2 families. Single F_2 families were chosen randomly and 75, 45, and 53 plants self-pollinated to generate F_3 families from ST8-5 x SMR18, ST8-5 x MM76, and ST8-5 x TMG-1, respectively.

For each family, at least two replications of 10 plants were evaluated for CMV resistance. Cucumber plants were germinated in vermiculite for 5 days and single seedlings transplanted to 12.5- cm plastic pots with steamed compost:field-soil:peat:sand (1:1:1:1 on a volume basis) mixture. Ten to 14 days after transplanting, expanding first or second true leaves were dusted with carborundum and inoculated by three gentle strokes of the pestle over the leaf. Two weeks after the first inoculation, the newest approximately one-half expanded true leaf was dusted with carborundum and re-inoculated with the same virus. Two weeks later, the plants were scored as 1 = no symptoms to a slight mosaic on lower leaves; 3 = slight mosaic limited on lower leaves; 5 = slight mosaic on upper leaves; 7 = mosaic on upper leaves, and; 9 = severe mosaic on upper leaves.

Results and Discussion. CMV resistance in SMR18 and MM76 originates from 'Tokyo Long Green' (H. Munger, personal communication). Observations by cucumber breeders indicate that MM76 possesses a higher level of resistance to CMV than SMR18. For segregating families using SMR18 and MM76 as sources of CMV resistance, too many susceptible families were observed to allow adequate fit to the single dominant gene hypothesis proposed by Wasuwat and Walker (4) (Figure 1). TMG-1 appeared to be a better source of resistance than SMR18 or MM76 and a greater frequency of resistant F_3 families were observed (Figure 1). This is indirect evidence that TMG-1 may possess a CMV resistance different from either SMR18 or MM76. Because further characterization and tests of allelism are required, genotypes at putative CMV-resistance loci were not assigned.

Literature Cited

1. Cohen, S., E. Gertman and N. Kedar. 1971. Inheritance of resistance to melon mosaic virus in cucumber. Phytopathology 61:253-255.

2. Kooistra, E. 1969. The inheritance of resistance to *Cucumis* virus 1 in cucumber. Euphytica 18:326-332.

(A)
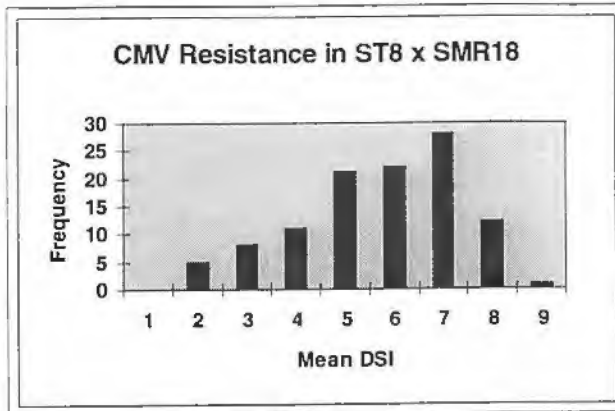
(B)
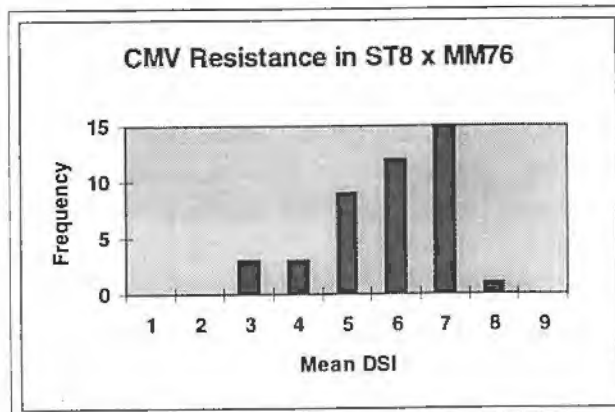
(C)
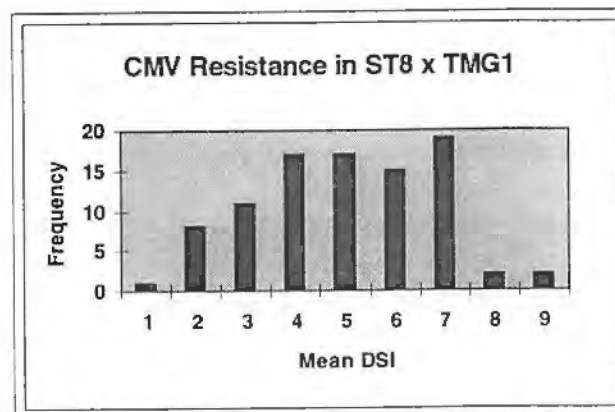
Figure 1. Frequencies of F_3 families plotted against the mean disease severity index (DSI) for reaction to CMV in (A) Straight 8 × SMR18, (B) Straight 8 × Marketmore 76, and (C) Straight 8 × TMG-1. For description of DSI, see Materials and Methods.

3. Provvidenti, R. 1985. Sources of resistance to viruses in two accessions of *Cucumis sativus*. Cucurbit Genet. Coop. Rpt. 8:12.

4. Wasuwat, S., and J. Walker. 1961. Inheritance of resistance in cucumber to cucumber mosaic virus. Phytopathology 51:423-428.

Regeneration of Interspecific Hybrids of *Cucumis sativus* L. x *C. hystrix* Chakr. by Direct Embryo Culture

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Introduction. *Cucumis hystrix* is a wild species of *Cucumis* subgen. *Cucumis*, which originated in Asia (Kirkbride, 1993). It has a taste and flavor typical of cucumber ($2n = 14$) (Chen et al., 1994), but its diploid chromosome number is 24 (unpublished data). Previous work employing isozyme analysis hypothesized a triangular phylogenetic relationship among *C. hystrix*, *C. sativus* and *C. melo* (Chen et al., 1995). However, the genetic distance between *C. hystrix* and *C. sativus* was smaller (0.50) than that calculated between *C. hystrix* and *C. melo* (0.71).

Materials and Methods. Interspecific crosses were made by conventional crossing of two types of Chinese cucumbers (maternal parents) with *C. hystrix* (paternal parent) in Japan between October 8 to 12, 1995, in the field. The fruits were harvested 50 days after pollination and then stored for 25 days at room temperature to improve maturity. The embryos were recovered and immediately cultured on MS hormone free solid medium with 3% sucrose, 0.8% agar, pH 6.0, at 25 C. Plantlets with four true leaves were then transferred to containers of vermiculite covered with a plastic bag for 5 days, followed by a 5-day period of gradual exposure in a greenhouse. When the plants had grown to about 30 cm in height, they were transferred to pots containing soil and fertilized weekly with a commercial nutrient formulation.

Results. All pollinations resulted in mature fruit. The embryo halted its development at the "rabbit-ear" stage. Of 235 dissected seeds, 158 embryos ("heart" to rabbit-ear stages) were obtained. Embryos began to grow within 3 days and turned green in 5 days on MS medium. Roots developed in 8 days and embryo growth and development increased. A total of 59 normal plants were obtained. The regeneration rate was 37.3%. Hybrid plants grew vigorously and were relatively uniform for such traits as the diameter and internode length of stem,

shape and size of leaf, and shape and size of flower when compared to the parents. However, some characteristics, such as multiple branching, densely brown hairs (especially on flowers), orange yellow corolla, and ovate fruit were obviously inherited from the paternal parent. The flower position in the stem was similar to that of the maternal parent. Some characteristics of flower structure, such as three separately elongated degenerated stigma on staminate flower and elongated stigma with brown hair on pistillate flower were not apparent in either parent.

The somatic chromosome number of these hybrid plants was $2n=19$. Malate dehydrogenase banding patterns observed using starch gels and glutamate oxaloacetate transaminase banding patterns present on polyacrylamide gels also confirmed the hybrid nature of these plants. Preliminary observation showed that the meiosis in PMCs was irregular. The staminate flower was highly sterile. No pollen grains and only a few tetrads were observed at anthesis. Backcross to the female parent resulted in 60% fruit set, but embryos were not present in the seeds which were produced. The check (without pollination) developed fruit parthenocarpically.

Discussion. The importance of wild *Cucumis* species for cucumber and melon breeding has long been recognized because *Cucumis* crops are susceptible to a number of devastating fungal, bacterial, viral and insect diseases (Kirkbride, 1993). In several instance, attempts have been made to produce interspecific hybrids between cucumber, melon and other species. However, thus far these attempts have been without repeatable success (Dane, 1991). Although the interspecific hybrid we obtained is not horticulturally acceptable (it can not produce offsprings naturally), this development should be viewed as an important first step in the process of interspecific hybridization in *Cucumis* species.

Previous investigations on the taxonomy and evolution of *Cucumis* have been based on the theories suggesting a basic chromosome number of 2n=14 (Asian group) or 2n=24 (Africa group). Researchers have found no justification keeping melon and cucumber in the same genus because of their different chromosome number, centers of origin, morphological characters, and strong cross incompatibilities (Pangalo, 1950; Ramachandran, 1986; Sujatha and Seshadri, 1989). It has been difficult to establish the taxonomic and evolutionary relationship between these two subgenera. The cucumber was found to be the most distantly related group among *Cucumis* species, i.e., having a large D value with all other species (Perl-Treves et al., 1985). *C. hystrix*, which is cross compatible with cucumber, might have a close taxonomic and evolutionary relationship with cucumber, and therefore melon. As such, it might be used as a bridge species in the genus *Cucumis* and therefore enrich our knowledge of this genus. We believe some answers about the evolution of *Cucumis* species might be obtained through further study of the relationship between *C. hystrix* and other species in *Cucumis*.

Literature Cited

1. Chen, J.F., S. Isshiki, Y. Tashiro, and S. Miyazaki. 1995. Studies on wild cucumber from China (*Cucumis hystrix* Chakr.). I. Genetic distances between *C. hystrix* and two cultivated *Cucumis* species (*C. sativus* L. and *C. melo* L.) based on isozyme analysis. Jour. Japan Soc. Hort. Sci. 64(suppl. 2):264-265.

2. Chen. J.F., Sh.L. Zhang and X.G. Zhang. 1994. The xishuangbanna gourd (*Cucumis sativus* var. *xishuangbannesis* Qi et Yuan), a traditionally cultivated plant of the Hanai people, xishuangbanna, Yunnan, China. Cucurbit Genet. Coop. Rpt. 17:18-20.

3. Dane, F. 1991. Cytogenetics in genus *Cucumis*. Plant Genetics and Breeding. 2B. 201-214.

4. Kirkbride, J.H., Jr. 1993. Biosystematics monograph of the genus *Cucumis* (Cucurbitaceae). Parkway Publishers, pp: 84-88.

5. Pangalo, K.I. 1950. Melons and independent genus *Melo* Adans. Botanichesky Zhurnal. 35:571-580.

6. Perl-Treves, R., D. Zamir, N. Navot and E. Galun. 1985. Phylogeny of *Cucumis* based on isozyme variability and its comparison with plastome phylogeny. Theor. Appl. Genet. 171:430-436.

7. Ramachandran, C. and V.S. Sehadri. 1986. Cytological analysis of the genome of cucumber (*C. sativus* L.) and muskmelon (*C. melo* L.). Z. Pflanzenzuchtg. 96:25-38.

8. Sujatha, V.S. and V.S. Seshadri. 1989. Electrophoretic examination of *Cucumis sativus* L. and *Cucumis melo* L. Cucurbit Genet. Coop. Rpt. 12:18.

Efficiency of Haploid Production in Cucumber

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Introduction. A method of double haploid production induced by irradiated pollen, used routinely in melon breeding programs is promising for use in cucumber (3,4,5,6). Since 1992 we have recovered haploid plants from several cucumber genotypes using an adaptation of this method. Initially, we determined the optimal irradiation dose and pollination season for cucumber. The success of this method, however, can be criticized because the criteria regarding the success in these studies seemed insufficient. Therefore, we designed a study which utilized the "Haploid Production Efficiency" (HPE 1) concept which takes into account "the number of haploid plants regenerated per fruit" as well as its components.

Material & Methods. Eight F_1 hybrid cultivars and 2 inbred lines were used in 1995. Young, rapid developing and healthy plants were used for pollinations. Female flowers of the plants were pollinated on the 23rd and the 28th of June with 300 Gy gamma irradiated pollen (2). The following variables were recorded: S/F (the number of seeds/fruit), E/S (the number of embryos/100 seeds), E/F (the number of embryos/fruit), P/E (the number of haploid plants regenerated/100 embryos), P/S (the number of haploid plants regenerated/100 seeds) and HPE (the number of haploid plants regenerated/fruit).

Results & Discussion. The number of fruits, seeds, embryos, haploid plants and HPE with its components are presented in Table 1. Variability was observed among genotypes for fruit number. This variation might be the result of pollination and/or genotype differences.

The mean S/F was low (115.2). This was due to parthenocarpy recorded in the gynoecious F_1 hybrids when grown in a greenhouse. The mean E/S was 2.9, and was as high as 5.36 in 'Passandra'. Generally, E/Ss in most cultivars were considered to be relatively high when compared with the previous studies (4,6). The mean E/F

was low (3.30). This was due to high number of soft embryos. Accordingly, P/Ss of the cultivars were adversely affected (with a mean of 0.9). The mean HPE was 1.1 and as high as 1.7 in some cultivars. These data indicate the utility and efficiency of the method used.

High values of S/F and E/S are desirable. Normally, high P/S and HPE values are expected when E/S and/or E/F values are high. However, this may not always be the case since these two components are also affected by P/E which, in turn, is closely related to quality of embryos. Therefore, S/F, E/S, and P/E should be high in order to reach a sufficient HPE.

Literature Cited

1. Chen, F. and P. Hayes. 1991. Effect of exogenous plant growth regulators on *in vitro* seed growth, embryo development, and haploid production in a cross between *H. vulgare* L. and *H. bulbosum* L. Plant Cell, Tissue and Organ Cult. 20:179-184.

2. Çaglar, G. 1995. Hiyarda (*Cucumis sativus* L.) Isinlanmis Polenlerle Tozlama yoluyla *in situ* Haploid Embriyo Uyartimi Ve Haploid Embriyolardan *in vitro* Bitki Eldesi Üzerinde Arastirmalar. Doktora Tezi. Çukurova Üniversitesi, Fen bil. Ens. Bahçe Bitkileri Ana Bilim Dali, Adana, 227 p.

3. Çaglar, G. and K. Abak. 1995. Farkli hiyar genotiplerinde isinlanmis polenlerle uyartim yoluyla haploid embriyo ve bitki eldesi. Tükiye II. Ulusal Bahçe Bitkileri Kongresi, 3-6 Ekim, Adana Cilt 11, p.159-162.

4. Przyborowski, J. and K. Niemirowicz-Szczytt. 1994. Main factors affecting cucumber (*Cucumis sativus* L.) haploid embryo development and haploid plant characteristics. Plant Breeding 112:70-75.

Table 1. The number of cucumber fruits, seeds, embryos, plants and S/F (seed/fruit), E/S (embryos/100 seeds), E/F (embryos/fruit) and P/S (haploid plants regenerated/100 seeds), P/E (haploid plants regenerated/100 embryos), HPE (haploid plants regenerated/fruit) observed during haploid production.

Cultivars	No. of fruits	No. of seeds	No. of embryos	No. of plants	S/F	E/S	E/F	P/S	P/E	HPE
Efes	9	1238	31	11	137.6	2.50	3.44	0.89	35.5	1.2
Ceren	9	652	29	4	72.4	4.45	3.22	0.67	13.8	0.4
Passandra	7	616	33	5	88.0	5.36	4.71	0.81	15.2	0.7
Nile	7	787	13	5	112.4	1.65	1.86	0.64	38.5	0.7
Ece	7	717	20	7	102.4	2.79	2.86	0.98	35.0	1.0
Alara	6	626	24	10	104.3	3.83	4.00	1.60	41.7	1.7
1404	6	846	21	10	141.0	2.48	3.50	1.18	47.6	1.7
Melisa	6	1002	9	8	167.0	0.90	1.50	0.80	88.9	1.3
F6[z]	6	731	20	7	121.8	2.74	3.33	0.96	35.0	1.2
F4[z]	5	620	24	4	124.0	3.87	4.80	0.67	16.7	0.8
Total	68	7835	224	72	-	-	-	-	-	-
Mean	-	-	-	-	115.2	2.90	3.30	0.90	32.1	1.1

[z]Inbred lines

5. Sauton, A. 1989. Haploid gynogenesis in *Cucumis sativus* induced by irradiated pollen. Cucurbit Genetic Coop. Rpt. 12:22-23.

6. Truong-Andre, I. 1988. *In vitro* haploid plants derived from pollination by irradiated pollen on cucumber. In: Proceedings of the Eucarpia Meeting on Cucurbit Genetics and Breeding. May 31 - June 2, Avignon-Montfavet, France, p.143-144.

Transfer and Expression of the Firefly Luciferase Gene in Cucumber

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Introduction. Cucumber can be infected with *Agrobacterium* and through this infection can be genetically transformed. Transgenic cucumber plants of the cultivar Straight 8 expressing the selection neomycin phosphotransferase II (*npt II*) gene were regenerated through inoculation with a strain of *Agrobacterium rhizogenes* (9). The transfer and expression of Cucumber Mosaic Virus coat protein gene in the genome of cultivar Poinsett 76 was obtained using an *Agrobacterium* disarmed strain (3). Also the factors influencing *Agrobacterium tumefaciens* mediated transformation and expression of neomycin phosphotransferase II were studied in two inbred lines of pickling cucumber (8).

The present report describes the use of a disarmed strain of *Agrobacterium tumefaciens* to mediate the transfer and expression of a reporter gene coding for luciferase in the genome of the cucumber hybrid Bambina. It also describes the use of regeneration via shoot organogenesis to obtain transformed plants that express the luciferase gene.

Materials and Methods. A modified *Agrobacterium tumefaciens* strain LBA 4404 which contains the disarmed Ti plasmid pAL 4404 (4) was used. This strain contained an additional plasmid (pAQ2) which was constructed by modification of the binary Ti vector BIN 19 (1) through addition of the plasmid T-DNA with the firefly luciferase reporter gene supplemented with CaMV promoter (6). The T-DNA region also contained the kanamycin-resistance selectable marker gene (*npt II*) driven by the nopaline synthase (*nos*) promoter (2). A bacterial kanamycin-resistance gene (*npt III*) driven by a bacterial promoter was cloned outside the borders of the T-DNA region.

Seeds of the Bambina (De Ruiter Seeds) cucumber hybrid were soaked in a Triton x 100 (Merck) 0.01% v/v solution for 20 min. They were subsequently surface sterilized in 20% v/v solution of commercial bleach (5% sodium hypochlorite) for 15 min. The seeds were then rinsed three times with sterile distilled water and placed under aseptic conditions in petri dishes containing 0.8% agar-agar (Sigma) in darkness at 25 C for five to seven

days to germinate. Unless otherwise stated all media were solidified with 0.8% agar. The pH of the media was adjusted to 5.7 before autoclaving at 121 C for 20 min.

Five- to seven-day-old *in vitro* grown seedlings were used as donors of cotyledon explants. Cotyledons were divided in two parts: proximal and distal to the embryonic axis ones. The distal parts were discarded and the proximal were divided in two parts with a cut across the central vein. These explants were submerged overnight in an MS (Murashige and Skoog) (5) liquid medium supplemented with 4 mg/l 6-benzylaminopurine (BA) in which bacterial cells of the previously referred to strain were diluted. Bacterial cells for infections were produced after an overnight culture in a liquid LB medium containing 100 mg/l kanamycin at 28 C and vigorous shaking. An aliquot of 1.5 ml of this culture was then centrifuged for 7 min at 10,000 rpm and the bacterial pellet was diluted in a 50 mm diameter petri dish containing 5ml of the previous referred to liquid medium supplemented with BA. Eight cotyledon segments were then added to this petri dish.

Twenty-four hours later the cotyledon explants were rinsed five times with distilled sterile water, blot dried on sterile filter papers and cultured upside down in sterile petri dishes on solid MS medium supplemented with 4 mg/l BA. This medium was found to be optimum for the regeneration of parthenocarpic cucumber hybrids such as 'Bambina' and 'Brunex' (7). After two days the explants were transferred on a MS medium supplemented with 4 mg/l BA, 50 mg/l kanamycin and 250 mg/l cefotaxime, sodium salt. One week later the cotyledonary segments were transferred on MS + 4 mg/l BA + 100 mg/l kanamycin + 500 mg/l cefotaxime. Every ten days the explants were re-transferred to petri dishes with the previously referred to medium. Kanamycin was used to select the transformed plant cells and cefotaxime to control the growth of bacteria which remained on the cotyledon surface after the co-cultivation period. The shoots which were produced (Fig. 1) were transferred in magenta vessels (Sigma) on MS media supplemented

Fig. 1. Putative transformed shoot regenerated from cotyledon segment of the cucumber hybrid Bambina three weeks after co- cultivation with recombinant bacteria.

Fig. 2. Putative transformed plantlet of the cucumber hybrid Bambina three months after co-cultivation with recombinant bacteria.
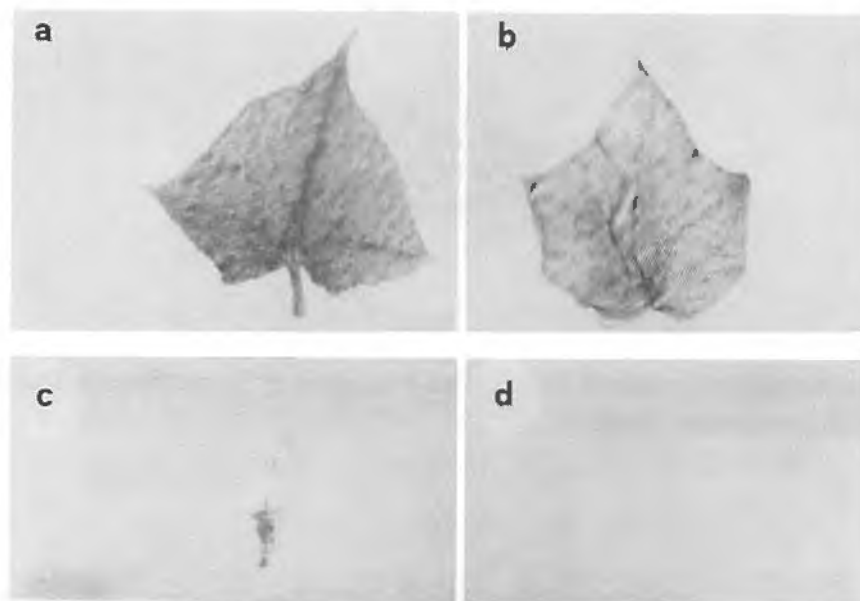
Fig. 3. Leaves from a putatively transformed (a) and a non- transformed plant (b) of the cucumber hybrid Bambina and their images on an x-ray film on which luminescence was recorded. This was accomplished after exposition by contact, from the leaf of the putative transformed (c) and the non-transformed plant (d). Luminescence was recorded only from the leaf of the transformed plant.

with 100 mg/l kanamycin and 500 mg/l cefotaxime, where they were elongated and rooted (Fig. 2).

Leaves from the putative transformed plants were soaked for 2.5 h in 0.1 mM luciferin solution containing 100 mM sodium citrate (pH 5) and 20% v/v dimethyl sulfoxide and then exposed by contact to x-ray film (Kodak OG) (6) for 24h. The leaf luminescence was recorded on the x-ray film (Fig. 3) to document the transfer and expression of the CaMV promoter and the firefly luciferase gene in the genome of the cucumber hybrid Bambina.

Results and Discussion. The transfer and expression of an easily manipulated and early detectable reporter gene was performed to standardize a protocol for cucumber genetic transformation. This procedure may be used for transferring genes which code for economically important traits (e.g., resistance to viruses and pests).

Literature Cited

1. Bevan, M. 1984. Binary *Agrobacterium* vectors for plant transformation. Nucleic Acid Res. 12:8711-8721.

2. Bevan, M.W., R.B. Flavell and M.D. Chilton. 1983. A chimeric antibiotic resistance gene as a selectable marker for plant cell transformation. Nature 304:184-187.

3. Chee, P.P. and J.L. Slightom. 1991. Transfer and expression of Cucumber Mosaic Virus coat protein gene in the genome of *Cucumis sativus*. J. Amer. Soc. Hort. Sci. 116:1098-1102.

4. Hoekema, A., P.R. Hirsch, P.J.J. Hoykaas and R.A. Schilperoort. 1983. A binary plant vector strategy based on separation of vir- and T- region of the *Agrobacterium tumefaciens* Ti plasmid. Nature 303:179-180.

5. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

6. Ow, D.W., K.V. Wood, M. DeLuca, J.R. De Wet, D.R. Helinski and S.H. Howell. 1986. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science 234:856-859.

7. Sapountzakis, G., E. Nianiou-Obeidat and A.S. Tsaftaris. In vitro shoot regeneration from cotyledon explants of the parthenocarpic cucumber (*Cucumis sativus* L.) hybrids 'Brunex' and 'Bambina'. (in preparation).

8. Sarmento, G.G., K. Alpert, F.A. Tang and Z.K. Punja. 1992. Factors influencing *Agrobacterium tumefaciens* mediated transformation and expression of kanamycin resistance in pickling cucumber. Plant Cell, Tissue and Organ Cult. 31:185-193.

9. Trulson, A.J., R.B. Simpson and E.A. Shahin. 1986. Transformation of cucumber (*Cucumis sativus* L.) plants with *Agrobacterium rhizogenes*. Theor. Appl. Genet. 73:11-15.

Selection and Multiplication of Transgenic Embryogenic Tissues of Cucumber Using a Suspension Culture Procedure

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Introduction. A few methods for initiating cell suspension cultures, development of somatic embryos, and regeneration of plantlets in several fresh market cultivars of cucumber have been described (e.g. 2,3). A procedure for initiation and maintenance of suspension cultures of a pickling cucumber cultivar, Endeavor, for high-frequency regeneration of normal-appearing plantlets has recently been developed in our laboratory (9). The objective of this paper is to describe the incorporation of cell suspension culture and *Agrobacterium*-mediated transformation procedures to select and multiply transformed embryogenic aggregates in order to recover a high frequency of transformed plantlets.

Materials & Methods. The cucumber cultivar used throughout this study was Endeavor, a pickling type, which is an F_1 hybrid (seed provided by Campbell Soup Co., Davis, CA). The decoated seeds were surface-sterilized by dipping in 70% ethanol for 30 sec, followed by soaking in a 10% solution of commercial bleach (Javex, 6.25% sodium hypochlorite) for 5 min, and rinsing three times in sterile distilled water. The seeds were germinated and seedlings maintained in Magenta culture vessels (Magenta Corp., Chicago, IL) containing 50 ml of half-strength Murashige and Skoog (MS) basal medium (5).

Agrobacterium tumefaciens strain EHA105, a supervirulent leucinopine type, with a binary vector pMOG196 (provided by Dr. L. Melchers, MOGEN Int. nv, The Netherlands) was used. The vector contained a petunia acidic chitinase gene (Linthorst et al., 1990), in addition to the neomycin phosphotransferase (*NPT II*) gene as a selectable marker and the cauliflower mosaic virus (CaMV) *35S* promoter for constitutive expression. The *Agrobacterium* was maintained on Laura-Bertaini (LB) medium with 100 mg/l kanamycin, pH 5.4. The bacterial density was adjusted to 10^8 cells/ml and acetosyringone was added to a final concentration of 100 µM, 1h prior to infection of explants.

The explants used for infection were petiole segments (4 to 5 mm long) taken from the first and second true leaves of 10 to 21 day-old seedlings. Inoculation was done by dipping the explants into the bacterial suspension for 5 min, followed by blotting with sterile paper towels. The explants were transferred onto cocultivation medium, which was MS medium with 2,4-D/BA (5.0/5.0 µM), pH 5.4, and cocultivated at 27 C in the dark for 2 to 4 days.

Following cocultivation, the explants were rinsed with MS medium and transferred onto selective medium, i.e., MS basal medium with 2,4-D/BA (5.0/5.0 µM), kanamycin (50 mg/l) and carbenicillin (500 mg/l). Calli which developed from the explants were subcultured onto fresh medium of the same composition 4 to 5 weeks after infection. Following one to two subcultures, embryogenic calli formed were used to initiate a suspension culture to further screen and multiply putative kanamycin-resistant cell aggregates. The procedures for the initiation and maintenance of the suspension cultures of pickling cucumber have been described (6) (Fig. 1).

Genomic DNAs from putatively transformed plants and from non- transformed (negative control) plants were extracted using previously described procedures (4). PCR amplification for the identification of the transgene in the genomic DNAs was conducted using two specific primer sequences (courtesy of Dr. M.M. Moloney, University of Calgary) of the *NPT II* coding region. This PCR was run for 30 cycles. The PCR products were analyzed by electrophoresis on 2.0% agarose gels. The oligomers were first tested by amplifying the characteristic 800 bp region of the *NPT II* gene by using 10 ng of pMOG196 as positive control template.

Results. Cocultivated petiole explants swelled and began to develop callused areas after 3-4 weeks on selective callus initiation medium. The calli surviving this selection step were characterized by an increase in size and development of pale yellow color and no browning. Explants which did not increase in size or did not form calli on the surface were considered to be nonviable. Surviving calli were subcultured onto fresh medium of the same composition where they would develop embryogenic (yellow and friable) sectors. In comparison,
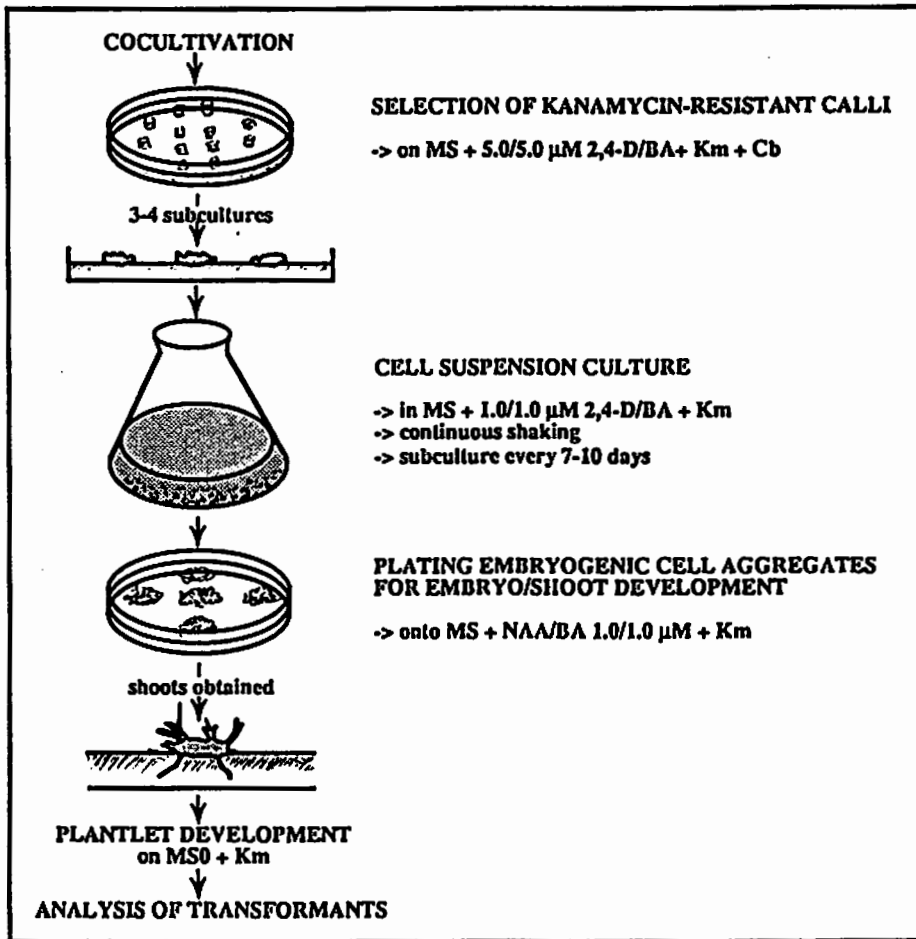
Figure 1. Procedure used to obtain transgenic cucumber plants through multiplication and selection of embryogenic aggregates/clumps in suspension culture.
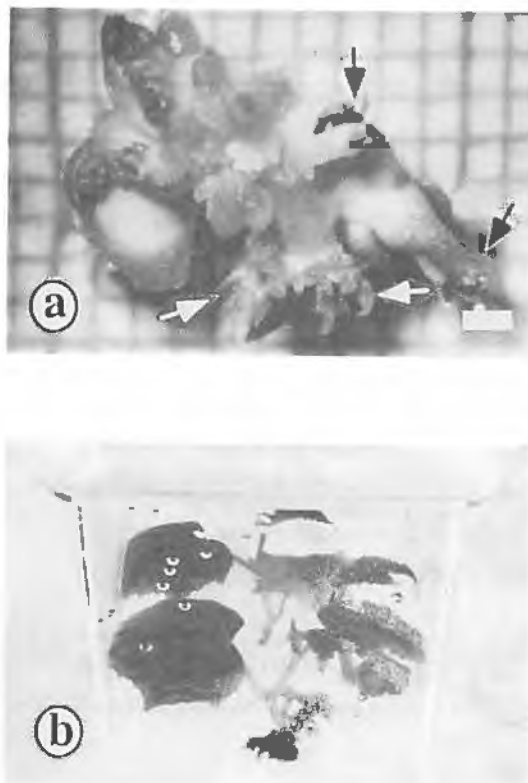
Figure 2. Development of transgenic shoots and plantlets from plated clumps: a) Shoots (arrows) formed from clumps plated on MS medium with NAA/BA (1.0/1.0 µM), 3-4 weeks after plating (bar = 1 mm); b) Plantlets developed from the shoots, 3 weeks after transfer to MS medium with no plant growth regulator.
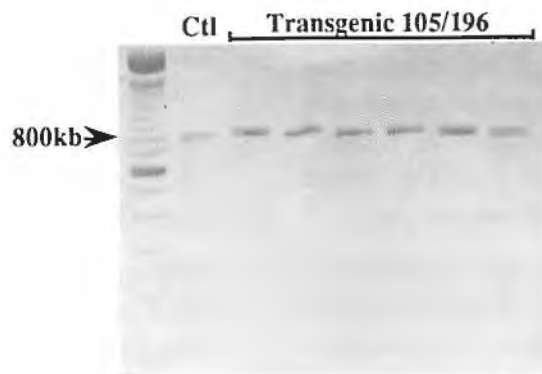
Figure 3. PCR amplification of the NPT II gene from genomic DNA isolated from positive control/vector pMOG196 (Ctl) and 6 randomly-selected transgenic plants transformed with petunia chitinase gene (EHA 105/pMOG 196).

most non-cocultivated explants were bleached and did not develop further. The frequency of embryogenic calli which developed further and grew on kanamycin-containing medium, recorded 8 weeks after cocultivation, was approximately 12%.

Small portions of the calli growing on kanamycin-containing medium that appeared embryogenic (yellow in color and granular or friable in appearance) were dissected and transferred into liquid MS medium containing 2,4-D/BA (1.0/1.0 μM) and 50 mg/l kanamycin to initiate the suspension culture. After 2 to 3 weeks of shaking on a gyratory shaker, the calli started to break apart, forming a suspension of cells and aggregates. In the suspension culture, the kanamycin-resistant embryogenic aggregates grew faster than on solid selective medium.

Following plating of kanamycin-resistant embryogenic aggregates onto solid medium containing NAA/BA (1.0/1.0 μM) and 50 mg/l kanamycin, shoots were obtained within 3 to 4 weeks (Fig. 2a). When the shoots were excised and transferred onto MS medium without growth regulators and with 50 mg/l kanamycin, they elongated to form plantlets (Fig. 2b) and developed roots after 2-3 subcultures (2-3 weeks) onto the same medium. When the rooted shoots were 5 cm or higher, they were transferred to pots where they developed into plants.

Proof of transformation was confirmed by PCR amplification of the *NPT II* gene using two specific primer sequences of the *NPT II* coding region. Six randomly selected plants from three separate flasks of suspension cultures produced a band of the expected size of 800 bp for the *NPT II* fragment at the same position as those of positive control, amplified DNA from the binary vector pMOG196 (Fig. 3).

Discussion. This report describes the incorporation of a liquid suspension culture with genetic transformation in cucumber. In previous reports on cucumber transformation, regeneration of plantlets was either not achieved or not reported. Direct shoot regeneration from kanamycin-resistant cucumber calli following *Agrobacterium*-mediated transformation has been reported to occur at a low frequency (10). Medium with optimal combinations and concentrations of plant growth regulators for direct plantlet regeneration of cv. Endeavor, i.e. 2,4-D/BA or NAA/BA (5.0/5.0 μM) (8) failed to induce regeneration from cocultivated explants. Repetitive divisions of embryogenic aggregates in suspension culture, however, bryogenic aggregates in suspension culture, however, has been shown to be a useful means to multiply the embryogenic tissues and to achieve an increased regeneration rate (1,2,7).

Suspension cultures proved to be useful for multiplying embryogenic aggregates and for production of cell clumps which were capable of shoot formation upon transfer onto MS medium devoid of plant growth regulators. This procedure may be useful in transformation studies of pickling cucumber conducted in other laboratories.

Literature Cited

1. Bergervoet, J.H.W., F. Van der Mark and J.B.M. Custer. 1988. Organogenesis versus embryogenesis from long-term suspension cultures of cucumber (*Cucumis sativus* L.). Plant Cell Rept. 8:116-119.

2. Chee, P.P. and D.M. Tricoli. 1988. Somatic embryogenesis and plant regeneration from cell suspension cultures of *Cucumis sativus* L. Plant Cell Rept. 7:274-277.

3. Malepszy, S. and E. Solarek. 1986. In vitro culture of *Cucumis sativus* L. IV. Conditions for cell suspension. Genetica Polonica 27:249-253.

4. Mettler, I.J. 1987. A simple and rapid method of DNA isolation from tissue cultured plant cells. Plant Mol. Biol. Rept. 5:346-349

5. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497

6. Punja, Z.K., N. Abbas, G.G. Sarmento and F.A. Tang. 1990. Regeneration of *Cucumis sativus* var. *sativus* and *C. sativus* var. *hardwickii*, *C. melo*, and *C. metuliferus* from explants through somatic embryogenesis and organogenesis. Plant Cell Tissue Organ Cult. 21:93-102.

7. Raharjo, S.H.T. and Z.K. Punja. 1994. Regeneration of plantlets from embryogenic suspension cultures of pickling cucumber (*Cucumis sativus* L. cv. Endeavor). In Vitro Cell. Dev. Biol. 30P:16-20

Phenylurea Cytokinin (CPPU) More Effective than 6-Benzyladenine in Promoting Fruit Set and Inducing Parthenocarpy in Melon

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The cytokinin N^6-benzyadenine (BA) promotes fruit set in hand- pollinated melons (3). Because fruit set in hand-pollinated melons is often low, many breeders have adopted the use of BA to enhance fruit set. In our program 200 ppm BA is conveniently applied in a lanolin- water paste (7:3) to the base of ovaries with a disposable syringe (minus needle). Hayata et al. (3) reported that 200 ppm of the synthetic cytokinin, 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU), increased fruit set in watermelon from 26.9% to 95%. The same concentration of CPPU also induced parthenocarpy in 89.5% of treated fruit. Likewise, in an earlier report, Hayata et al. (2) found that CPPU enhanced fruit set in melon. In the present study we compared the effects of CPPU and BA on fruit set and parthenocarpy in melon, cv. Delicious 51.

Delicious 51 seeds were sown in Jiffy mix in 50 plug trays in Jan 1995. Seedlings were transplanted into 21.6 x 21.6 cm (dia./ht.) pots (#600XL nursery pots, Nursery Supply, Inc.) on 10 Feb and grown in groundbeds in a greenhouse (27 C day and 17 C night). Plants were vertically trellised, pruned to one central leader, and lower laterals were removed. For the first experiment there were 18 replications of the following treatments: (1) control (pollination + lanolin), (2) pollination + BA (200 ppm), (3) pollination + CPPU (200 ppm), (4) no pollination + BA, (5) no pollination + CPPU. For all hand pollinations, anthers were carefully emasculated just prior to applying pollen.

Growth regulators were applied in a lanolin water paste (7:3). BA (10 mg) was dissolved in 2 ml 0.1 N KOH and then brought up to 15 ml distilled water. CPPU (10 mg) was solubilized in 1.5 ml dimethylsulfoxide (DMSO) and brought up to 15 ml distilled water. The 15 ml aliquots were each added to 35 ml lanolin paste and stirred vigorously to form uniform lanolin paste emulsions. The emulsions were loaded into 3 ml disposable syringes (minus needles) for treatment applications to the base of ovaries of pollinated flowers. A ring of approximately 0.05 ml was applied to each ovary. Treatments

began on 6 April and were completed on 19 April. During most pollination days, complete sets of treatments were made. Following fruit harvest, seeds were removed, and if less than 100 viable (well filled) seeds per fruit was indicated, seeds were counted. Seed viability was confirmed directly by germination tests.

In spite of the seemingly favorable growth conditions and weather during pollination, no fruits set with the control treatment. Both cytokinins enhanced fruit set with 14 out of 18 (77.7%) set with BA + pollination and all fruits set with CPPU + pollination. Without pollination, 44% of the BA-treated flowers set fruit and 100% of the CPPU-treated flowers set fruit. Although both hormones markedly stimulated fruit set, seed set or fill was low in many of the pollinated fruits (Table 1), presumably reflecting the apparently unfavorable conditions for fruit set and/or pollen viability. Because of the small sample size, it is not possible to ascertain if seed set was significantly different between the BA and CPPU treatments.

One concern is that BA was solubilized in KOH, whereas CPPU was solubilized in dimethylsulfoxide (DMSO), a solvent known to increase the absorption of chemicals through biological membranes (4). Therefore, we cannot rule out that the greater effectiveness of CPPU was due to increased absorption. Secondly, it is possible that higher concentrations of BA would elicit the same response as 200 ppm of CPPU. Previous studies (Zack and Loy, unpublished) with higher concentrations of BA suggest that this is not the case, but in view of our current findings, this aspect needs to be reinvestigated. In a second experiment we compared fruit set with pollination and with or without lanolin application. With lanolin, 6 of 15 flowers set fruit; without lanolin, 7 of 15 flowers set fruit, indicating that lanolin neither stimulated nor inhibited fruit set.

Because of its strong propensity to induce parthenocarpy in melons, we cannot wholeheartedly endorse CPPU treatment in a breeding program without further seed set data. Overall, however, we have been more satisfied

Table 1. Counts of filled seed in pollinated and unpollinated (NP) melon fruits.

Treatments	Fruit examined[z]	No seed	1-20 seed	20-100 seed	100+ seed
Control	0	-	-	-	-
BA	4	-	2	2	-
CPPU	13	3	2	3	5
BA-NP	7	7	-	-	-
CPPU-NP	18	17	1 (3 seed)	-	-

[z]Seed data for 10 fruit from BA + pollination treatment and 5 fruit from CPPU + pollination treatment were not recorded, but fruit appeared to have mostly well-filled seed.

with our pollination results with CPPU than with BA, especially in lines that exhibit poor fruit set with hand pollination.

Literature Cited

1. Hayata, Y., Y. Niimi, R. Isoda, and K. Makita. 1990. Effect of cytokinin on the fruit setting and sugar accumulation of melon fruit. Abstr. Jpn. Soc. Hort. Sci. Spring Meeting 59:178-179.

2. Hayata, Y., Y. Niimi, and N. Iwasaki. 1995. Synthetic cytokinin--1- (2-chloro-4-pyridyl)-3-phenylurea (CPPU)--promotes fruit set and induces parthenocarpy in watermelon. J. Amer. Soc. Hort. Sci. 120:997-1000.

3. Jones, C.M. 1965. Effects of benzyladenine on fruit set in muskmelon. Amer. Soc. Hort. Sci. 87:335-340.

4. Norris, L.A. and V.H. Freed. 1963. Dimethyl sulfoxide as an absorption and translocation aid. Western Weed Control Conf., Res. Prog. Rpt., pp. 85-86.

Selection and Characterization of Known Genetic Marker Accessions for Hybridization with Commercial Varieties of *Cucumis melo* L.

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Introduction. The available cultivars of melon (*Cucumis melo* L.) require improvement in their resistance to diseases like powdery mildew, Fusarium wilt and CGMMV, in addition to other agronomic, vegetative, and fruit characters.

The improvement of available cultivars require broadening the genetic base by exploitation of existing genetic markers, for various characters such as disease resistance, in addition to other horticultural characters (1,3). This can be achieved by interspecific or intervarietal hybridization, which has number of limitations - the interspecific incompatibility being the most serious barrier (2). Somatic hybridization/protoplast fusion or normal selection followed by inbreeding can also be adopted to overcome this barrier. Thus, it becomes necessary to study the field performance of known genetic markers (1). In the present study, known genetic marker lines (1) obtained from Montfavet, France, have been characterized in order to incorporate the marker gene(s) in somatic hybridization and conventional hybridization studies based on their agronomic, vegetative, and fruit characters.

Materials and Methods. Melon is a summer crop and the ideal time for sowing in Delhi is February. The crop was sown in the third to fourth week of February. Preparation of the field was undertaken during the first two weeks of February, which requires proper manuring and irrigation of the field. Horizontal rows were made in the field and the seeds were directly sown on the edge of one side. Spacing of hills was kept as 1.0 m and row to row spacing was 2 m. Four to five seeds were sown per hill in an irrigated field.

The seven genetic marker lines (1) and the indigenous cvs were directly sown in the field in 1992, 1993 and 1994. The accessions resistant to powdery mildew, Fusarium wilt and CGMMV (EC-327434, EC-327435, and EC-327440, respectively) were scored for vegetative characters. EC-327434 did not reach the fruiting stage because of Fusarium wilt infection during flowering. Various fruit characters listed in Table 1 and 2 were scored for accessions EC-327435, EC-327440, for Fusarium wilt-resistant lines Harela, Kakri, NC 62963, M4 and for 'Pusa Madhuras' (PM) and 'Pusa Sharbati' (PS).

Results and Discussion. Vegetative and fruit data for the accessions and cultivars are summarized in Tables 1 and 2.

Genetic marker line EC-327435 and EC-327440 can be utilized in conventional/somatic hybridization studies for incorporation of *Pm-1*, *Pm-2*, and *Fom-3* genes in cultivated/commercial varieties (1). These lines performed well under Delhi field conditions, producing fruit with good size, weight, and seed number.

Literature Cited

1. Jain, J. and T.A. More. 1994. Preliminary screening of indigenous cultivars and a few known marker lines of *Cucumis melo* for Fusarium wilt and CCMMV resistance. Cucurbit Gen. Coop. Report. 17:69-71.

2. M. Chatterjee and T.A. More. 1991. Interspecific hybridisation in *Cucumis* spp. Cucurbit Gen. Coop. Report. 14:69-70.

3. Nuez, F., J. Prohens, M.J. Diez and P. Fernandez de Cordova. 1994. *Cucumis melo* L. accessions of the gene bank of the Polytechnical University of Valencia. Cucurbit Gen. Coop. Report. 17:57-60.

Table 1. Vegetative and fruit characters of melon accessions and cultivars under field conditions in Delhi, India.

Accession/cultivar	Vegetative characters	Fruit characters			
		Size and shape of the fruit	Color of the fruit skin	No. of ribs	Flesh color
'Arka Jeet'	Yellowish green leaves with smooth leaf surface	No flowering and fruit production			
EC-327434 PI 124112 (*Pm-4*, *Pm-5*)	Dark green leaves, leaf margin, inundated, somewhat rough leaf surface	No flowering and fruit production			
EC-327435 ('PMR-5') (*Pm-1*, *Pm-2*)	Small, closely placed green leaves with smooth linear margin, hairy petiole	Small size, round to oval shape	Yellowish green, wrinkled skin	10	Orangish yellow
EC-327440 ('Perlita FR') (*Fom-3*)	Large leaves with rough surface and hairy petiole	Flat round to oblong in shape, medium sized fruits	Greenish grey, rough rectum pattern of the skin	10	Pale green
Harela (*Fusarium* wilt resistant)	Fast growing plant with small yellowish green leaves, smooth leaf surface, fragile	Fruits not available			
Kakri (*Fusarium* wilt resistant)	Yellowish green leaves, smooth leaf surface with linear margin, hairy petiole	Long, cylindrical, with tapering ends	Ridged, light green	-	Whitish green

Table 1 (Contd.)

Accession/cultivar.	Vegetative characters	Fruit Characters			
		Size and shape of the fruit	Color of the fruit skin	No. of ribs	Flesh color
M4 (developing line) *Fusarium* wilt resistant	Small, closely placed yellowish green leaves with smooth leaf surface	Round, large sized	Greenish yellow, smooth skin	10	Greenish yellow
NC 62963 (*Fusarium* wilt resistant line)	Small, less spreading plant	Long, oblong	Yellowish to green with patchy skin	Not present	Whitish orange
'Pusa Sharbati'(PS)	Small, fast growing plant	Flat round, small sized	Orange green with rough reticulate pattern of skin	10	Dark orange
'Pusa Madhuras' (PM) (Control)	Fast growing vine, yellowish green small leaves with smooth serrated leaf margins, tendrils present	Flat round	Yellowish green, smooth skin with dark green ribs	10	Orangish yellow

Table 2. Fruit characters of some melon genetic marker lines and cultivars.

Accession/cultivar	Fruit weight (g)	Flesh thickness (cm)	Cavity (cm)	Fresh seeds wt. (g)	Soluble solids (%)
EC-327435	350.00±95.39[z]	2.18±0.36	5.40±0.00	60.00±33.16	4.00±0.00
EC-327440	840.00±84.85	2.38±0.15	5.63±0.15	115.00±7.07	5.25±0.35
Kakri	700.00±214.47	NR[y]	6.38±0.95	NR	NR
NC 62963	383.33±104.08	2.66±0.40	3.45±0.33	133.33±28.85	4.00±1.00
M4	1331.50±143.77	3.04±0.29	7.21±1.67	230.62±83.96	6.72±1.44
PS	575.00±35.35	3.50±0.70	5.25±0.35	56.00±7.07	6.50±0.70
PM	588.13±50.37	2.36±0.45	7.26±0.24	142.25±35.00	8.57±0.17

[z] mean ± standard deviation.

[y] not recorded.

Identification and Selection of Genetic Marker Donor Lines for Incorporation of Disease Resistance in Cultivars of *Cucumis melo* L.

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Introduction. Some of the major diseases of muskmelon (e.g., cucumber green mottle mosaicism, powdery mildew and Fusarium wilt) have been reported in Delhi and surrounding areas, thereby highlighting the need to develop multiple disease resistant varieties. This can be achieved by adopting conventional breeding methods of interspecific hybridization, and overcoming interspecific incompatibility (5). In the present study, a step towards somatic hybridization/protoplast fusion involved identifying and selecting donor lines/sources of resistance with suitable genetic marker(s), outlining their regeneration potential, and determining their vegetative and fruit characteristics.

Materials and Methods. Seven marker lines of melon were selected as donor lines based on the information on genetic markers in the *Cucumis melo* L. gene list (3,5). Two cultivated varieties of melon, Pusa Sharbati (PS) and Pusa Madhuras (PM), and a good performing developing line M4 were selected as recipient lines (5).

Since the availability of somatic hybrids is based upon the regeneration potential of the two parent cells/lines, it became necessary to outline the regeneration potential of donor and recipient lines in the present research work.

Regeneration response of the seven donor and three recipient lines was studied in 1990 and 1991, on a pre-standardized callus formation medium of MS + 0.5 mg/l benzyl adenine (MB) and a differentiation medium of MS+1.0 mg/IAA and 5.0 mg/l kinetin (MIK) (1). Two of the recipient lines, PM and M4, had already been reported in 1991 and 1992 to possess high regeneration potential governed by dominant G (2).

Characterization for vegetative and fruit characters involved percentage germinability under controlled conditions in a net house on a soil mixture of FYM:sand:soil in the ratio of 1:1:1 (v/v/v). Donor and recipient lines were screened for Fusarium wilt and cucumber green mottle mosaicism (CGMMV) under field conditions at various stages of development up to fruit harvest, and for significant vegetative character and fruit characters (Tables 2 and 3) during the cropping period from February to the middle of June in 1992, 1993 and 1994.

Results. Seven marker lines were chosen based on their genetic resistance to powdery mildew and Fusarium wilt. EC-327435 and EC-327434 are known to possess genes for powdery mildew resistance (*Pm-1* and *Pm-2*, and *Pm-4* and *Pm-5*, respectively). EC-327437 and EC-327440 possess the Fusarium wilt resistance genes *Fom-1* and *Fom-3*, respectively, and EC-327438 and EC-327439 possess the vegetative characters dissected leaf (*dl*) and green leaves (*gl*), respectively (Table 1).

Based on regeneration studies, it was found that except for EC-327434 no other donor line differentiated on a standardized medium (Table 1). Among the recipient lines, PM and M4 possess high regenerability; PS was found to be nearly non-regenerative and therefore cannot be involved in somatic hybridization studies.

Germination of EC-327434 and EC-327440 was 100 percent, while that of EC-327435 was 83% (Table 2). EC-327434 was found to be sensitive to CGMMV and did not reach the flowering stage, hence there was no fruit formation. However, EC-327435 and EC-327440 reached the fruit harvest stage, and hence were characterized for vegetative and fruit characters along with the recipient lines.

Tables 3 and 4 describe the vegetative and fruit characteristics of the donor and recipient accessions. Fruits of EC-327435 were round to oval in shape with orange-yellow pulp, and weighed approx. 350 g with a T.S.S. of approx. 4%. Fruits of EC-327440 were flat-round to oblong in shape with pale green pulp, weighed approx. 840 g and had a T.S.S. of 5.3%. Among the recipient lines, fruits of PM were small sized with a high T.S.S. of 8.6%, fruits of M4 were large sized with a T.S.S. of 6.7%., and fruits of PS (which was non-regenerative in culture) were small at 575 g and had a T.S.S. of 6.5%.

Discussion. EC-327434 can be utilized as a donor line for marker genes *Pm-4* and *Pm-5* with the indigenous cultigens (PM and M4) which have been identified for a

Table 1. Regeneration response of marker donor lines and recipient cultivars of *Cucumis melo* L.

Marker lines or cultivars	Genetics	Explant	Regeneration Response (%)			
			Callus formation	Callus differen- tiation into shoot buds	Callus differen- tiation into roots	No change
Donor lines						
EC-327434	PI 124112	Cot. leaves	9.1	72.7	18.2	0
	(*PM-4, PM-5*)	Epicotyl	81.8	9.1	0	9.1
EC-327435	PMR-5	Cot. leaves	-	-	-	100
	(*PM-1, PM-2*)	Epicotyl	-	-	-	100
EC-327436	Va 435 (*nsv*)	Cot. leaves	-	-	-	100
		Epicotyl	-	-	-	100
EC-327437	Charentais	Cot. leaves	-	-	-	100
	(*Fom-1*)	Epicotyl	-	-	-	100
EC-327438	Marker (*dl*)	Cot. leaves	-	-	-	100
		Epicotyl	-	-	-	100
EC-327439	Marker (*gl*)	Cot. leaves	-	-	-	100
		Epicotyl	-	-	-	100
EC-327440	Perlita FR	Cot. leaves	-	-	-	100
	(*Fom-3*)	Epicotyl	-	-	-	100
Recipient lines						
Pusa Madhuras	*aa G_*	Cot. leaves	83.3 ± 28.9	16.7 ± 2.9	0.0	0.0
		Epicotyl	33.3 ± 7.2	66.7 ± 7.2	0.0	0.0
Pusa Sharbati	*aa G_*	Cot. leaves	0.0	1.7 ± 2.9	0.0	98.3 ± 2.9
		Epicotyl	0.0	0.0	0.0	100
Developing M4	*A_G_*	Cot. leaves	56.1 ± 20.3	30.5 ± 6.8	0.0	13.4 ± 4.2
		Epicotyl	75.2 ± 12.3	23.8 ± 10.8	0.0	1.0 ± 0.7

Table 2. Seed germinability and sensitivity to *Fusarium oxysporum* f.sp. *melonis* and cucumber green mottle mosaic virus (CGMMV) in marker donor lines and recipient cultivars of melon.

Accession/cv.	Germination (%)	Sensitivity to Fusarium wilt	Sensitivity to CGMMV	Fruits with seeds harvested
EC-327434	100.0 ± 0.0	++[y]	NAS[w]	No
EC-327435	83.3 ± 14.4	-[x]	-	Yes
EC-327436	41.7 ± 14.4	++	NAS	No
EC-327437	83.3 ± 14.4	++	NAS	No
EC-327438	4.0 ± 1.5	++	NAS	No
EC-327439	41.7 ± 14.4	++	NAS	No
EC-327440	100.0 ± 0.0	-	-	Yes
Pusa Madhuras[z]	100.0 ± 0.0	-	-	Yes
Pusa Sharbati	37.5 ± 4.2	-	-	Yes
M4	83.3 ± 0.8	-	-	Yes

[z]Control
[y]Not sensitive
[x]100% sensitive
[w]NAS = not available for scoring

Table 3. Descriptive vegetative and fruit characters of marker donor lines and recipient cultivars of melon.

Accessions/cvs.	Vegetative characters	Size and shape of the fruit	Color of the skin	No. of vein tracts	Flesh color
EC-327434	Dark green leaves. leaf margin inundated, somewhat rough leaf surface	*- - Fruits Not Available - -*			
EC-327435	Small, closely placed green leaves with smooth linear margin, hairy petiole	Small size, round to oval shape	Yellowish green, wrinkled skin	10	Orangish yellow
EC-327440	Large leaves with rough surface and hairy petiole	Flat round to oblong in shape, medium sized fruits	Greenish gray, rough rectum, pattern of the skin	10	Pale green
M4 (developing line)	Small, closely placed yellowish green leaves with smooth leaf surface	Round, large sized	Greenish yellow, smooth skin	10	Greenish yellow
Pusa Sharbati	Small, fast growing plant	Flat round, small sized	Orange green with rough and reticulate pattern of skin	10	Dark orange
Pusa Madhuras	Fast growing, yellowish green leaves with smooth serrated leaf margin, tendrils present	Flat round small skin with dark green vein tracts	Yellowish green, smooth	10	Orangish yellow

Table 4. Fruits characteristics of donor marker lines and recipient cultivars of melon.

Accessions/cvs.	Fruits harvested	Fruit weight (g)	Flesh thickness (cm)	Cavity (cm)	Fresh seeds weight (g)	T.S.S. (%)
EC-327434	-	-	-	-	-	-
EC-327435	+	350.0 ± 95.4	2.2 ± 0.4	5.4 ± 0.0	60.0 ± 33.2	4.0 ± 0.0
EC-327440	+	840.0 ± 84.9	2.4 ± 0.2	5.6 ± 0.2	115.0 ± 7.1	5.3 ± 0.4
M4	+	1331.5 ± 143.8	3.0 ± 0.3	7.2 ± 1.7	230.6 ± 84.0	6.7 ± 1.4
Pusa Sharbati	+	575.0 ± 35.4	3.5 ± 0.7	5.3 ± 0.4	56.0 ± 7.1	6.5 ± 0.7
Pusa Madhuras	+	588.1 ± 50.4	2.4 ± 0.5	7.3 ± 0.2	142.3 ± 35.0	8.6 ± 0.2

genetic marker G for high regeneration potential (2). Further work is required in outlining the feasibility of interlineal hybridization between identified and donor selected lines with the isogenic lines of available indigenous cvs., in addition to somatic hybridization.

Literature Cited

1. Jain, J. and T.A. More. 1992. *In vitro* regeneration in *Cucumis melo* cv. Pusa Madhuras. Cucurbit Genetics Coop. Rept. 15:62-64.

2. Jain, J. and T.A. More. 1993. Genotypic control of regeneration potential in *Cucumis melo*. Cucurbit Genetics Coop. Rept. 16:53-54.

3. Jain, J. and T.A. More. 1994. Preliminary screening of indigenous cultivars and a few known marker lines of *Cucumis melo* for Fusarium wilt and CGMMV resistance. Cucurbit Genetics Coop. Rept. 17:69-71.

4. Jain, J. and T.A. More. 1995. Regeneration response of a few genetic marker lines and commercial cultivars of *Cucumis melo* L. Cucurbit Genetics Coop. Rept. 18:48-49.

5. Jain, J. and T.A. More. 1991. Contribution in: Research achievements in muskmelon. In: H.S. Gill, T.A. More, N. Singh and A.S. Kataria, eds. Vegetable Research -A 'resume' (1956-90). Ankur Publishers, New Delhi. pp. 17-25k.

6. Pitrat, Michael. 1990. Gene list of *Cucumis melo* L. Cucurbit Genetics Coop. Rept. 13:58-68.

Host Range of a Melon Yellowing Virus Transmitted by *Bemisia tabaci* (Gennadius) in Southern Spain

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To study the host range of a melon yellowing virus transmitted by *Bemisia tabaci* (Gennadius) in Spain (4), plant species most commonly cultivated in the area, weeds growing near the greenhouses, and two species of *Nicotiana* were tested.

- **Cucurbitaceae**: *Cucumis sativus* L. cv. Bellpuig, *Cucurbita maxima* Duch., *Citrullus lanatus* (Thunb) Matsum. et Nakai cv. Sugar Baby, *Cucurbita pepo* L. cv. Negro Belleza

- **Solanaceae**: *Lycopersicon esculentum* Mill. cv. Daniela, *Solanum melongena* L. cv. Redonda Negra Lisa, *Capsicum annuum* L. cv. Italico, *Nicotiana tabacum* L. cv. Xanthi, *Nicotiana benthamiana* L.

- **Compositae**: *Lactuca sativa* L. cv. Summer Bibb and cv. Romana, *Sonchus oleraceus* L.

- **Leguminosae**: *Phaseolus vulgaris* L., *Pisum sativum* L. cv. Alderman

- **Cruciferae**: *Capsella bursa-pastoris* (L.) Medicus

- **Malvaceae**: *Malva parviflora* L.

- **Portulacaceae**: *Portulaca oleracea* L.

- **Chenopodiaceae**: *Chenopodium album* L.

Cucumis melo cv. Piel de Sapo was used as a susceptible control. Because the virus was not isolated when the host range was evaluated, controlled inoculations using *B. tabaci* as the virus vector were carried out in two steps. In the first one, ten seedlings of each species were inoculated using *C. melo* as inoculum source. A second step was necessary to determine if the virus was present in the inoculated plants of the different species tested. In this step the inoculum sources used were the inoculated plants of the species being tested. Ten seedlings of each species and ten seedlings of melon were inoculated. Controlled inoculations were carried out by placing 60 individuals of *B. tabaci* on the inoculum source, where they were allowed to feed for 48 hours. Then, the whiteflies were transferred to healthy seedlings and allowed to feed for 72 hours. Whiteflies were then killed and plants were moved to an insect-proof glasshouse to await the appearance of symptoms. Five plants of each species that had never been in contact with whiteflies were used as indicators of possible undesirable inoculations.

Results indicated that the host range of melon yellowing virus transmitted by *B. tabaci* appears to be restricted to Cucurbitaceae (Table 1). Within this plant family, almost all plants of each species tested showed symptoms of infection in the first and the second steps (Table 1). Symptoms of this yellowing viral disease in the cucurbit species were similar to the ones described in *C. melo* (2).

Regarding host range and other preliminary results concerning molecular aspects of this virus (Rodríguez-Cerezo, unpublished data), melon yellowing virus in South Spain differs from viruses or strains described in other melon growing areas, such as lettuce infectious yellows virus (LIYV) (1) or cucurbit yellow stunting disorder virus (CYSDV) (3).

Literature Cited

1. Duffus, J.E., R.C. Larsen and H.Y. Liu. 1986. Lettuce infectious yellows virus - A new type of whitefly-transmitted virus. Phytopathology 76:97-100.

2. Gómez-Guillamón, M.L. y R. Camero. 1993. ¿Es *Bemisia tabaci* el vector de un nuevo virus de amarilleo?. II Congreso Ibérico de Ciencias Horticolas. Zaragoza. Actas de Horticultura 10:1356-1358.

3. Hassan, A.A. and J.E. Duffus. 1991. A review of a yellowing and stunting disorder of cucurbits in the United Arab Emirates. Emir. J. Agr. Sci., 2: 1-16.

4. Sesé, A.I.L., M.L. Gómez- Guillamón and J.R. Díaz-Ruiz. 1994. Appearance of a possible new melon yellowing disease in Spain. Cucurbit Genetics Cooperative Report, 17: 72-73.

Table 1. Incidence of melon yellowing disease in the Cucurbitaceae using different inoculum sources and hosts.

Species	A[z]	B	C	
Citrullus lanatus	10/10[y]	10/10	10/10	
Cucurbita pepo	10/10	---	9/10	
Cucurbita maxima	8/10	8/10	10/10	
Cucumis sativus	10/10	10/10	10/10	
Cucumis melo	10/10	10/10	10/10	

[z] Inoculum source and host genotype: A = *C. melo* on each species, B = each species on itself, C = each species on *C. melo*. *B. tabaci* was used as the virus vector.

[y] a/b: a = number of plants with symptoms, b = total plants inoculated; --- = data not recorded.

Temperature-Conditioned Response to *Sphaerotheca fuliginea* Race 1 in the Spanish Melon Cultivar ANC-57

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The response of a Spanish melon cultivar, ANC-57, to *Sphaerotheca fuliginea* race 1 is conditioned by temperature in such a way that it was resistant at 26 C and susceptible at 21 C (2). The resistance of this cultivar is due to one dominant gene different than *Pm-1* (Gómez-Guillamón et al., unpublished data). The behavior of this cultivar against *S. fuliginea* race 1 was observed under artificial conditions at different temperature regimes.

The following temperature regimes were studied: 1) 19 C constant temperature, 2) 26 C constant temperature, 3) 19 C from sowing until inoculation, and 26 C after inoculation; 4) 26 C from sowing until inoculation, and 19 C after inoculation; and 5) alternating periods of 12 hours at 19 C and 12 hours at 26 C. Relative humidity was 70% and the photoperiod was 16h light/8h darkness in all cases. The inoculations were carried out by spraying the second leaf of seedlings at the third true-leaf stage with a suspension of spores in an aqueous solution of Triton X-100 (10^{-3} ml/l) (1). The suspension was made from a monosporic isolation of *S. fuliginea* race 1 taken from melon (SF26A). Three genotypes were inoculated: 'Bola de Oro', known to be susceptible to the fungus, 'PMR-45', resistant to race 1 of *S. fuliginea*, and the Spanish cultivar ANC-57.

Data were collected 10 days after inoculation. The percentage of surface area of the leaf covered with powdery mildew and the number of spores per unit of surface were recorded. Two discs were cut out of each inoculated leaf and were placed in a known volume of Triton X-100 solution (with the same characteristics as that used for spore solution) and the spores were counted with a haemacytometer. Two readings were made of the samples from each disc.

All plants of 'Bola de Oro' showed symptoms of powdery mildew in all temperature regimes. 'PMR-45' was always resistant and its resistance was independent of experimental temperatures (Table 1).

Plants of 'ANC-57' cultivated at a constant 19 C showed slight symptoms of powdery mildew, and none of the plants maintained at 26 C constantly showed symptoms of infection (Table 1). When plants of 'ANC-57' were cultivated at 19 C and maintained at 26 C after inoculation, they were resistant to race 1 of *S. fuliginea*; however, when infected plants were cultivated at 26 C and maintained at 19 C after inoculation, they were susceptible. Plants cultivated under the alternating temperature regime 12h 19 C/12h 26 C showed some powdery mildew tolerance: only 4 % of the leaf surface was infected and the number of conidia per mm^2 was very small (Table 1).

Temperatures of 26 C or more were needed for the resistance gene to be expressed; nevertheless, when inoculated plants were exposed to the alternating temperature regime 12 h 19 C/12h 26 C, they showed only slight symptoms of infection. The resistance gene in 'ANC-57' is allelic to the one in other Spanish melon cultivars which do not show differential temperature response to *S. fuliginea* race 1 (Gómez-Guillamón et al., unpublished data). Therefore, modifier genes that affect the expression of the resistance gene could be present in 'ANC-57', as suggested earlier (2). These genes would have a temperature-conditioned response which could stimulate or inhibit the resistance gene action.

Literature Cited

1. Floris, E. 1993. Oidopsis en melón. Doctoral Thesis. Universidad de Zaragoza, Spain.

2. Gómez-Guillamón, M.L., and J.A. Torés. 1992. Influence of temperature on the resistance of *Sphaerotheca fuliginea* in Spanish melon cultivars. Proceedings of the Fifth EUCARPIA Cucurbitaceae Symposium, Skierniewice (Poland):155-159.

Table 1. Response of three melon genotypes to *S. fuliginea* race 1 under five different temperature regimes.

	19°C[z]						
	19°C[y]				26°C[y]		
Cultivar	p/p[x]	Leaf area infected (%)	Spore no.[w]		p/p[x]	Leaf area infected (%)	Spore no.[w]
Bola de Oro	5/5	60	99		5/5	46	129
PMR 45	0/7	0	0		0/5	0	0
ANC-57	4/4	35	44		0/5	0	0
	26°C[z]						
	19°C[y]				26°C[y]		
Bola de Oro	4/4	100	292		4/4	95	295
PMR 45	0/9	0	0		0/3	0	0
ANC-57	6/6	53	108		0/4	0	0
	12 h 26°C / 12 h 19°C[z]						
	12 h 26°C / 12 h 19°C[y]						
Bola de Oro	5/5	52	193				
PMR 45	0/8	0	0				
ANC-57	5/5	4	18				

[z]Temperature before inoculation.
[y]Temperature after inoculation.
[x]p/p : plants infected / plants inoculated.
[w]Per mm^2.

Potential Utility of RAPD Markers Linked to *Fom 2* Gene in Melon (*Cucumis melo* L.)

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Molecular marker identification and utilization in marker-assisted-selection (MAS) can be a valuable tool for the plant breeder, and has been an active research area the past several years. Recently, two research groups have identified RAPD markers linked to the dominant gene conferring resistance to Fusarium wilt races 0 and 1, *Fom 2*. Wechter et al. (6) used bulked segregant analysis to identify one primer (596) 2.2 centimorgans from *Fom 2* in 'MR-1'. In the first published molecular-marker map in melon, Baudracco-Arnas and Pitrat (2) found two RAPD primers closely linked to *Fom 2* in an F$_2$ population derived from 'Songwhan Charmi' (PI 161375) and 'Vedrantais'. E07 and G17 are flanking markers 1.6 and 4.5 cm, respectively, from the *Fom 2* gene.

Screening young plant populations for Fusarium wilt resistance is a fairly simple, straightforward test. Based on this criteria, molecular markers appear to have little practical utility in breeding for resistance. An exception to this would be in the case of selecting for resistant genotypes where the pathogen is not native, and its entry is restricted. In this situation, a marker for *Fom 2* would be valuable. In addition, a linked molecular marker would facilitate simultaneous multiple-disease screening that would otherwise be difficult, and could facilitate cloning of the resistance gene.

The objective of this study was to assess the applicability of these markers for use in marker-assisted selection of Fusarium wilt race 1 resistance in melon. The initial question of interest is, are these primers specific to populations derived from the source genotype of the identified primer, or are they usable in other populations? The results may indicate the ancestry and relatedness of *Fom 2* genes in different melon genotypes.

Materials and Methods. Young leaves were harvested from greenhouse grown plants and DNA was extracted according to the protocol of Baudracco-Arnas (1). Along with primer source genotypes ('MR-1', 'Vedrantais') other known susceptible ('Ananas Yokneum' [AY], 'Topmark') and resistant genotypes (PI 161375) were screened with the 3 RAPD primers (Table 1). In addition, a subsample from a segregating backcross population (MD 8654 used as the *Fom 2* source) was also screened with the primers. The PCR reactions followed the general protocol of Giovannoni et al. (3). Reactions (25 µl volumes) were carried out in a Perkin Elmer 480 thermocycler. Each reaction contained 2 µl DNA (5 ng/µl stock), 17 µl sterile distilled H$_2$0, 2.5 µl 10x PCR buffer, 1.2 µl GATC nucleotides, 0.1 µl MgCl$_2$ (100 mM), 0.1 µl Perkin-Elmer Ampli*Taq* polymerase, and 2 µl of the 10-mer primer (3 ng/µl stock) synthesized by Gibco BRL Life Technologies (Grand Island, New York). The following thermocycles were used: 1 cycle, 94 C 10 min; 44 cycles, 94 C 1 min / 36 C 1 min / 72 C 2 min; 1 cycle: 94 C 1 min / 36 C 1 min / 72 C 10 min. Amplified products were separated on an 2.0% agarose gel run at 5v/cm for 4 hours. Gels were scored for the presence or absence of the linked fragment of each primer: 595 - 1.6 kb, E07 - 1.3 kb, G17 - 1.0 kb.

Results and Discussion. Several gels were run with each primer to evaluate the utility of each for selecting resistant genotypes. Of the 3 primers, we had the most difficulty in producing consistent amplification of the linked fragment with 596. Therefore, no scoring could be done with primer 596. PCR conditions will have to be tested to optimize the reactions. The unstable nature of PCR based RAPDs is well documented, and has been a problem in *Cucumis* (5).

The other two primers identified in 'Vedrantais' did produce the expected fragment profile on most gels. Scoring G17 was difficult because of a fragment in both resistant and susceptible genotypes that was only 50 bp in size smaller than the linked fragment. This fragment (1.0 kb) is linked to the susceptible allele (*fom 2*) and was seen in the susceptible 'Vedrantais' (source) and 'Ananas Yokneum', but was absent in the resistant genotypes PI 161375 and 'MR-1'. In a small sample of lines segregating for *Fom 2* from the test population, the susceptible band appeared in some, but not all susceptible genotypes. Primer E07 performed like G17 in the parental lines, and with a subset of segregating lines, did accurately predict phenotype. The linked fragment,

Table 1. Characteristics of RAPD primers linked to the dominant, resistant gene conferring resistance to Fusarium wilt races 0 and 1 in melon (*Fom 2*).

Primer	Sequence 5'-3'	Reference	Source Genotype/phenotype	Fragment size (kb)
596	CCC CTC GAA T	(6)	'MR-1'/Resist.	1.6
E07	AGA TGC AGC C	(2)	'Vedrantais'/Susc.	1.3
G17	ACG ACC GAC A	(2)	'Vedrantais'/Susc.	1.0

however, was not consistently amplified in every gel. E07 is more tightly linked to *Fom 2* than G17 (1.6 vs. 4.5 cm), and therefore would more consistently identify the correct phenotype.

Wechter et al. (6) did not find the 1.6 kb fragment from primer 596 in 3 other resistant genotypes tested. Their data indicate that this primer may be utilized only in populations derived from MR-1. We were not able at this time to get consistent data with this primer, and therefore cannot contribute further data on this primer.

Data from the two primers linked to the susceptible allele in 'Vedrantais' indicate potential success in identifying susceptible melon individuals from diverse backgrounds. In the small sample thus far tested, these primers have co-segregated with phenotype. Assuming that susceptibility is the older, ancestral form of the *Fom 2* gene, a linked marker to the susceptible allele may be usable over a wider array of genotypes than one linked to the resistant allele. This hypothesis can only be tested after evaluating many known susceptible and resistant genotypes with these primers, and determining co-segregation with phenotype.

The fragment from E07 and G17 is linked to the susceptible allele, thus the resistant genotype has no band. This is not desirable when using MAS, since a null band could result from a failed PCR reaction, and not the absence of the linked fragment (i.e., a true, resistant plant). Contrarily, the advantage of a linked susceptible marker is that homozygous dominant individuals could be identified from heterozygous individuals. Wechter et al. (6) identified a second primer which was linked to the susceptible allele; however, they did not pursue this further because of the above mentioned problem. It would be interesting to test this primer along with the other two from 'Vedrantais'.

The problems of RAPD consistency and difficulty in scoring (multiple fragments per single primer) could be overcome if RAPDs are converted to SCAR (sequence characterized amplified regions) markers (4). This would become attractive economically if identified RAPD primers could be utilized across diverse genotypes. The preliminary data presented here shows that two primers linked to the susceptible allele may work across melon genotypes, although more data is needed to confirm this.

Literature Cited

1. Baudracco-Arnas S. 1995. A simple and inexpensive method for DNA extraction from *Cucumis melo* L. Cucurbit Genetics Coop. Rept. 18:50-51.

2. Baudracco-Arnas, S. and M. Pitrat. 1996. A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor. Appl. Genet. (In Press).

3. Giovannoni, J.J., R.A. Wing, M.W. Ganal and S.D. Tanksley. 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucl. Acid. Res. 19:6553-6558.

4. Paran I. and R.W. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85:985-993.

5. Staub J., J. Bacher and K. Poetter. 1996. Sources of potential errors in the application of random amplified polymorphic DNAs in cucumber. HortScience 32:262-266.

6. Wechter W.P., M.P. Whitehead, C.E. Thomas and R.A. Dean. 1995. Identification of a randomly amplified polymorphic DNA marker linked to the *Fom 2* Fusarium wilt resistance gene in muskmelon MR-1. Phytopathology 85:1245-1249.

Micropropagation of the Melon Hybrid 'Galia'

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'Galia' is one of the most important melon genotypes cultivated in greenhouses in the Mediterranean basin. *In vitro* techniques for clonal micropropagation have been used with good results in species of the Cucurbitaceae such as cucumber (*Cucumis sativus*; 2,5), pumpkin (*Cucurbita pepo*; 4) and watermelon (*Citrullus lanatus*; 1). The objective of this work was to test different growth regulator combinations for micropropagating the melon (*Cucumis melo*) hybrid 'Galia'.

Seeds of 'Galia' (Hazera, 1939, L.T.D., Israel) were soaked in a 75% v/v ethanol solution for 1 minute, subsequently in a Triton x 100 (Merk) 0.01 % v/v solution for 15 minutes, and finally surface sterilized in a Milton (Procter & Gamble L.T.D.) solution (1% w/v NaOCl and 16.5% w/v NaCl) for 25 minutes. The seeds were then rinsed three times with sterile water and placed under aseptic conditions in vessels containing Murashige and Skoog (MS) medium (3) solidified with 0.8% w/v agar (Sigma). Cultures were placed in a growth chamber to allow germination and shoot growth until the 6-8 leaf stage. Axillary buds were used as the explant source.

All the experiments were conducted in a growth chamber maintained at 25 C provided with 16 hours photoperiod by cool white fluorescent light at 1500 lux. Media were adjusted to a pH of 5.7, autoclaved at 121 C for 20 minutes, and solidified with 0.8% w/v agar. Three experiments were conducted to estimate the optimum level of growth regulators and to quantify the rate of propagation. In every experiment eight replications were used. Four weeks after the establishment of each experiment the number of the shoots at least 0.5 cm long were measured.

Experiment 1 - Estimating the optimum level for cytokinin: Axillary buds derived from *in vitro* grown shoots were established in vessels containing MS medium supplied with 0.0, 0.5, 1.0 or 2.0 mg/l 6-benzylaminopurine (BA).

The means of the shoots produced per axillary bud are presented in Table 1. It is obvious that BA level affects the number of the shoots produced, and the optimum level is 2.0 mg/l (2.37 shoots per explant).

Experiment 2 - Estimating the optimum level for auxin: Axillary buds derived from *in vitro* grown shoots were transferred in vessels containing MS medium supplied with the optimum BA level found in the previous experiment (2.0 mg/l) and 0.0, 0.1 or 0.5 mg/l indole-3-acetic acid (IAA).

The means of the shoots produced per axillary bud are presented in Table 2. Best results were obtained with 0.1 mg/l IAA, yielding 2.50 shoots per explant. A cluster of shoots produced from a developed axillary bud, which was cultivated four weeks in the above medium, is shown in Fig. 1.

Experiment 3 - The effect of callus removal from the explants: Axillary buds produced *in vitro* were cultivated on MS medium supplemented with the optimum levels of BA and IAA (2.0 mg/l BA, 0.1 mg/l IAA). Two weeks after culture establishment, the formed callus was removed from the explants base, and the explants were transferred to fresh media of the same composition.

In the previous experiments, a significant mass of callus formed at the explants base, which possibly depressed growth. This experiment was aimed to study this problem. The explants which had callus removed two weeks after the initial establishment showed significantly more shoots per explant (4.75 vs. 3.62, LSD:0.89, at P=0.05).

Elongation and rooting of the produced shoots, after their excision from the clusters, was carried out on MS medium with no growth regulators within 4 weeks (Fig. 2).

In conclusion, the data indicated that:

a) The presence of cytokinin (BA) in the culture media increased the shoot propagation rate, and the optimum level was 2.0 mg/l. This level is higher than ones reported for others cucurbits. BA levels of 0.5 and 1.0 mg/l have been proposed for two cucumber genotypes (5), and 1 mg/l for pumpkin (4). Optimum levels of kinetin raged from 0.1 mg/l in cucumber (2) to 1.0 mg/l for watermelon (1).

b) The presence of auxin (IAA) in the media affected the number of shoots produced, with the best results

Figure 1. Cluster of shoots from an *in vitro* produced and developed axillary bud which was cultivated four weeks on an MS medium supplemented with 2 mg/l BA and 0.1 mg/l IAA.
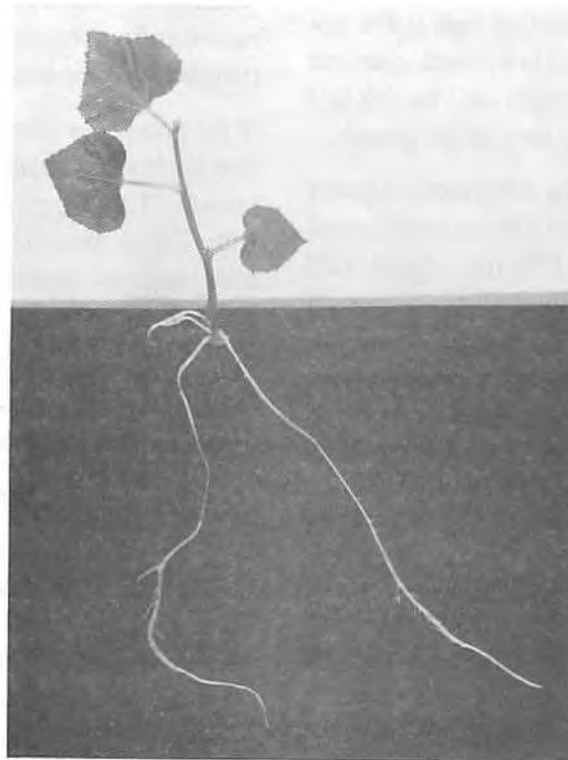

Figure 2. Plantlet derived after cultivation of a shoot, which was excised from a cluster formed in conditions as described in Fig. 1, for four weeks on growth regulator free MS medium.

at 0.1 mg/l IAA. This auxin level was similar to those proposed for one cucumber genotype (0.1 mg/l IAA) (2) and for watermelon (0.04 mg/l IAA) (1). For two other cucumber genotypes (5) and for pumpkin (4), best results were obtained in media lacking auxin.

Literature cited

1. Barnes, L.R. 1979. *In vitro* propagation of watermelon. HortScience 11:223-227.

2. Handley, L.W. and O.L. Chambliss. 1979. *In vitro* propagation of *Cucumis sativus* L. HortScience 14:22-23.

3. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

4. Pink, D.A.C. and D.G.A. Walkey. 1984. Rapid propagation of *Cucurbita pepo* L. by culture of meristem tips. HortScience 24:107-114.

5. Sapountzakis, G. and A.S. Tsaftaris. 1994. Micropropagation of the cucumber hybrids 'Brunex' and 'Bambina'. Cucurbit Genetics Coop. Rep. 17:50-53.

Table 1. The mean number of shoots produced, per *in vitro* grown axillary bud, of the melon hybrid 'Galia', four weeks after cultivation on MS media supplemented with different levels of BA.

BA (mg/l)	0.0	0.5	1.0	2.0	
Shoots	1.00	1.87	1.75	2.37	

Sample Size: 8 explants per treatment.
LSD: 0.95 (at P=0.05).

Table 2. The mean number of shoots produced, per *in vitro* grown axillary bud, of the melon hybrid 'Galia', four weeks after cultivation on MS media supplemented with 2 mg/l BA and different levels of IAA.

IAA (mg/l)	0.0	0.1	0.5	
Shoots	1.87	2.50	2.25	

Sample Size: 8 explants per treatment.
LSD: 1.29 (at P= 0.05).

Breeding and Production of Watermelon for Edible Seed in China

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Introduction: Edible seed watermelon has been grown in China for several hundred years, mainly as a source of edible seed. This is the same species as common watermelon grown for flesh (*Citrullus lanatus* (Thunb.) Matsum and Nakai). The area under cultivation is increasing, up to 140,000 ha with seed yields of over 200,000 t, as the economy develops and demand for seed to be exported increases. Edible seed watermelon is grown mainly in the Provinces of Gansu, Xingjiang, Neimen, Ningxia, and Anhui, and is a very important economic crop in China.

Varieties and Type: Cultivated edible seed watermelon are divided into two classes: those with large black seeds, and those with red seeds. The edge of the large black-seeded type is black with a white center. Commercially, it is most desirable that the black and white sectors are sharply contrasted. Seed width is the important measure and is used as the standard for seed size, with narrower seeds bringing less profit than wider seeds. Before 1989, seed width was less than 10 mm, and seed yield was less than 1,500 kg/ha. After 1989, breeders, using various selection techniques, developed varieties with an increased seed width to approximately 11 mm. Over two to three years the larger-seeded varieties progressively replaced the smaller-seeded varieties, and yields increased to about 2,250 kg/ha. Representative varieties are 'GN- 1' and 'Jingyuan Daban'.

There are also varieties that are used for both flesh and seeds. In traditional Chinese medicine the flesh has been found to be good for the stomach, and in the edible seed production area, many people eat the flesh of these watermelons. The flesh of most edible seed varieties is poor, with soluble solids between 4 and 5%. However, there are varieties bred for both flesh and edible seeds, with soluble solids between 6 and 7%, the flavor is good, and the seed width is about 10 mm. A representative variety is 'Lanzhou Daban'. The skin of the fruit is thick and the flesh is pliable so there is little water loss and fruits can be preserved until November or December. The flesh has a distinctive flavor when eaten in winter.

Red-seeded edible seed varieties are cultivated over much less area than black-seeded varieties. The seed color is completely red and the width is about 8 mm. A representative variety is 'Ningxia Red'.

Seeds: In recent years, breeders have used larger red-seeded varieties grown for flesh, and crossed them to large black-seeded edible seed lines, and developed new lines through selection and inbreeding. These new strains have a seed width of about 10 mm, and their production has been well received in the expanding watermelon market.

Problems: Though there are many large black-seeded edible seed watermelon varieties, most are closely related, and they all are very susceptible to anthracnose, powdery mildew, and other diseases. The problem is that there are few larger-seeded edible seed accessions in the available watermelon germplasm. When edible seed varieties are crossed with disease-resistant varieties developed for flesh, the progeny have smaller seed and lower seed yield than is desirable. It has proven to be very difficult to breed varieties for disease-resistance and high seed yield.

Red-seeded varieties of edible seed watermelon are welcome in commerce, but they have only 50 to 70% of the yield of black-seeded varieties. The problem is that the inheritance of seed color is complicated, with three genes, r (red), t (tan), and w (white) interacting, and black-dotted seeds dominant over all three genes. Thus, if you cross red seed with any other seed color, the F_1 seeds are not red. It is difficult to breed for large-seeded, high yielding, red-seeded edible seed varieties.

The key to solving these problems is to find germplasm with desirable characteristics that can be used either directly or indirectly in edible seed watermelon breeding programs.

Literature cited

1. Li, J., Y. Zhai, and Y. Zhang. 1995. New variety of red-seed edible seed watermelon -- QJ-25. China Watermelon and Muskmelon 1:21-22.

2. Li, J., Y. Zhang, and Y. Zhai. 1995. Present situation of production and development of edible seed watermelon. Northwestern Horticulture 1:2-3.

3. Liu, Z. 1987. Varieties of edible seed watermelon in Ningxia. Bulletin of Melon Science and Technology 2:30.

4. Rhodes, B. and X. Zhang. 1995. Gene list for watermelon. Cucurbit Genet. Coop. Rpt. 18:69-84.

5. Wei, D. and D. Wu. 1986. Melon in Northwest of China. P: 79-80, 242.

6. Xiong, D. 1994. Present situation of production of red-seed edible seed watermelon -- Xingfeng. China Watermelon and Muskmelon 2:28-29.

7. Zhang, Z. and X. Zeng. 1992. New varieties of edible seed watermelon -- Jingyuan Daban. Chinese Vegetables 3:52-53.

Inheritance of Seed Size from Diverse Crosses in Watermelon

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Introduction: Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) is divided into two types of crops in China: common watermelon for flesh; and watermelon grown for seeds for human consumption (edible seed). Considerable diversity for seed size is found among the available germplasm. The largest varieties have seed in excess of 11.5 x 17.0 mm (width x length, mean of 10 seed), and the smallest varieties have mean seed width and length of 2.6 x 4.2 mm, respectively. The expression of this character (seed size) is controlled by genetic differences between varieties, and is affected by environment and cultural conditions. In this experiment, two varieties, expressing extreme phenotypes for seed size, were crossed and the inheritance of the trait analyzed. These results will provide help to breeders working on seed size, especially for edible seed watermelons.

Materials and Methods: Parents and progeny: P_1: tomato seed watermelon (ts) (furnished by Dr. B. Rhodes, Clemson University, Clemson, SC, USA); mean of 10 seeds, width x length = 2.6 x 4.2 mm. P_2: 'GN-1' (GN); edible seed watermelon; self-pollinated; mean of 10 seeds, width x length = 11.5 x 17.6 mm. Progeny included: $F_1 = P_1$ x P_2, and the reciprocal cross, P_2 x P_1; $F_2 = F_1$ self-pollinated; BC_1 (F_1 x P_1); and BC_2 (F_1 x P_2). Field plots were grown from 1993 to 1995 at the Tiaoshan Farm in Gansu. Seed size (width and length) were recorded in September, 1995.

Results and Discussion: The results are presented in Table 1, and graphically in Figure 1. A *t*-test showed no statistical difference between the F_1 progeny from the reciprocal crosses [P_1(ts) x P_2(GN) and P_2(GN) x P_1(ts)], negating cytoplasmic inheritance and allowing the progeny from the two crosses to be analyzed as one. The regression analysis showed that width and length are controlled by the same genes ($r = 0.9898$). The F_1 seeds were all medium in size between the two parents; the F_2 progeny segregated in a ratio of 1:2:1 (small: medium: large); BC_1 (ts x F_1) segregated 1:1 (small: medium); and BC_2 (GN x F_1) segregated 1:1 (medium: large). These results fit a pattern of inheritance of a single gene with two alleles, and incomplete dominance between alleles. In addition, when the F_2, BC_1, and BC_2 progeny are compared in Figure 1, the group of small seeds in BC_1 (F_1 x ts) are smaller than the corresponding group in the

Figure 1: Segregation for seed size in the F2 and the reciprocal backcross population of the cross ts x GN in field plots at the Tiaoshan Farm, Ganshu, P. R. China, 1995.
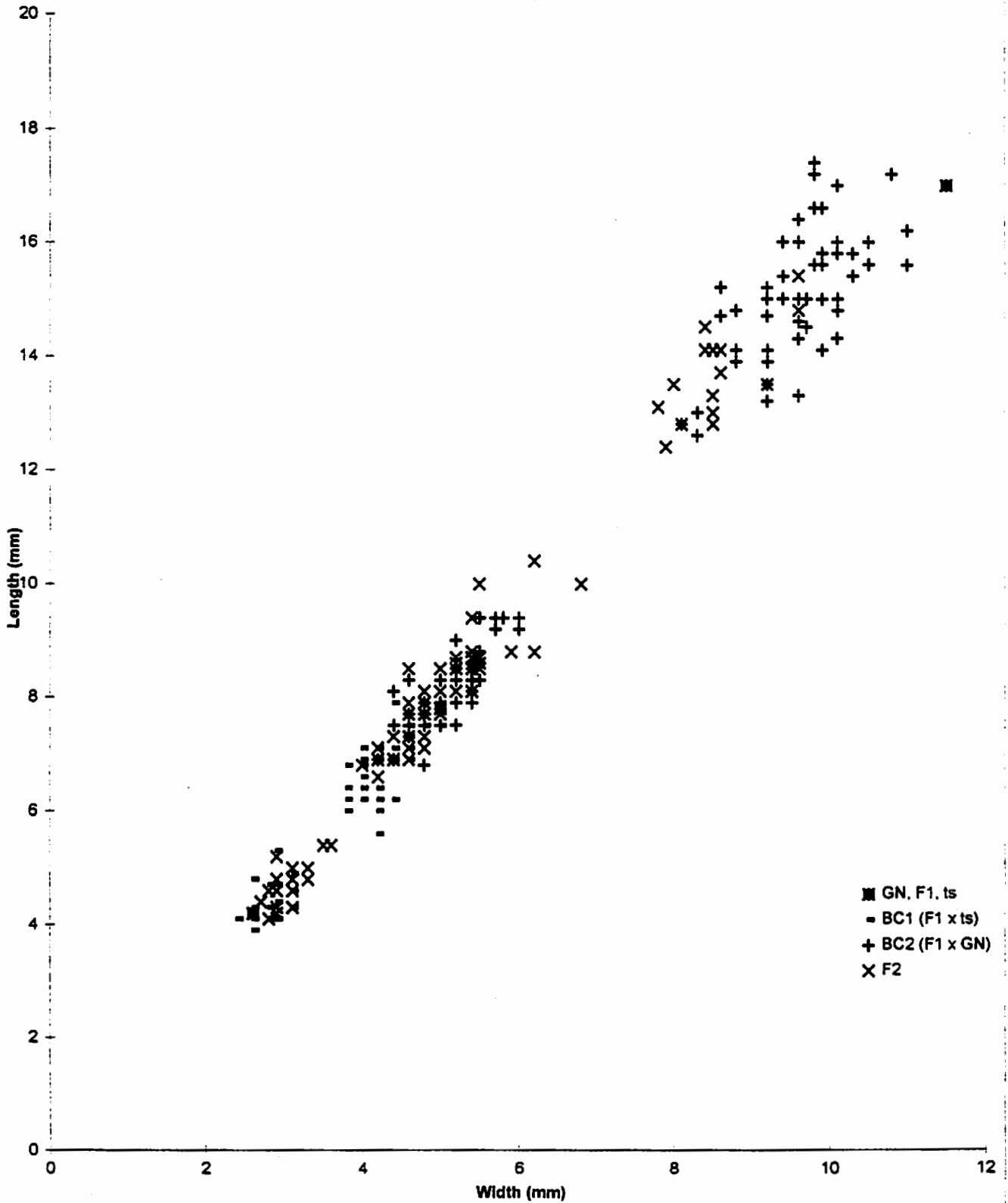
F$_2$ population, and the group of large seeds in BC$_2$ (F$_1$ x GN) are larger than the corresponding group among the F$_2$ progeny. These comparisons suggest that several modifier or minor genes influence seed size, depending upon the parents involved in each cross.

Literature cited

1. Rhodes, B. and X. Zhang. 1995. Gene list for watermelon. Cucurbit Genet. Coop. Rpt. 15:69-84.

2. Suzuki, D. T., A. J. F. Griffiths, and R. C. Lewontin. 1981. Genetic Analysis, 2nd Edition, p. 123-162.

Table 1. Segregation of seed size in the F$_2$ and backcross populations of the cross ts x GN, from field plots at the Tiaoshan Farm, Gansu, P. R. China, 1995.

| | Observed number | | | Ratio | | | | |
	Small	Medium	Large	Small	Medium	Large	X^{2z}	P
P$_1$[y]	20	0	0	all			--	--
P$_2$[x]	0	0	20			all	--	--
F$_1$[w]	0	40	0		all		--	--
F$_2$[v]	24	48	15	1	2	1	2.7931	0.05
BC$_1$[u]	32	42	0	1	1	0	1.3514	0.05
BC$_2$[t]	0	57	60	0	1	1	0.0342	0.05

[z] $X^2_{0.05} = 3.84$ (v=1), = 5.99 (v=2)

[y] Tomato seed watermelon = ts

[x] GN-1 = GN

[w] ts x GN and the reciprocal cross

[v] F$_1$ self-pollinated

[u] F$_1$ x ts

[t] F$_1$ x GN

Watermelon Fruit Blotch Infection Rates in Diploids and Triploids

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Watermelon fruit blotch (WFB) caused by the bacterium now known as *Acidovorax avenae* subsp. *citrulli* was first noted in South Carolina in 1989. The pathogen from an infected 'Prince Charles' fruit was used to inoculate fruit of SC-7, an inbred diploid line. Infested SC-7 seed were grown out at the Pee Dee Research and Education Center (PDREC) in 1994 at Florence, SC. In a triploid trial where SC-7 was the pollenizer (3), these plants exhibited a fruit infection frequency of 95% with severe symptoms. The 20 triploid varieties exhibited a range of resistance to secondary infection by the pathogen from SC-7, but all triploids were more resistant than the diploids.

In 1990, we made selections from genotypes identified by Sowell and Schaad (8) as having resistance in the seedling stage to the bacterium that Schaad et al. (7) identified as *Pseudomonas pseudoalcaligenes* subsp. *citrulli*. A selection of PI 299378 identified by Sowell et al. (8) as resistant to *P. pseudoalcaligines* subspecies *citrulli*, but exhibiting large lesions in our seedling trial (5), was also saved. Seed were saved from fruit selfed in the greenhouse after inoculation with an isolate of *Acidovorax avenae* subsp. *citrulli* provided by Hopkins (University of FL, Leesburg). We reported possible resistance to WFB in certain seed lots of 'Congo' and in PI 295843 (5), but field observations by Hopkins et al. (3) did not confirm this resistance.

We planted these selections to test the hypothesis that WFB resistance existed in these germplasms. We also sought to evaluate more triploids for WFB resistance.

Materials and Methods: In 1995 we set up a trial similar to the 1994 triploid trial, including the selections saved from the 1990 experiment. We also included a seed lot of 'TRITEN' triploid that had been artificially inoculated by soaking in a 10^8 cfu WFB solution for ten minutes (4).

Selections from 'Congo', PI 295843 and PI 299378, all inoculated with WFB in 1990, infested SC-7 seed from 1989 and 'TRITEN' seed artificially infested with WFB in 1994, and clean triploid seed were seeded into flats in April in the greenhouse at PDREC. These seeds were grown until May in a greenhouse with overhead watering

before they were transplanted to the field. The SC-7 from 1989 which became 95% infected with severe symptoms in the same field in 1994 was transplanted in every other plot. After transplanting of 5 hills or less of each genotype in three randomized complete blocks, the blocks were watered with overhead irrigation weekly. Ammonium nitrate was used to sidedress the plants in early July near the time of anthesis, and leaf burn was evident on all the plots, in some cases enough to kill the plant. Almost all of the plants recovered completely from fertilizer burn. A month later, all the fruit in the field were rated for WFB. Percent infection (percentage of fruit infected in each plot) and severity of symptoms (rating of each fruit by size of lesions, rating scale in Table 1) were scored. Chi-square analysis was used to test the effect of genotype and ploidy level(1). Computation was performed using FREQ on SAS (6).

Results and Discussion: The artificially infested 'TRITEN' seed germinated poorly, and most seedlings died during germination from the inoculation with WFB. Although all but one of the 'TRITEN' seed infested with WFB did not survive at the seedling stage, the single plant that survived produced fruit free of WFB. In contrast to the trials of 1994, where infection frequency of SC-7 fruit at maturity was 95% and infection was in the most severe category, infection of SC-7 in this test was only 22.5%, with less severity. However, a bulked lot of seed from the pollenizers of the 1994 experiment, SC-7 and 'Crimson Sweet', were infected at a rate of 47.6%. Infection levels varied significantly among replications. The sparse infection on SC-7 and the significant difference among replications suggest that the pathogen did not spread quickly and uniformly across the field. The high levels of infection on all three replications of bulk 1994 PD indicate that the disease was expressed when it was present on susceptible fruit.

Infection frequency was significantly higher in the diploids, originating from infested seed, than in the triploids (P <0.0004). With the exception of the single 'TRITEN' plant, the triploids were secondarily infected in the field.

Table 1. Percentage and severity of fruit blotch in diploid and triploid watermelons[1]

Genotype[2]	Ploidy	Total # Fruit	% Infection ± std. dev.	Severity ± std. dev.	Rind Color Pattern
Primary Infection (seed infestation)					
Bulk '94 PD	2N	19	47.6 ± 4.1	3.0 ± 0.5	mix
SC-7	2N	392	22.5 ± 22.4	2.0 ± 1.0	light green/green stripe
C-1 Congo	2N	22	63.9 ± 12.7	4.4 ± 0.7	two types3
C-2 Congo	2N	25	28.2 ± 8.2	2.3 ± 0.4	two types3
H-1 Congo	2N	14	17.5 ± 10.6	1.7 ± 0.4	two types3
H-2 Congo	2N	25	0	-	dark green/darker stripe
S Congo	2N	22	9.5 ± 16.5	1.4 ± 0.7	two types3
WR Congo	2N	13	6.3 ± 8.8	1.4 ± 0.5	two types3
M Congo	2N	33	3.3 ± 8.8	1.1 ± 0.2	dark green/darker stripe
PI 295843	2N	9	0	-	cream
PI 299378	2N	21	0	-	cream
TRITEN	3N	3	0	-	light green/green stripe
Secondary Infection (acquired from diploids and TRITEN)					
AC 2532	3N	34	13.9 ± 17.3	1.6 ± 0.7	light green/green stripe
AC 3731	3N	22	9.5 ± 16.5	1.6 ± 1.0	light green/green stripe
AC 5244	3N	26	4.2 ± 7.2	1.1 ± 0.1	light green/green stripe
ACR 94W001	3N	12	4.2 ± 7.2	1.1 ± 0.1	light green/green stripe
AC 3521Y	3N	31	2.8 ± 4.8	1.1 ± 0	light green/green stripe
AC 5032	3N	22	0	-	light green/green stripe
AC 5444	3N	35	0	-	light green/green stripe
ACR 94W003	3N	19	0	-	light green/green stripe

Factor	X^2 Value	P Value
Genotype	98.00	<0.0001
Ploidy	24.25	<0.0001

[1]Three replications of 5-hill plots were planted. 'SC-7' contaminated with WFB was transplanted in every other plot. Per cent infection was calculated from the number of fruit infected and total number of the fruit in the plot. Severity ratings on individual fruit were as follows: No lesions = 1; lesions < 6.45 cm^2 = 3; lesions > 6.45 cm^2 but < 100 cm^2 = 5; lesions > 100 cm^2 and open wounds = 7.

[2]Seed sources of Congo: H = Hollar, Inc., Rocky Ford, CO; C = Coffey Seed Co., Plainview, TX; WR = US Vegetable Lab, Charleston, SC; M = Musser Seed Company, Twin Falls, ID; S = Shumway Seed Co., Graniteville, SC. Seed sources of PIs: Southern Regional PI Station, Griffin, GA. Seed sources of triploids: TRITEN - Xinjiang Western China Seed Company, Changji, Xinjiang. AC triploids- Abbott & Cobb, Inc., Feasterville, PA.

[3]These lots were segregating for rind color: dark green background and darker stripes vs. light green background with dark green stripes.

In 1994 and 1995, we observed resistance of triploids to secondary infection during fruit development. Even so, in both tests there was significant variation in infection levels among triploid varieties.

Apparent differences existed among and within 'Congo' seed lots (Table 1). For example, one selfed 'Congo' selection within lot 109991(H-2) was free of WFB. The "resistant" selection had a dark green/ darker green striped fruit. The "susceptible" selfed selection (H-1) of lot 109991 was segregating for light green/dark green striped fruit and dark green/darker green striped fruit. This association of infection with light green background was 90% in all the plots of the 'Congo' selections and is consistent with the observations of Hopkins et al. (2) that susceptibility is associated with light green fruit color.

There is now agreement that the WFB pathogen, *Acidovorax avenae* subsp. *citrulli* and the pathogen *Pseudomonas pseudoalcaligenes* subsp. *citrulli* that Sowell and Schaad (8) used to identify seedling resistance in 'Congo' and PI 295843 and 299378 are essentially the same (3). Thus, it is reasonable to expect that WFB resistance may exist among the genotypes where Sowell and Schaad (8) found seedling resistance to *Pseudomonas pseuodoacalignes* subsp. *citrulli*.

Seed were saved from lesion-free fruit of 'Congo' adjacent to contaminated plots of SC-7. Seed were also saved from infected SC-7 and from the two PIs.

Literature cited

1. Eskridge, K.M. 1995. Statistical analysis of disease reaction data using nonparametric methods. HortScience 30:478-480.

2. Garrett, J.T., B.B. Rhodes and Xingping Zhang. 1995. Triploid watermelons resist fruit blotch organism. Cucurbit Genetics Coop. Rpt. 18:56-58.

3. Hopkins, D.L., C.M. Thompson and G.W. Elmstrom. 1993. Resistance of watermelon seedlings and fruit to the fruit blotch bacterium. HortScience 28:1-2.

4. Latin, R.X. and D.L. Hopkins. 1995. Bacterial fruit blotch of watermelon: the hypothetical exam question becomes reality. Plant Disease 761- 765.

5. Rhodes, B.B., N.V. Desamero and X.P. Zhang. 1991. A strategy toward varietal resistance to watermelon fruit blotch. Cucurbit Genetics Coop. Rept 14:102-103.

6. SAS Institute. 1958. SAS/STAT Users Guide. SAS Institute. Cary, NC.

7. Schaad, N.W., G. Sowell, Jr., R.W. Goth, R.R. Colwell, and R.E. Webb. 1978. *Pseudomonas pseudoalcaligenes* subsp. *citrulli* subsp. *nov*. Int. J. Systemic Bacteriol. 28:117-125.

8. Sowell, G., Jr. and N.W. Schaad. 1979. *Pseudomonas pseudoalcaligines* subsp. *citrulli* on watermelon: Seed transmission and resistance of plant introductions. Plant Dis. Rept. 63:437-441.

The *B* Genes and their Phenotypic Expression in *Cucurbita*: an Overview

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The *B* genes are nuclear mutants, and each *B* gene conditions precocious depletion of chlorophyll. In fruits, these genes can be expressed in all known genetic backgrounds. But in some backgrounds they can also be expressed in one or more other plant organs. Historically, symbol *B* represented a genetic potentiality for *bicolor* fruits in which the non-green portion is depleted of chlorophyll.

Two unlinked *B* genes were identified, *B1* and *B2*. *B1* originated in *C. pepo* L. and *B2*, in *C. maxima* Duch. ex Lam. Gene *B2* is not an uncommon mutation in open-pollinated populations of some standard $B2^+$ cultivars. Therefore, one may conclude that such a mutation was the origin of all our *B2* cultivars. The literature hints to the past existence of a few edible *B1* cultivars in *C. pepo*, but no clue is given as to their origin. Nonetheless, it is evident from genetic data that the presently utilized *B1* had a two-step origin in the inedible ornamental gourds of *C. pepo* var *ovifera* (L.) Alef. The first step was a mutation from $B1^+$ to $B1^w$, the latter being a gene for a weak expression of chlorophyll depletion and bicolor fruits. The second step was a mutation from $B1^w$ to *B1*, a gene for stronger expression and uniformly pigmented yellow fruits. There is also evidence indicating that the *B1* locus is unstable in some backgrounds and stable in others. $B1^w$ and *B1* were transferred to more than ten edible cultivars of *C. pepo*. In addition, *B1* and *B2* were transferred to several cultivars of *C. moschata* Duch. ex Poir, a species that has not been reported to generate *B* mutations. Recent field observations suggested that 'Gold Striped Cushaw' of *C. argyrosperma* Huber carries a weak *B* gene whose phenotype resembles that of *B2*. The seed of this cultivar was obtained from Glenn Drowns.

Potentially, each *B* gene brings about a profound change in the ontogenetic timing of chlorophyll depletion during fruit development. As a result, three distinct developmental phases of fruit pigmentation are recognized in *Cucurbita*: the standard phase, the precocious phase #1 and the precocious phase #2 (Figure 1).

The standard phase of pigmentation represents the post-anthesis phase of fruit development in all B^+B1^+ $B2^+B2^+$ (B^+) cultivars. Prior to anthesis the ovaries of these cultivars are consistently green. From anthesis and on, the fruits are either persistently green or turn to other colors (white, tan, yellow or golden) depending upon the presence of specific pigment-controlling genes such as *w* and *y* for persistent green and *W* and *Y* for white or yellow colors. Whether the change in color occurs at anthesis or on approaching maturity the fruits are uniformly pigmented. Furthermore, the change in color and chlorophyll depletion appears to occur simultaneously .

The precocious phase #1 and the precocious phase #2 represent cultivars whose genotypes are $B1B1$ $B2^+B2^+$ (*B1*) and $B1^+B1^+$ $B2B2$ (*B2*) respectively. Chronologically, phase #1 ends a few days prior to anthesis and phase #2, a few days following anthesis. Within these phases, *B1* and *B2* can be expressed at different times: the later the expression the lesser is the extent of yellow pigmentation of the ovary. Consequently, *B1* and *B2* plants can differentiate either uniformly yellow ovaries or bicolor (green-yellow) ovaries. As these ovaries grow through and beyond anthesis, the color of their maturing fruits is affected by the pigment-controlling genes that are active at this stage of development. For example, the ovaries of Atlantic Giant, *B2B2*, are bicolor whereas the mature fruits are uniformly white. If the *B* genes fail to "turn-on" during their limited phases for potential action, the color of the *B* fruits would be indistinguishable developmentally from that of B^+ fruits.

The ontogenetic timing of *B* expression is governed by several factors: the strength of the *B* allele, the dosage of *B*, a group of nuclear timers (regulators) and the environment. The nuclear timers were formerly known as modifier genes that affect the *extent* of precocious *pigmentation* over the surface of the fruit: the *Ep* and *ep* modifiers of *B* expression. The heterozygote $B1^wB1^+$ of some ornamental gourds is particularly prone to delay the expression of $B1^w$ and is apt to exhibit a remarkable phenotypic plasticity, bearing variable bicolor fruits as

well as green fruits in an unpredictable order. The homozygote *B2B2* of PI 165558 is associated with one of the earliest known expression of *B2* and is highly stable phenotypically, bearing uniformly golden fruits exclusively.

While bicolor fruits vary greatly in extent of green and non-green (yellow or golden) areas, the distribution pattern of these areas is subject to distal-proximal polarity, green in the lower or distal portion of the fruit and non-green in the upper region, towards the proximal end. The bicolor pattern is further affected by other factors including fruit shape, e.g., the "ring" phenomenon is limited to long-necked (ladled) fruits as in 'Spoon'. In a rare case, the common polarity radically moves upward: the fruit being essentially green and the peduncle, uniformly golden (Figure 2). In another rare case, the common polarity is completely reversed, golden in the distal portion and green in the upper region of the fruit.

Interactions between the *B* genes and other genes lead to numerous phenotypic effects most of which are horticulturally "bad" but some are "good." The basic biological mechanisms controlling these manifold effects are not understood. Yet, pragmatically, one can be guided by the results of breeding experiments. These results imply that the *Cucurbita* genome is highly resourceful and that through genetic recombination the "bad" effects can often be eliminated while the "good" effects are sustained or enhanced.

The ovaries of many *BB* homozygotes develop poorly and the size of their fruits is smaller than that of comparable B^+B^+ fruits. But the ovaries of 'Atlantic Giant', *B2B2*, develop normally and their fruits are the largest on this planet.

In B^+ cultivars of Winter Squash, young fruits are low and mature fruits are high in levels of flesh carotenoids. In B^+ cultivars of Summer Squash, young and mature fruits are low in flesh carotenoids. The external fruit color in these two groups of cultivars may be green, tan, yellow, or golden. The genetic mechanism that controls the level of flesh carotenoids in B^+ cultivars is not known. A new system for high levels of flesh carotenoids was developed through a particular interaction between the *B* genes and the *L* genes. In this *B-L* system, both young and mature fruits contain high levels of flesh carotenoids and their external color is persistently golden. The *L* genes (*L-1* and *L-2*, Paris) condition a high rate of pigment accumulation during fruit development.

And the best sources of *L* genes are cultivars whose fruits are dark green, gradually becoming solid black at maturity.

The yellow and golden fruits of all presently known B^+ cultivars and most *B* cultivars are subject to green discoloration due to virus infection. But fruits of some *BB* lines of *C. pepo* and *C. maxima* are resistant in varying degrees. Although resistant lines are usually homozygotes, *BB*, not all homozygotes are resistant. The decisive factor appears to be the ontogenetic timing of *B* expression: the earlier the expression of *B* the higher is the probability for resistance. From this perspective, the operative system is the interaction between the *B* genes, their nuclear timers, and the environment. Field records showed that the fruits of PI 165558, *B2B2*, possess the highest known level of resistance to virus-induced greening. Do the *B* genes carry virus information?

Some interactions between the *B* genes and other genes appear to enhance female expression. Other interactions appear to affect fruit quality in diverse ways. But the evidence for these interactions has not been critically examined.

The environment plays an important role in the expression and regulation of the *B* genes. Among potential influences, the effects of temperature have been demonstrated in growth-chamber studies and confirmed by field observations. Low temperatures (e.g., 12 C), high temperatures (e.g., 35 C) and intermediate temperatures can have different effects on the expression of the *B* genes, depending on the genetic background. Light, may also be an important influencing factor but I am not aware of any experimental data on the effect of light on the expression of the *B* genes.

Apart for some exceptions, the exposure of *B* cultivars, *BB* or BB^+, to a progressive increase of temperature, from low to high, is associated with a corresponding decrease in extent of precocious fruit pigmentation.

When young plants of $B1^+B1^+$ and *B1B1* inbreds are exposed to low temperatures, the leaf-blades of the two groups of inbreds exhibit yellow or golden spots. But the incidence of spots is 3- to 10-fold higher in *B1B1* than in $B1^+B1^+$ leaves. No such spots occur in $B1^+B1^+$ and *B1B1* leaves at high temperatures. Are the induced spots in $B1^+B1^+$ leaves responses to "residual heredity" that is genetically related to *B1*?

Figure 1. Illustrating three phases of fruit development in *Cucurbita*. Fruit pigmentation other than green can occur during either one of these phases depending on the genotype and the environment.
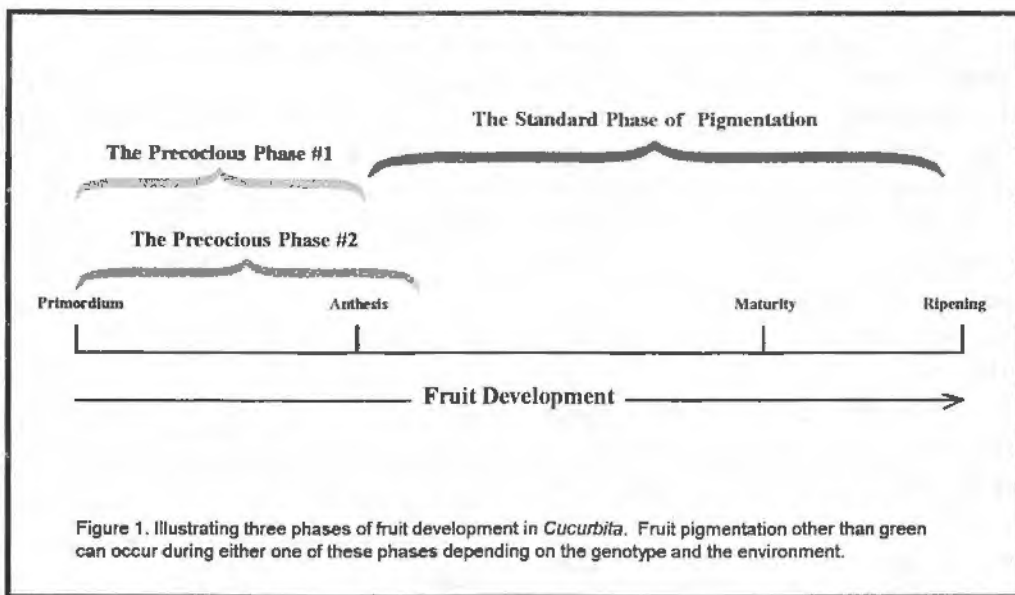
Figure 2. Illustrating a fruit in which precocious depletion of chlorophyll largely affects the peduncle. Fruits of this pattern were found in a rare segregate obtained from the interspecific cross: *C. maxima* ('Pink Banana') x *C. moschata* ('Chirimen').

Figure 3. Illustrating complete association between precocious depletion of chlorophyll in stems and predominantly-female expression. This association exists in a rare inbred of *C. pepo*.

Figure 4. Illustrating the 'Golden Crown' mutant of *C. pepo*.

When young plants of genetically diverse $B1B1$ inbreds are exposed to low temperatures, the leaf-blades of some inbreds are yellow whereas the leaves of other inbreds are green. The yellowing is expressed either as diffusion affecting the entire leaf surface or as variable patterns, often as "vein designs." No such yellowing occurs at high temperatures. A study of inheritance, based on one re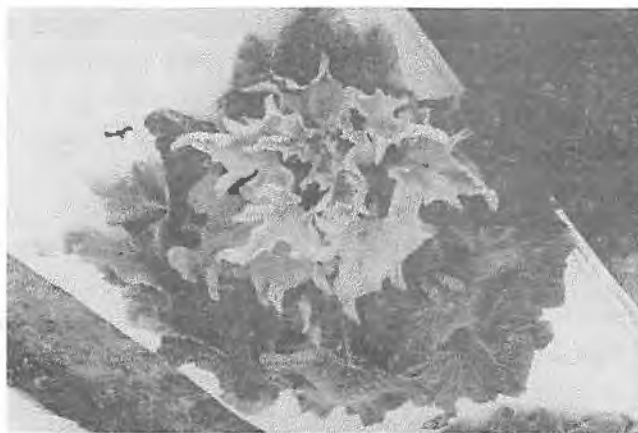levant cross, showed that these alternative responses are conditioned by a pair of nuclear genes. The recessive gene behaves as a a selective activator that selectively activates the expression of $B1$ in leaves in response to a low-temperature signal. The partially dominant gene behaves as a selective suppressor. This monogenic inheritance is probably an over- simplification because temperature-sensitive inbreds differ in degree of their sensitivity. In sensitive inbreds, both spotting and yellowing occur in different leaves of the same plant.

Selective regulation of $B1$ expression is limited to leaf-blades. However, there exist rare cases in which stems and petioles are yellow. One of these cases originated as mutation in a green-stemmed, predominantly-female (PF) $B1B1$ inbred. Like its parent, the mutant inbred is PF and $B1B1$. But unlike its parent, the mutant stems and petioles gradually turn yellow in proximity to well-developed pistillate flowers and fruits (Figure 3; some of the lower pistillate flowers were removed in order to expose the main stem). According to present interpretation: (a) precocious chlorophyll depletion is preceded by a diffusible substance; (b) the mutant produces a larger amount of this substance than its parent; (c) the substance is diffused from pistillate flowers to adjacent regions; and (d) a high concentration of pistillate flowers promotes the flow.

Selective regulation of $B2$ expression can affect leaf-blades, petioles, stems and staminate flower buds. But the precise regulatory mechanism is not yet understood.

The American $B2B2$ cultivars such as 'Boston Marrow', 'Golden Delicious', 'Golden Nugget' and 'Pink Banana' produce green stems and green leaves in diverse environments of low, intermediate and high temperatures. By contrast, PI 165558, $B2B2$, a cultivar from Almora, India, is more sensitive to temperature fluctuations. When this cultivar is grown in an environment of intermediate temperatures, it produces precociously yellow stems and green leaves. If, in the same environment, this cultivar is exposed to a few cold nights (2-3) of low temperatures, it subsequently continues to produce yellow stems but it also produces, for a short period, some yellow-spotted leaves as well as some completely yellow leaves. But when this cultivar is grown in an environment of high temperatures, it produces green stems and green leaves. Unlike the yellowing of stems in the $B1B1$ mutant described above, stem yellowing in PI 165558 occurs during both the vegetative and reproductive stages of plant development, and it is clearly not associated functionally with pistillate flowers.

When some yellow-stemmed $B2B2$ inbreds -- derivatives of crosses between $B2^+B2^+$ cultivars and PI-165558, $B2B2$ -- are grown under field conditions of intermediate temperatures, all their leaves gradually turn yellow. These inbreds can be reproduced by seed, but with great difficulty. When the same inbreds are grown in an environment of low temperatures, their populations consist entirely of lethal seedlings.

The $B2$ of PI 165558 was transferred to the $B2^+B2^+$ cultivar of 'Green Delicious' (Munger's strain). The new precocious $B2B2$ inbred of 'Green Delicious' background -- PGRD -- produces green stems and green leaves in environments of diverse temperature variations as do other $B2B2$ cultivars. But unlike most American $B2B2$ cultivars, which are essentially bicolor-fruited, the PGRD inbred produces uniformly golden fruits. Is the $B2$ allele of PI 165558 stronger than that present in most other $B2$ cultivars?

The transfer of $B2$ to $C.$ $moschata$ revealed two facts. First, $C.$ $moschata$ is an exceptionally rich source of genetic elements that selectively activate the expression of $B2$ in stems. Second, some of these genetic elements impart great pheontypic stabilty to the expression of $B2$ in stems even at high temperatures. If we consider both $C.$ $maxima$ and $C.$ $moschata$, we see a continuous series of variation in the expressivity of $B2$ in stems, from zero expressivity to 100% expressivity in diverse environments. All intermediate grades are environmentally sensitive in different degrees. The simplest hypothesis is that the expression of $B2$ in stems is governed by numerous genetic regulators that interact with the environment.

In 1983, a mutant was found in an F2 of 'Bicolor Spoon', $B1^wB1^w$, x 'Table King', $B1^+B1^+$. This mutant was named 'Golden Crown' (Figure 4): it differentiates a number of strictly green leaves along the main stem followed by a transitional phase towards completely yellow or golden leaves at the top (the golden crown). Phenotypically, this mutant resembles many ornamental

cultivars including *Amaranthus tricolor* var. *spendens* and the so-called "ornamental cabbage" or "ornamental kale" cultivars (*Brassica oleracea*, acephala group). One can easily obtain a true-breeding line of 'Golden Crown'. But serious difficulties were encountered for a long time in attempts to decipher the genetic basis for this mutant. With some temerity one can now claim that 'Golden Crown' is conditioned by a single nuclear gene.

Recently, a cross was made between 'Fordhook Zucchini' (Superak's strain) as seed parent and 'Golden Crown'. The F_1 plants produced green leaves exclusively. Among 95 F_2 plants, not a single 'Golden Crown'

individual was found, but one bicolor-fruited plant was clearly identified. If these results can be confirmed, (a) did 'Golden Crown' originate as transposition of some element related to or part of Bl^w?, and (b) did the bicolor-fruited plant in the above F_2 originate as reverse transposition?

High Yields of Summer Squash Lines and Hybrid Combinations

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Cucurbita moschata landraces commonly found in Mexico exhibit a high degree of variability (4). In our collection we have observed useful traits such as attractive butternut fruit shape and field resistance to a virus disease which clearly infects other species, particulary *C. pepo* and *C. maxima* (1,5). Interspecific crosses between *C. pepo* and *C. moschata* were performed in 1983 seeking improvement of the cultivar Gray Zucchini (GZ) which is preferred in Mexico over other cultivars of summer squash. Crosses using GZ were unsuccessful but by using cv. Classic as female parent, viable F_1 seeds were obtained. An F_2 generation consisting of 380 plants was obtained in 1986 (2). Eight of these plants free of any viral symptom were used to start a backcross program to GZ and after 4 backcrosses and 3-5 selfing generations, GZ-type lines were developed. Other lines were developed starting in 1987 by the cultivation of small weak embryos obtained from crosses between the two species and then by backcrossing and selfing. This paper reports on the behavior of such lines and hybrid combinations in comparison to commercial materials.

Materials and Methods. In the summer-fall of 1993 14 lines, 8 F_1 hybrids between lines, 12 line x GZ hybrids, the open pollinated cultivars GZ and Black Zucchini (BZ), and the F_1 commercial hybrids 'Corsair', 'Raven', 'Classic', and 'Onyx' were established by direct seeding followed by conventional cultural practices for summer squash production. Treatments were arranged in a randomized complete block design with 4 replications. Plots measured 4.3 m with 3 plants per meter. Yield, degree of virus infection on foliage, and plant size and morphology were determined.

Results and Discussion. The highest single yield (32,674 kg/ha) was obtained for the F_1 hybrid of the cross 2-6-4 x 7-1-1, whereas the lowest (4,872 kg/ha) was for BZ. The standard GZ produced 10,384 kg/ha (HSD value was 12,674 kg/ha). Six of the line x GZ hybrids and two line x line hybrids showed positive heterosis. Figure 1 shows the average commercial yield obtained for all hybrids between lines (21,174 kg/ha), line x GZ

hybrids (19,948 kg/ha), lines (15,800 kg/ha), commercial hybrids (14,114 kg/ha), and open pollinated cultivars (7,628 kg/ha).

Symptoms of viral infection were very high for commercial materials as shown in Table 1. On the other hand, lines and hybrids had healthier plants. Although virus identification for this test was not possible, symptoms on foliage indicated that SLCV was present. Four of the lines were inoculated in 1995 with CMV, ZYMV, WMV, and PRSV and were suceptible to all. One line had delayed symptoms for CMV indicating that it may possess some field resistance (3). In similar materials established in the spring of 1995, ZYMV and SMV were found. During the fall of 1995 a SLCV test was performed in one of the lines and was positive despite plants showing no symptoms of the disease.

Stem length varied from 34.2 cm to 64.1 cm and number of stems from to 1 to 4 (data not shown).

These results show that as reported for interspecific crosses in *Cucurbita* (6), local *C. moschata* landraces may be used to improve *C. pepo* cultivars. Once an improved line has been obtained, it may be used as a parent to produce better offspring.

Literature Cited

1. Fierro Quintero, A. 1987. Evaluación de 22 líneas de calabaza Cehualca Cucurbita moschata (Duch) Duch. ex Poir. B.S. Thesis, Univ. de Sonora, México.

2. Garza Ortega, S. and J.D. Castro Encinas. 1987. Observaciones sobre segregación de algunas características de una generación F_2 de una cruza interespecífica en Cucurbita. Soc. Mexicana de Ciencias Hortícolas. II Congreso Nacional. Irapuato, México (Abstract).

3. Kyle, M.M. 1995. Personal communication.
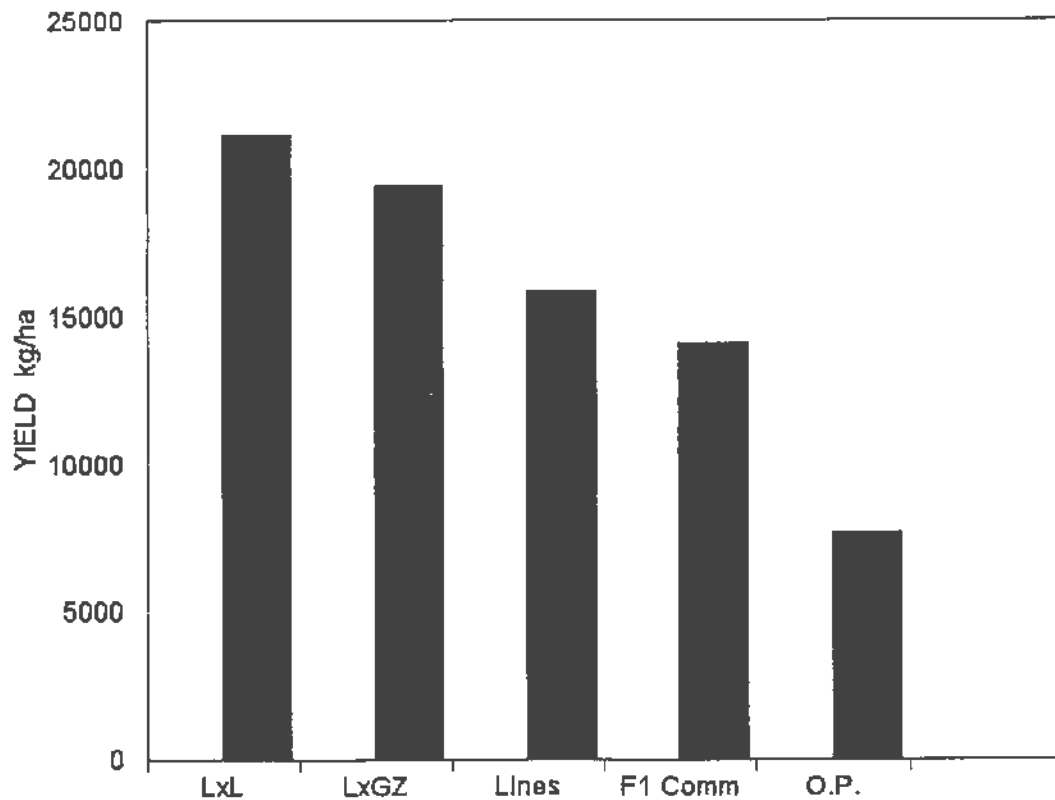
Figure 1. Average commercial yield of lines (14) and hybrids compared to commercial materials. LxL = hybrids between lines (8); LxGZ = hybrids of lines x 'Gray Zucchini' (12); F₁ Comm = commercial hybrids (4); O.P. = open pollinated cultivars (2).

Table 1. Degree of symptoms of virus infection on foliage. Data show average of 8 line x line hybrids, 12 line x 'Gray Zucchini' hybrids, 14 lines, 2 open pollinated cultivars, and 4 commercial hybrids.

Materials tested	Virus infection index[z]
Line x Line	0.02
Line x Gray Zucchini	0.27
Lines	0.30
O.P. cultivars	3.50
Commercial hybrids	3.97

[z]Infection index was rated on a 0 to 4 scale: 0 = no infection, 2 = slight, 3 = strong, 4 = severe.

4. Merrick, L.C. 1991. Systematics, evolution, and ethnobotany of a domesticated squash, *Cucurbita argyrosperma*. PhD Dissertation, Cornell University. USA.

5. Rodríguez Duarte, M.R. 1988. Evaluación de 23 líneas de calabaza Cehualca *Cucurbita moschata* (Duch) Duch. ex Poir. B.S. Thesis, Univ. de Sonora, México.

6. Whitaker, T.W. and R.W. Robinson. 1986. Squash breeding. In: Bassett, J.M., ed. Breeding vegetable crops. AVI Publ. Co., Westport, Conn. 584 p.

Resistance to Three Isolates of Zucchini Yellow Mosaic Virus (ZYMV) in squash (*Cucurbita pepo* L.)

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Zucchini yellow mosaic virus (ZYMV) is a very serious disease in squash, particularly in the central region of the Sudan. A recent survey reported a high incidence and widespread occurrence of the disease in different cucurbit species (3). In many occasions it causes a total crop loss (1). This project aimed at identifying a source with a sufficiently high level of resistance for incorporating into the popular susceptible cv. Eskandarany (ESK).

Twenty one accessions obtained from the North Central Plant Introduction Station (Ames, Iowa), and 15 breeding lines obtained from Cornell University were evaluated for zucchini yellow mosaic resistance (ZYMR). A scale of 1 to 9 was used, where 1 indicates that a plant is severely infected (shoestringing) and 9 indicates no apparent symptoms of the virus. The tissue immuno-blot assay (TIBA) technique was used to detect the virus in the F_1 populations (4). The F_2 plants were tested at the seedling stage at INRA (Montfavet, France) with the help of Pitrat and Lecoq. Three ZYMV isolates, Su19 and Su4 from central and eastern Sudan, respectively, prepared by Ali (2), and a French isolate, E9, prepared by Lecoq, were used.

None of the PI accessions tested showed an acceptable level of ZYMR. Only two plants in two of the Cornell breeding lines showed a high level (=8) of ZYMR. The first was one out of 7 plants in row 94-38 of the pedigree (91-757 sib PMR + ZYMR *pepo*). The second was one out of 5 plants in row 94-37 of the pedigree (91-720-2⊗, Nigerian Local.BC$_2$ cas. F$_5$, ZYMR.EPS). This level of resistance was retained in the F_1 progenies when crossed with the popular susceptible cv. ESK. The TIBA test gave negative results for row 95-13 and 95-14, while row 95-24 results ranged from negative to high in different plants. Approximately 10% of the F_2 plants had ZYMR levels similar to that of the donor parents. This indicates some level of dominance of ZYMR which will permit selection of resistant plants during successive backcrossing to the cv. ESK. Judging by the overall mean infection rating for three isolates, it appears that plants inoculated

with Su19 and E9 had the lowest means, 4.4 and 4.3, respectively, while plant infected with Su4 had a slightly higher overall mean of 4.8. This suggests that Su4 is slightly milder than the other two isolates (Table 1). The F_2 of 95-14 appears to be the most promising among the F_2's for selection of highly resistant plant with a ZYMR level similar to that of the donor parent.

Literature cited

1. Ali, H.M. 1993. Studies on some mosaic-inducing viruses in cucurbits in the Gezira. M.S. Thesis, University of Gezira, Sudan.

2. Ali, H.M. 1994. Personal communication.

3. Lecoq, H., G.A. Daffalla, Y.F. Mohamed, H.M. Ali, C. Wipf-Scheibe, C. Desbiez, A.E. El Jack, S.K. Omara and M. Pitrat. 1994. Survey of virus diseases infecting cucurbit crops in eastern, central and western Sudan. J. Agri. Sci., Univ. Khartoum, V. 2, No. 2 (July).

4. Lin, N.S., Y.H. Hsu and H.T. Hsu. 1990. Immunological detection of plant viruses and mycoplasmalike organisms by direct tissue blotting on nitrocellulose membrane. Phytopathology 80:824-828.

Table 1. Zucchini yellow mosaic resistance of F$_2$ populations [(ESK x 94-38-1)⊗ and (ESK x 94-37-2)⊗] to three isolates of ZYMV.

Isolates	Row No.[z]	ZYMR rating scale for F$_2$ populations									Mean
		1	2	3	4	5	6	7	8	9	
		Number of F$_2$ plants in each rating class									
Su 19	95-13			6	10	-	3				4.0
	95-14			5	4	7	2	2			4.6
	95-24			1	11	2	5				4.6
	RC[y]					2	1				5.3
	SC[x]			2	2						3.5
Su 4	95-13			6	5	5	3	1			4.4
	95-14			2	4	5	6	1			5.0
	95-24			2	6	3	4	1			5.1
	RC[y]					3	6				5.7
	SC[x]			4							3.0
E 9	95-13			5	11	2	1				3.9
	95-14			2	3	8	5	1			5.0
	95-24			4	11	1					3.6
	RC[y]						6				6.0
	SC[x]			1	3						3.8

[z]95-13 and 95-14 are selfed progenies of two different ESK plants crossed with 94-38-1; 95-24 is a selfed progeny of ESK x 94-37-2.

[y]RC = resistant check, 9505 F$_1$ hybrid tolerant to ZYMV.

[x]SC = susceptible check, cv. Eskandarany.

A Taiwan Strain of Papaya Ringspot Virus from *Carica papaya* Causing Prominent Symptoms on Cultivated Cucurbits

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Papaya ringspot virus (PRV), an economically important viral agent, usually occurs in tropical and semitropical areas of the world. Its particles are flexuous rods about 780 x 12 nm containing a single strand of RNA, which in nature are spread by a number of aphid species in the non-persistent manner. This potyvirus is not seed-borne, but can be easily transferred mechanically. Most of the strains and variants of PRV are serologically related and belong to one of two groups: Type W (PRV-W)(= watermelon mosaic virus 1) and Type P (PRV-P) (papaya-infecting) (6).

PRV-W occurs in warm regions of the world, but occasionally it has been found in temperate zones. It is one of the major viruses affecting Cucurbitaceae, but it cannot infect any member of the Caricaceae. Symptoms incited by PRV-W include severe plant stunting, foliar mosaic, and extreme reduction of leaf lamina. Fruits are prominently affected by knobby overgrowths and color break. Resistance has been located in *Cucumis melo* (9), *Cucumis sativus* (8), *Cucurbita* species (6), *Lagenaria siceraria* (2), *Luffa acutangula* and *L. aegyptica* (1), and in a few other cucurbit species, including *Cucumis metuliferus* (5).

PRV-P is mainly confined to tropical areas and the major hosts are Caricaceae, and particularly affected is the cultivated papaya (*Carica papaya*). Symptoms include plant stunting, leaf mosaic, distortion, necrotic streaks on stem and petioles, and concentric rings on fruits. In nature, however, PRV-P is not of common occurrence in Cucurbitaceae, in which most of the strains cause mild to moderate symptoms. Summer squashes and other cultivated cucurbits infected with PRV-P were found to be inadequate for the production of large amounts of antiserum to this virus. However, a susceptible line (Acc. 2549) of *Cucumis metuliferus* (4) is presently used extensively for this purpose. Conversely, other accessions of this cucurbit from South Africa were reported to be resistant to both PRV-P and PRV-W (3).

Recently an isolate of PRV-P from Taiwan (PRV-P/T) was found to cause on cucurbits (particularly on zucchini) more prominent foliage symptoms than those incited by PRV-W. Extensive greenhouse studies have also demonstrated that PRV-P/T is controlled by the same genes conferring resistance to PRV-W in cucurbits (1,2,5,6,8,9). PRY-P/T has been maintained in the Hawaiian papaya 'Solo', which responds with the typical symptoms incited by other strains of PRV-P. However, young plants of this cultivar initially develop some foliage necrosis and prominent stunting. With special care, infected plants slowly recover from this acute stage, but during the chronic stage foliage symptoms are still prominent. Other cultivars should be tested to find one that is less sensitive than 'Solo'. PRSV-P/T can be kept in papaya plants for many months.

One of the major inconveniences in working simultaneously with several cucurbit viruses is possible accidental mixtures. By losing the purity of a given virus, breeding for resistance can encounter severe problems. Viral contamination can be eliminated if each virus is kept in a host that is highly resistant to, or is not infected by other cucurbit viruses. In breeding cucurbits for resistance to PRV, this PRV-P/T from Taiwan is very valuable and a good substitute for PRSV-W. It can be kept free of contaminants if maintained routinely in papaya. Extracts from infected papaya leaves usually have a low virus titer and are not suitable for screening a large number of cucurbit plants for resistance. Thus, the best results can be obtained by transferring PRSV-P/T from papaya to zucchini (or any other summer squash) and using them as sources of virus inoculum. Infected zucchini can also provide large amounts of the virus to produce an effective antiserum. However, at 28-30 C the incubation period for zucchini inoculated with PVR-W is 5-6 days, whereas for PRV-P/T it is longer, 8-10 days.

Literature cited

1. Bhargava, B. 1977. Some properties of two strains of watermelon mosaic virus. Phytopath. Z. 88:199-208.

2. Provvidenti, R. 1981. Sources of resistance to viruses in *Lagenaria siceraria*. Cucurbit Genet. Coop. Rept. 4:38-40.

3. Provvidenti, R. and D. Gonsalves. 1982. Resistance to papaya ringspot virus in *Cucumis metuliferus* and its relationship to resistance to watermelon mosaic virus 1. J. Hered. 73:239-240.

4. Provvidenti, R. and R.W. Robinson. 1974. Resistance to squash mosaic virus and watermelon mosaic virus 1 in *Cucumis metuliferus*. Plant Dis. Reptr. 58:735-738.

5. Provvidenti, R. and R.W. Robinson. 1977. Inheritance of resistance to watermelon mosaic virus 1 in *Cucumis metutiferus* (Naud.) May. J. Heredity 68:56-57.

6. Provvidenti, R., R.W. Robinson and H.M. Munger. 1978. Resistance in feral species to six viruses infecting *Cucurbita*. Plant Dis. Reptr. 62:326-329.

7. Purcifull, D., J. Edwardson, E. Hiebert and D. Gonsalves. 1984. Papaya Ringspot Virus. In: CMI/AAB Descriptions of Plant Viruses. Ferry Lane, Kew, Surrey, England. p 8.

8. Wang, Y-J., R. Provvidenti, and R.W. Robinson. 1984. Inheritance of resistance to watermelon mosaic virus 1 in cucumber. HortScience 19:587-588.

9. Webb, R. E. 1979. Inheritance of resistance to watermelon mosaic virus 1 in *Cucumis melo*. HortScience 14:265-266.

Post-harvest Treatments for Producing Sponges from Immature Fruits of Luffa Gourd

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Luffa gourd (*Luffa aegyptiaca* Mill) is grown in small areas for the production of sponges for cosmetics and cleaning products. In eastern North Carolina, we usually plant in mid April, and harvest weekly from mid October to mid November. Sponges are produced by harvesting fruits that have turned brown at the end of the season, then removing the seeds and skin from the fruit, and drying the sponge.

Fruits that are green at harvest can be divided into two categories. Fruits that have a dry, leathery skin that is beginning to turn brown will be referred to as mature-green. Those can be used in the same way as fruits that are brown at harvest. Fruits that are still soft and have a bright green skin will be referred to as immature-green. Luffa gourd is of tropical origin and requires a long growing season. As a result, luffa growers may harvest immature-green fruits when frost kills the plants at the end of the season (usually October in eastern North Carolina).

Currently, there are no published data on the handling and processing techniques of immature-green fruits for optimum sponge quality. The objective of this experiment was to determine how to use immature-green fruits.

Methods: In order to evaluate the handling of immature-green fruits, 4 treatments were used: air drying, forced and heated air drying, soaking in water, and soaking in a 10% bleach solution. Those treatments will be hereafter referred to as air, dryer, water, and bleach, respectively. Air involved hanging the sponges on wires in a greenhouse (30 C day / 20 C night). Dryer treatment involved putting the sponges in a dryer with heated air (32 C) forced through drying racks. The dryer was designed as a cucumber seed dryer (Wehner and Humphries, 1994). Water treatment involved soaking the sponges in tanks of water, with the water changed daily. Bleach treatment involved soaking the sponges in tanks of 10% chlorine bleach, with the solution changed daily.

The experiment was a randomized complete block design with 2 years, 2 replications, and 1 fruit per treatment combination. The fruits were treated for 1 to 18 days, and evaluations were made 1, 4, 6, 8, 13, 15, and 18 days after harvest. A rating system of 1 to 9 was used for evaluation of skin brownness (1-3 = light green, 4-6 = brownish green, 7-9 = dark brown), peeling ease (1-3 = difficult, 4-6 = intermediate, 7-9 = easy), and sponge quality (1-3 = poor, 4-6 = intermediate, 7-9 = excellent). In the first year, data were not taken on the treatments involving water soaking and bleach soaking after 13 days, because the fruits were disintegrating. In the second year, data were not taken on the fourth day due to scheduling difficulties.

Results: In the first year, skin brownness and ease of peeling generally increased as the treatment time increased (Table 1). Of the 4 treatments tested, air drying for at least 13 days provided the most usable sponges. The data were variable due to the small number of fruits tested

In the second year, water was consistently the best treatment for ease of peeling (Table 2). Air was the best treatment for promoting skin brownness. Dryer treatment was the best for sponge quality. In general, at least 13 days in the dryer provided the most usable sponge. Once again, the data were variable due to the small number of fruits tested.

The differences between years can be explained by the different stages of immature-green fruits used. In the first year, the immature-green fruit were consistently more mature in development than were those in the second year.

In conclusion, immature-green fruits can be used as sponges if they are air dried for 2 to 3 weeks in a warm, dry location. That treatment will cause the fruit skin to turn brown, and it can then easily be peeled. Care should be taken to avoid the immature-green fruits where the fibrous network has not begun developing, or the dried fruit will not be usable as a sponge. Future studies are needed to evaluate sponges produced from immature-green fruits for strength and usable life relative to mature fruits.

Literature cited

1. Wehner, T. C. and E. G. Humphries. 1994. A seed dryer for cucumber seeds. Cucurbit Genet. Coop. Rpt. 17: 54-56.

Table 1. Sponge usability in 1993 for immature-green fruits of luffa sponge gourd after 4 post-harvest treatments on skin brownness, ease of peeling, and sponge quality 1 to 18 days after fruits were harvested from the vine.[z]

Treatment	Days after harvest						
	1	4	6	8	13	15	18
Skin brownness rating							
Air	1	1	1	4	9	9	9
Dryer	3	5	2	6	6	5	6
Water	1	2	4	6	6	-	-
Bleach	2	2	3	5	5	-	-
Ease of peeling							
Air	1	7	8	5	8	8	8
Dryer	3	2	6	6	6	6	7
Water	6	6	7	8	8	-	-
Bleach	7	6	5	8	6	-	-
Sponge quality							
Air	1	5	4	5	8	6	6
Dryer	4	3	3	6	5	4	5
Water	5	6	6	6	6	-	-
Bleach	2	5	6	6	5	-	-

[z]Data are means of 2 replications of 1 fruit each. LSD (5% for row- column comparisons = 3).

Table 2. Sponge usability in 1995 for immature-green fruits of luffa sponge gourd after 4 post-harvest treatments on skin brownness, ease of peeling, and sponge quality 1 to 18 days after fruits were harvested from the vine.[z]

Treatment	Days after harvest						
	1	4	6	8	13	15	18
Skin brownness rating							
Air	1	.	7	5	6	6	6
Dryer	1	.	5	4	5	5	5
Water	2	.	2	3	3	3	3
Bleach	4	.	4	4	6	6	6
Ease of peeling							
Air	2	.	5	5	4	5	8
Dryer	2	.	4	4	6	6	6
Water	2	.	6	7	8	8	9
Bleach	2	.	2	4	3	3	4
Sponge quality							
Air	2	.	5	6	4	6	8
Dryer	2	.	6	6	6	6	7
Water	2	.	4	5	6	8	8
Bleach	3	.	2	4	4	4	5

[z]Data are means of 2 replications of 1 fruit each. LSD (5% for row- column comparisons = 2).

Post-harvest Bleaching of Luffa Sponges for Reduced Stains Without Reduced Strength

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Sponges obtained from luffa gourds (*Luffa aegyptiaca* Mill.) have many uses ranging from household cleaning to personal hygiene. Fruits are harvested from the vines after the skin of the fruit has started to dry and turn brown. The seeds are shaken out and the skin removed to reveal the sponge, which is the fibrous skeleton from mesocarp of the fruit. The sponges may absorb brown pigment from the skin causing discoloration of the sponge. Stained sponges may have a lower market value. The objective of this study was to evaluate bleach treatment to improve sponge appearance and reduce staining without reducing sponge strength.

The experiment was a randomized complete block with 10 bleach soak durations and 2 replications. Sponges were harvested from field performance trials at the Horticultural Crops Research Station near Clinton, NC. After seeds and skin were removed from the fruits, the sponges were grouped into stained and normal. After rating the percentage of the total fruit surface that was stained brown, they were submerged in a 10% bleach solution for 0, 1, 2, 4, 8, 16, 32, 64, 128, or 256 minutes. The bleach solution in the soaking tank was replaced every 30 minutes to assure its strength. After bleaching, the sponges were rinsed in clear water for 30 seconds. The sponges were rated for whiteness after 3 days of drying, and whiteness and strength after 127 days of aging to determine the long-term effects of the bleach.

Each sponge was cut transversely into 3 pieces approximately 100 mm long, the length often used by sponge manufacturers. Two of the sections were evaluated by 2 judges for sponge fiber strength, each transversely tearing a separate section in 3 places. The third section was evaluated for strength by a third judge using a knife to make a transverse cut in two places. Strength ratings were based on a scale of 1-9 (1-3=low, 4-6=moderate, 7-9=high strength). The average of the three ratings was used to determine the strength of the sponge (Table 1).

For heavily stained sponges, sponge strength decreased as whiteness increased. However, there was a lot of variation among sponges for strength, so the trend was not clear. Our recommendation for sponges that are heavily stained is bleaching for 30 to 60 minutes. Bleaching for periods longer may decrease sponge strength. Shorter bleaching time did not whiten the sponge adequately. There was a tendency for the heavily stained sponges to be white after bleaching (3 days), but then to darken later (127 days).

For the normal (unstained) sponges, whiteness increased with bleaching time, and sponge strength had a slight tendency to decrease after 64 minutes of bleaching (Figure 1). The recommendation for sponges that are not heavily stained is bleaching for 30 to 60 minutes. Bleaching for longer periods of time decreases the strength of the sponge.

Table 1. Sponge whiteness[z] and strength of unstained luffa sponge gourds after 11 post-harvest treatments rated before treatment and at 3 and 127 days after treatment.[y]

Bleach treatment (minutes)	Normal sponges				Stained sponges			
	Sponge whiteness			Strength rating	Sponge whiteness			Strength rating
	Before	3 days	127 days		Before	3 days	127 days	
0	80	80	72	5	45	45	20	6
1	90	90	90	9	5	5	10	2
2	85	95	95	8	35	75	90	6
4	85	97	100	5	20	65	60	5
8	95	100	100	7	55	90	70	6
16	75	100	100	9	10	100	70	4
32	85	100	100	7	10	100	35	8
64	80	100	100	8	35	100	100	8
128	90	100	100	4	0	100	65	6
256	85	100	100	5	5	100	95	4

[z] Percentage white, unstained area on the whole sponge surface.

[y] Data are means of 3 ratings.

CGC on the World Wide Web

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In June 1995, CGC established its presence on the World Wide Web (WWW) at *http://probe.nalusda.gov:8000/other-docs/cgc* (Fig. 1) in an effort to provide additional services to CGC members, and to educate the broader public on issues dealing with the genetic and breeding of cucurbit crops.

The initial purpose in establishing a CGC web site was to provide archival access to back issues of the CGC Report. In the early 1990's, CGC was facing a dilemma in that the original CGC By-Laws required all back issues of the CGC Report be "available indefinitely" for sale "to active members" of CGC. This had not been a problem in CGC's formative years, but was increasingly so as CGC approached its third decade. Stocks of many past issues were close to depletion, and duplication costs for reprinting small quantities of specific reports were far in excess of what could reasonably be charged for back issues. Also, the sheer mass of paper which was accumulating in order to adhere to this policy was taxing the storage capacities of the CGC Chair.

In 1993, I proposed at the annual CGC Business Meeting that we pursue a change in the By-Laws such that the Chair only be required to retain sufficient copies of the last five years of the CGC Report for sale to active members. Reports older than five years whose supplies had become exhausted would no longer be reprinted. As per our charter, a mail ballot was conducted among the membership to approve this change, and the subsequent vote was overwhelmingly in favor of the proposal (2).

At the 1993 CGC business meeting, I also made the pledge that I would find an alternate means - probably electronic - to en-

Figure 1. Home page for the Cucurbit Genetics Cooperative site on the World Wide Web.

sure that research information from all CGC back issues remained available to cucurbit researchers. After the By-Laws change became official in 1994, I began pursuing the possibility of archiving the back issues on a CD-ROM using the facilities of the USDA National Agricultural Library (NAL). Unfortunately, the estimated cost for mastering and duplicating a CGC CD-ROM was far beyond our means (approximately $45,000 US) and the idea was abandoned.

Fortunately, around the same time the web was becoming increasingly popular as a means of disseminating information via the Internet, and the April 1995 version of NetScape Navigator became the first popular web browser to support the HTML table format, enabling scientific information to encoded in a much more effective manner for data presentation on the web.

I established the first website for CGC on my University of Maryland computer account in June 1995. (Those of you with e-mail addresses may have recalled receiving a e-mail message from me in June announcing the experiment.) Within several days, I had overrun the capacity of the Maryland account and sought a larger (and more permanent) home for the web activities. My CD-ROM contact at NAL put me in touch with the web administrators for the USDA
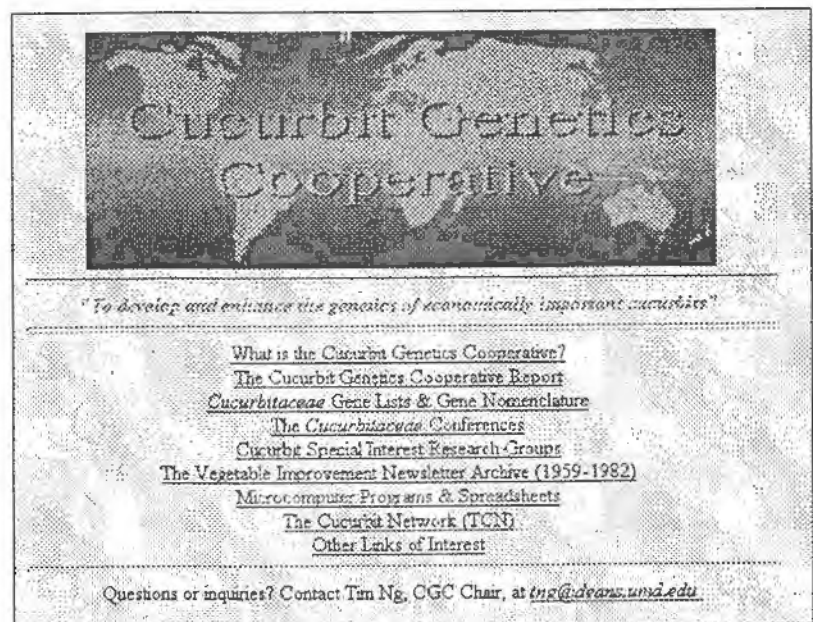
Plant Genome project, and they generously agreed to host the CGC website on the USDA Agricultural Genome Information Server (AGIS). I next digitized the table of contents of all of the CGC reports and placed them at the website for reference, then started building web pages for other resources which might be of interest to the CGC membership. Finally, I recruited a number of talented and dedicated CGC members (Jeff Adelberg, Thomas Andres, Jim McCreight, Bill Rhodes, Todd Wehner, David Wolff, and Xingping Zhang) to start a year-long project of digitizing all of the research articles from the first seven CGC Reports for posting on WWW. This project is now nearing completion.

The next step was to inform the world of the Internet of our existence. Yahoo placed a reference to the CGC homepage in their "Science/Agriculture" category on 11 July 1995. Subsequently, powerful web browsing and indexing search engines such as Altavista (*http://altavista.digital.com*) delved into the CGC site and indexed all of the existing pages regardless of their level within the CGC hierarchy. In fact, these search engines now provide a means of indexing key words within each of the CGC research articles that are HTML-encoded, and will (hopefully!) eliminate the need for updated index of articles in the CGC Report.

The CGC website is still growing in a number of areas. Henry Munger gave me permission to archive the Vegetable Improvement Newsletter (VIN) collection from 1959-1982, and I am currently in the process of converting all of that material to web documents. I've subsequently built in web hyperlinks to other cucurbit-related locations, and most recently incorporated the web efforts of "The Cucurbit Network" into our site.

Although only a year old as of this writing, the CGC website has a tremendous potential for furthering CGC's goal *"to develop and enhance the genetics of economically important cucurbits."* It provides a means of rapidly dispensing information about events of interest to cucurbit researchers, such as the *Cucurbitaceae* conferences. It allows CGC members to contact each other directly via the Internet by clicking onto their e-mail address in the web page CGC directory rather than looking up their e-mail address. By providing wider access to the public, it helps CGC recruit new members who work with cucurbit species but were previously unaware of CGC's existence. It allows us to provide access to binary electronic files such as the Joseph Kirkbride's *Cucumis* database program (1), which was included on disk in CGC Rept. 15, as well as computer programs written by other CGC members. And last, but not least, it allows us to archive back issues of the CGC Report for future reference, and use WWW search engines to provide the indexing needed for past articles.

As the web grows and more of our members gain access, it is not inconceivable that CGC will become completely electronic and dispense with the printed Report. Since this will eliminate the workload associated with creating a printed report, it is also possible that CGC Reports could be issued on a more frequent basis - perhaps twice or more a year! As with all of our other activities, I would be very much interested in hearing from the CGC membership what ideas you might have for future directions for the CGC website.

In closing, I believe it important to acknowledge the contributions of the wonderful folks at the Agricultural Genome Information Service (AGIS), USDA Beltsville Agricultural Research Center, who set up the CGC account and generously provided hard disk space for us on their web server. These individuals include *Pamela Mason* (now at the National Institute for Standards and Technology), *Gail Juvik, Doug Bigwood, John Barnett, Gary McCone* and *Marty Sikes*. Their help has been invaluable.

Literature Cited

1. Kirkbride, Joseph Jr. 1992. Interactive microcomputer database for identification of *Cucumis*. Cucurbit Genetics Coop. Rept. 15:48-50.

2. Ng, T.J. 1994. Changes to CGC By-Laws. Cucurbit Genetics Coop. Rept. 17:ix.

Update of Gene List for *Cucurbita* spp.

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Alf Christianson Seed Co.. Spencer NY 14883

The most recent complete gene list for *Cucurbita* species was published in CGC Rpt. 15:102-109 (1992). The following update includes only additions to that gene list and revisions of previous gene symbols. Before publishing a proposed new gene symbol for a *Cucurbita* species, researchers are urged to consult the gene lists in CGC 15 and CGC 19 in order to avoid using a symbol already assigned to another gene.

| Gene Symbol | | | | |
Preferred	(Synonym)	Character	Species	Reference
D^o		*Dark* stem *only*, fruit color not affected	*pepo*	9
fv		*fused vein*; fusion of primary leaf veins	*pepo*	2
gl		*glabrous*, lacking trichomes	*maxima*	4
•*I-mc*	(I_{mc})	*Inhibitor* of *mature* fruit *color*	*pepo*	3
l-lSt	(*St*)	*Striped* fruit allele of the *light* fruit color gene	*pepo*	7
mldg		*mottled light* and *dark green* immature fruit	*moschata*	1
•*ms-3*	(*ms-2*)	*male sterile-3*	*maxima*	4
•*n-2*		*naked seeds-2*	*moschata*	10
pl		*plain light* fruit color	*pepo*	8
sp		*spaghetti* flesh, breaking into strands after cooking	*pepo*	6
wc		*white corolla*	*maxima*	5
wyc		*white-yellow corolla*	*maxima*	5
yg		*yellow-green* leaves and stems	*maxima*	4

•recommended new or revised gene symbol

Literature Cited

1. Cardosa, A.I.I. and P.T. Della Vecchia. 1993. Inheritance of immature fruit color in *C. moschata*. Cucurbit Genetics Coop. Rpt. 16:68-69.

2. Carle, R.B. and J.B. Loy. 1996. Genetic analysis of the fused vein trait in *Cucurbita pepo* L. J. Amer. Soc. Hort. Sci. 121:13-17.

3. Clayberg, C.D. 1992. Reinterpretation of fruit color inheritance in *Cucurbita pepo* L. Cucurbit Genetics Coop. Rpt. 15:90-92.

4. Korzeniewska, A. 1992. New genes in *Cucurbita maxima* Duch. In: R.W. Doruchowski and K. Niemirowicz-Szczytt, eds. 5th Cucurbitaceae Symposium, Skierniewice, Poland, p. 75.

5. Korzeniewska, A. 1996. Two independent loci for white and white yellow corolla in *Cucurbita maxima* Duch. In: M.L. Gómez-Guillamón, C. Soria, J. Cuartero, J.A. Torés, and R. Fernández-Munoz, eds. Proc. 6th Eucarpia Meeting Cucurbit Genetics and Breeding, Malaga, Spain, pp. 78-81.

6. Mazurek, Z. and K. Niemirowicz-Szczytt. 1992. Inheritance of spaghetti traits in *Curcubita pepo*. In: R.W. Doruchowski and K. Niemirowicz-Szczytt, eds. 5th Cucurbitaceae Symposium, Skierniewice, Poland, pp 70-74.

7. Paris, H.S. 1989. Allelism for fruit striping at the l-1 locus in *Cucurbita pepo*. In: C.E. Thomas, ed. Cucurbitaceae '89, Charleston, SC, pp. 116-118.

8. Paris, H.S. 1992. A recessive, hypostatic gene for plain light fruit coloration in *Cucurbita pepo*. Euphytica 60:15-20.

9. Paris, H.S. 1996. Genes affecting stem and fruit color intensity of straightneck squash. In: M.L. Gómez-Guillamón, C. Soria, J. Cuartero, J.A. Torés, and R. Fernández-Munoz, eds. Proc. 6th Eucarpia Meeting Cucurbit Genetics and Breeding, Malaga, Spain, pp. 43-48.

10. Xianglin, Z. 1987. A study of the breeding of naked kernel pumpkin and its genetic behavior. Acta Hort. Sinica 14:115-118. (In Chinese with English summary).

Gene Nomenclature for the Cucurbitaceae

[From: Robinson, R.W., H.M. Munger, T.W. Whitaker and G.W. Bohn. 1976. Genes of the Cucurbitaceae. HortScience 11:554-568.]

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, and all letters of the symbol and name are in lower case if the mutant gene is recessive to the normal type. The normal allele of a mutant gene is represented by the symbol "+", or where it is needed for clarity, the symbol of the mutant gene followed by the superscript "+". The primitive form of each species shall represent the + allele for each gene, except where long usage has established a symbol named for the allele possessed by the normal type rather than the mutant.

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 reoccurrences 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.

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

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.

Release of Three Gynoecious Pickling Cucumber Inbreds: Gy 7, Gy 8 and Gy 9

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Three gynoecious pickling cucumber inbreds (Gy 7, Gy 8, and Gy 9) are now available for release. Descriptions of the inbreds follow:

Gy 7: Gy 7 is an advanced selection (F_{10}) of a determinate gynoecious pickling cucumber. The inbred was derived from a cross of Gy 4 by M21. Gy 7 has a dark green vine with one to three laterals at high density plantings (> 50,000 plants per acre) and two to five laterals at low density plantings (< 30,000 plants per acre). Fruit are longer than Gy 14A with white spines, dark green with medium size warts and slight stippling and striping. The fruit are tapered at both ends. Plants are resistant to scab, downy mildew, anthracnose and angular leaf spot under Wisconsin conditions. Some field tolerance is exhibited to both cucumber mosaic virus and powdery mildew in Wisconsin. Seedling tests were used to screen for resistance to those diseases not common in Wisconsin. Seed yields are equal to those of Gy 14.

Gy 7 has good combining ability for yield (fruit number) and hybrids with Gy 7 parentage have performed well in trials located in all major pickling cucumber growing areas in the United States. Staminate flower induction has been effective with silver thiosulfate.

Gy 8. Gy 8 is an advanced selection of an indeterminate, gynoecious pickling cucumber line derived from a cross of Gy 14A and a UW breeding line designated 70. UW 70 has a complicated pedigree involving the following breeding lines: MSU 713-5, MSU 7, New Hampshire PM #1 Bush, New Hampshire Tiny Dill, Chipper and SC 10. Gy 8 has moderately long, medium green vines with an indeterminate, branched habit similar to Gy 14A. Fruit are cylindrical with slightly rounded to blocky ends, light to medium green color, with white spines and moderate warts, moderate stippling and striping. Gy 8 is generally about 0.3 of an L/D unit longer than Gy 14A. Plants are resistant to scab, cucumber mosaic virus, downy mildew, anthracnose and angular leaf spot and have some field tolerance to powdery mildew under Wisconsin conditions. Seedling tests were used for those diseases that are not common in Wisconsin. Seed yields are equal to those of Gy 14A.

Gy 8 has good combining ability for yield (fruit number) and hybrids with Gy 8 parentage have performed well in trials located in all major pickling cucumber growing areas in the United States. Brining quality of hybrids made with Gy 8 has been very good. Staminate flower induction has been effective with silver thiosulfate.

GY 9. Gy 9 is similar to Gy 8 in all respects, including parentage, except for fruit color. Gy 9 fruit are dark green with moderate stippling and striping. Gy 8 and Gy 9 were separated on the basis of fruit color at an advanced generation (> F_9), otherwise they are similar for vine type, fruit type, disease resistance, combining ability and hybrid performance.

Seed requests should be directed to Richard L. Lower, Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison WI 53706 USA. A standard inbred line research and development agreement will be forwarded to all who request seed. Upon receipt of a signed agreement seed will sent free of charge. The agreement allows for the conduct of research and development.

Release of Watermelon Mosaic Virus (WMV) Resistant Watermelon Breeding Lines WM-1, WM-2, WM-3 and WM-4

The U.S. Department of Agriculture, Agricultural Research Service, has released WM-1, WM-2, WM-3 and WM-4 watermelon mosaic virus 2 resistant breeding lines of watermelon (*Citrullus lanatus*). These lines are unique in that they are resistant to infection by the FC-1656 strain of WMV prevalent in Florida and demonstrate resistance in the greenhouse to the prevalent isolates of the virus from Arizona, California, Israel, Italy, and New York. In addition, plants which do become infected demonstrate milder symptoms than those produced in more susceptible lines.

These lines were developed at the Plant Genetic Resources Conservation Unit at Griffin, Georgia. WM-1, WM-2, WM-3 and WM-4 were selected from PI's 189316, 189317, and 248178 and 'Egun', respectively. Initial selections were made from 670 accessions evaluated in field and greenhouse tests, and selected resistant plants were selfed for 3-5 generations.

Breeder seed of WM-1, WM-2, WM-3 and WM-4 is available on a pro- rata basis to both public and private breeders. Requests should be sent to the Vegetable Curator, USDA, ARS, 1109 Experiment St., Griffin GA 30223-1797 USA, or faxed to (770) 229-3324. It is requested that appropriate recognition be made if this germplasm contributes to the development of a new breeding line or cultivar.

Al Masoum, Ahmed A. P.O. Box 10355, Dubai, United Arab Emirates. Tel: (9713)-614430, Fax: (9713)-612662. Growth regulators on cucurbit crops.

Alvarez, Jose Ignacio S&G Semillas, S.A., Ctra. Malaga, km. 87,2, 04700 El Ejido, Almeria, Spain. Tel: 950-58 12 07, Fax: 950-58 12 79. Melon breeding.

Andres, Thomas C. 5440 Netherland Ave., # D24, Bronx, NY 10471-2321. Tel: (718) 601-7329, Fax: (718) 601-7329. E-mail: *tcandre@med.cornell.edu*.

Ayuso, Ma Cruz Petoseed Iber SA, Paraje San Nicolas S/N, 04710 La Mojonera, Almeria, Spain.

Barbercheck, Mary Dept. Entomology, Box 7634, North Carolina State Univ., Raleigh, NC 27695-7634. Tel: (919) 515-1651, Fax: (919) 515- 2824. E-mail: *mary_barbercheck@nscu.edu*. Spotted cucumber beetle, cucurbitacins.

Barham, Robert W. Barham Seeds, Inc., 10030 New Ave., Gilroy, CA 95020. Tel: (408) 847-7412, Fax: (408) 847-5877.

Beaver, Linda *see Wessel-Beaver, Linda*

Beekman, A.G.B. ROYAL SLUIS, P.O. Box 22, 1600 AA Enkhuizen, The Netherlands.

Bhargava, Yash Sandoz (India) Ltd., "Seeds House", 1170/27, Revenue Colony, Shivajinagar, Pune - 411 005, India. Agrochemicals, seeds, etc.

Bosma, Monique ENZA ZADEN, De Enkhuizer Zaadh. b.v., Postbox 7, 1600 AA Enkhuizen, The Netherlands. Tel: 02280-1-58-44, Fax: 02280-1-59-60.

Boyhan, George E. Auburn University, 101 Funchess Hall, Auburn, AL 36849. Tel: (205) 844-3041, Fax: (205) 844-3131. E-mail: *gboyhan@ag.auburn.edu*. Melon and watermelon breeding

Burkett, Al PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax: (916) 668-0219. Pickling cucumber breeding.

Çaglar, Gulat Alata Bahce Kulturleri, Arastirma Enstitusu, Erdemli, Icel-Turkey. Tel: 0324.5151049, Fax: 0324.5152527. Cucumber breeding.

Carey, Edward E. International Potato Center (ICP), P.O. Box 25171, Nairobi, Kenya. Tel: 592054. E-mail: *cgi801%nsfmail@inter*.

Carle, R. Bruce Univ. Florida, Central Florida R&E Center, 5336 University Ave., Leesburg, FL 34748-8232. Tel: (352) 360-6686, Fax: (352) 360-6691. Watermelon and squash breeding.

Carre, Monique INRA, AMPM BP 93, 84143 Montfavet, France.

Chen, Fure-Chyi Dept. Plant Industry, Natl. Pingtung Polytechnic Institute, Neipu, Pingtung 91207, Taiwan, Rep. China. Tel: 886-8-774- 0267, Fax: 886-8-770-4186. Gene transfer, breeding, tissue culture and isozymes.

Chung, Paul PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax: (916) 668-0219.

Close, Timothy J. University of California, Riverside, Dept. Botany & Plant Sciences, Riverside, CA 92521-0124. Tel: (909) 787-3318, Fax: (714) 787-4437. E-mail: *timclose@ucracl.ucr.edu*. Bacterial wilt, dehydration stress, and proteins.

Cohen, Ron A.R.O., Newe Ya'ar Expt. Station, P.O. Box 90000, Haifa, 31900, Israel. Tel: 04-833186, Fax: 04-836936. Plant pathology; root and foliar diseases of cucurbits.

Cohen, Yigal Department of Life Sciences, Bar-Ilan University, Ramat- Gan 52 100, Israel. Tel: + 9723-5318251, Fax: + 9723-6771088. Melon.

Cook, Kevin L. Rogers Seed Co. Research Station, 10290 Greenway Road, Naples, FL 33961. Tel: (813) 775-4090, Fax: (813) 774-6852. Breeding of summer squash.

Corella, Pilar Asgrow Seed Co., Apdo. 175, 04700 El Ejido (Almeria), Spain. Tel: 34-51-580012, Fax: 34-51-581162.

Coyne, Dermot P. Department of Horticulture, University of Nebraska, Lincoln, NE 68583-0724. Tel: (402) 472-1126, Fax: (402) 472-2853. E-mail: *dcoyne@unlinfo.unl.edu*. Breeding, genetics, physiology, plant development and disease resistance of squash.

Cramer, Chris North Carolina St. Univ., Box 7609, Raleigh, NC 27695- 7609. Tel: (919) 515-3178. E-mail: *chris_cramer@ncsu.edu*. Cucumber yield, yield components, combining ability, and recurrent selection.

Cui, Hongwen Department of Horticulture, Northwestern Agricultural Univ., Yangling, Shaanxi 712100, P.R. China.

Dane, Fenny Dept. Horticulture, Auburn University, Auburn, AL 36849. Tel: (205) 844-3047. E-mail: *fdane@ag.auburn.edu*.

Danin-Poleg, Yael A.R.O., Newe Ya'ar Expt. Station, P.O. Box 90000, Haifa 31900, Israel.

de Groot, Erik Breeding, Sementi Nunhems S.R.L., Via Ghiarone, 2, 40019 S. Agata Bolognese, Italy.

de Ruiter, A.C. de Ruiter Zonen CV, Postbus 4, 2665 ZG Bleiswijk, The Netherlands. Tel: 01892-16555, Fax: 01892-17890. Breeding and seed production of cucumbers.

Decker-Walters, Deena The Cucurbit Network, 11901 Old Cutler Road, Miami, FL 33156-4242. Tel: (305) 667-3800, Fax: (305) 661-5984. Communication via *The Cucurbit Network*; the whole family Cucurbitaceae.

Della Vecchia, Paulo T. Av Das Nacoes 68, Jardim Europa, CP 12900-000 Braganca, Paulista SP, Brazil. Tel: (011) 7810855, Fax (011) 4841599. Breeding & genetics, seed production and disease resistance of melon and squash.

Denlinger, Phil Mt. Olive Pickle Co., Inc., P.O. Box 609, Mount Olive, NC 28365. Tel: (919) 296-1996.

Dewei, Ma Department of Horticulture, Hebei Agricultural University, Baoding, Hebei 071000, P.R. China.

Dhaliwal, Major Singh Dept. of Vegetable Crops, L.S.&F. Punjab Agriculture University, Ludhiana-141004, Punjab, India.

Di Nitto, Louis Victor Sunseeds, 8850 59th Ave., N.E., Brooks, OR 97305. Tel: (503) 393-3243, Fax (503) 390-0982. Melon (*Cucumis melo*).

Dogimont, C. INRA, St. Maurice, BP 94, 84143 Montavet, France.

Drowns, Glenn Sand Hill Preservation Center, 1878 230th Street, Calamus, IA 52729. Tel: (319) 246-2299. Genetic preservation of all cucurbits. Taxonomy of *Cucurbita moschata* and *Cucurbita argyrosperma*.

Duangsong, Usa Peto Thailand, P.O. Box 171, 99 Moo 2, Wiang-Y, Mae Gorn, A Muang Chiang Rai 57000, Thailand.

Dunlap, James R. Texas Agric. Expt. Station, 2415 E. Highway 83, Weslaco, TX 78596. Tel: (210) 968-5585, Fax: (210) 968-0641. E-mail: *j-dunlap@tamu.edu*. Melon physiology - fruit development and ripening.

Eigsti, Orie J. 1602 Winsted, College Green, Goshen, IN 46526. Tel: (219) 533-4632. Polyploid *Citrullus lanatus*.

El Jack, Ali Elamin Dept. Horticulture, Fac. Agric. Sci., University of Gezira, Wad Medani, P.O. Box 20, Sudan.

El-Doweny, Hamdy Hassan Ali c/o A. El-Menshawy, Foreign Agric Rel, Min. Agriculture, Nady El-Seid Street, Dokki, Cairo, Egypt. Cucurbit breeding program, including: diseases (virus, fungal), salinity, greenhouses, hybrids.

Elmstrom, Gary Pioneer Vegetable Genetics, 18285 County Road 96, Woodland, CA 95695. Tel: (916) 666-6136. Triploid watermelon breeding.

Ezura, Hiroshi Plant Biotechnology Institute, Ibaraki Agricultural Center, Ago, Iwama, Nishiibaraki, 319-02 Ibaraki, Japan. Tel: 0299-45-8330, Fax 0299-45-8351.

Fanourakis, Nikolaos E. Technological Educational Institute, Heraklion Crete 71500, Greece.

Funakushi, Hisashi Mikado Seed Growers Co., Ltd., 1203 Hoshikuki, Chūo-ku, Chiba City 260, Japan. Tel: 81-43-265-4847, Fax 81-43-266- 6444.

G.A. Kaufmanns Buchhandlung, Aloys-Schulte-Strasse 2, D 5300 Bonn 1, Germany.

Gaba, Victor Dept Virology, Inst Plant Protection, A.R.O., Volcani Center, P.O.B. 6, Bet Dagan 50250, Israel. Tel: 972-3-9683568/9, Fax: 972-3-9604180. E-mail: *vpgaba@volcani.bitnet*. Tissue culture & transformation.

Gabert, August C. Sunseeds Genetics, Inc., 8850 59th Ave. NE, Brooks, OR 97305-9625. Tel: (503) 393-3243, Fax: (503) 390-0982. Cucumber and summer squash breeding and genetics.

Gaggero, James M. 8276 Canyon Oak Drive, Citrus Heights, CA 95610. Tel: (916) 721-9584, Fax: (916) 721-5352. Cucurbitacins.

Garrett, J.T. Pee Dee Res. & Educ. Center, 500 West Pocket Road, Florence, SC 29501. Tel: (803) 662-3526, Fax: (803) 661-5676. E-mail: *jtgrtt@prism.clemson.edu*. Production systems, especially stand establishment.

Gautier, Graines Boite Postale 1, 13630 Eyragues, France. Tel: 90.94.13.44, Fax: 90.92.83.96.

Gillian L. Maggs, Curator, National Herbarium (WIND), NBRI, Private Bag 13184, Windhoek, Namibia. Tel: + 264 61 3029111, Fax: + 264 61 3022177.

Gomez, Humberto IRTA, Centre de Cabrils, 08348 Cabrils (Barcelona), Spain. Tel: (93) 750 75 11, Fax (03) 753 39 54.

Gómez-Guillamón, M. Luisa Estacion Experimental "La Mayora", 29750 Algarrobo-Costa, M laga, Spain. Tel: (952) 51 10 00, Fax: (952) 51 12 52. E-mail: *guillamon@mayora.csic.es*.

Green, C. Ed PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax (916) 668-0219.

Groff, David Asgrow Seed Company, Rt. 1, Box 1907, Omega TyTy Road, Tifton, GA 31794. Tel: (912) 386-8701, Fax (912) 386-8805. Breeding of squash, cucumber, melon and watermelon.

Grumet, Rebecca Dept. Hort., Plant & Soils Building, Michigan State University, East Lansing, MI 48824-1325. Tel: (517) 353-5568, Fax: (517) 353-0890. E-mail: *grumet@msu.edu*. Disease resistance, gene flow, tissue culture and genetic engineering.

Hagihara, Toshitsugu Hagihara-Farm-Co., Ltd., 984 Hokiji, Tawaramoto-cho, Shiki-gun, Nara-ken, 636-03, Japan. Tel: 07443-3-3233, Fax 07443-3-4332.

Haim, Davidi Hazera Ltd., Mivhor Farm Doar, Sede Gat 79570, Israel.

Han, Sang Joo Seoul Seed Intl Co. Ltd, Chongill B/D, 736-17 Yeoksam-Dong, Kangnam-gu, Seoul, Korea. Tel: (2) 569-7147, Fax (2) 552-9439. Disease resistance.

Hassan, Ahmed Abdel-Moneim Department of Vegetable Crops, Fac. Agriculture, Cairo University, Giza, Egypt. Tel: 724107 & 724966. Cucumber, melon, squash & watermelon germplasm evaluation and breeding for disease resistance, incl. viruses.

Havey, Michael J. USDA/ARS, Department of Horticulture, University of Wisconsin, Madison, WI 53706. Tel: (608) 262-1830. E-mail: *mjhavey@macc.wisc.edu*.

Herman, Ran "Zeraim" Seed Growers Company Ltd., Department of Breeding, Gedera 70 700, Israel. Tel: 08-59 27 60, Fax 08-59 43 76.

Herrington, Mark Edward 21 Warner Street, Wellington Point, Queensland 4160, Australia. Tel: 07 2861488.

Hertogh, K. Nickerson-Zwaan b.v., Postbus 19, 2990 AA Barendrecht, The Netherlands.

Himmel, Phyllis Asgrow Seed Company, 500 Lucy Brown Lane, San Juan Bautista, CA 95045.

Hirabayashi, Tetsuo Nihon Horticultural Production Inst., 207 Kamishiki, Matsudo-shi, Chiba-ken 271, Japan. Tel: 0473-87-3827, Fax 0473-86-1455. Varietal improvement of cucurbit crops, especially melon, cucumber and pumpkin.

Hollar, Larry A. Hollar & Co., Inc., P.O. Box 106, Rocky Ford, CO 81067. Tel: (719) 254-7411, Fax (719) 254-3539. E-mail: *lahollar@iguana.ruralnet.net*. Cucurbit breeding and seed production.

Holle, Miguel CALCE 2, # 183 Urb. El Rancho, Miraflores - Lima 18, Peru. E-mail: *mholle@cipa.org.pe*. Plant genetic resources.

Hong, Kue-Hyon Vegetable Breeding Div., NHRI, 540 Tap-Dong, Suwon 441-440, Republic of Korea. Tel: 82-331-290-6181, Fax 81-331-295- 9548.

Humaydan, Hasib Ag Consulting International, 317 Red Maple Drive, Danville, CA 94506. Tel: (510) 736-1241, Fax (510) 736-1241.

Hung, Lih National Taiwan Univ., College Agric., Dept. Hortic., Vegetable Crops Lab., Taipei, Taiwan 107, Republic of China.

Hutton, Mark Chriseed Research Dept., 208 Bald Hill Road, Spencer, NY 14883. Tel: (607) 272-1255, Fax (607) 272-1255. Breeding and product development.

Ibrahim, Aly M. c/o Mo. Ibrahim, 701 S. Friendswood Dr., Apt. 504, Friendswood, TX 77546. Cucumber, melon, watermelon.

Ignart, Frederic Centre de Recherche TEZIER, Route de Beaumont, Domaine de Maninet, 26000 Valence, France. Tel: (33) 75575757, Fax (33) 75552681. Squash and melon breeding.

Iida, Akira Minowa Noen, 63-1 Ichieda-cho, Yamato-Kohriyama City, Nara Pref., Japan, T639-11.

Ikeda, Satoru Sakata Seed America, Inc., P.O. Box 1118, Lehigh, FL 33970-1118. Tel: (941) 369-0032, Fax (941) 369-7528.

Ito, Kimio Vegetable Breeding Laboratory, Hokkaido Natl. Agric. Expt. Sta., Hitsujigaoka, Sapporo 062, Japan. Tel: 011(851)9141, Fax: 011(859)2174.

Jain, Jaagrati B-149, M.P., Enclave, Pitampura, Delhi - 110034, India. Tel: 7183099. Muskmelon genetics and tissue culture.

Jiang, Jiping PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax (916) 668-0219. Developing disease screens for fungal diseases of cucurbits.

Johnston, Rob Jr. Johnny's Selected Seeds, Foss Hill Road, Albion, ME 04910- 9731. Tel: (207) 437-9294, Fax: (207) 437-2603.

Kamimura, Shoji 421-19 Furuichi-machi, Maebashi City, Gunma-ken 371, Japan.

Kampmann, Hans Henrik Breeding Station Danefeld, Odensevej 82, 5290 Marslev, Denmark. Tel: 65 95 17 00, Fax: 65 95 12 93.

Kanno, Tsuguo Dept Upland Farming, Tohoku Natl. AES, 50, Arai, Fukushima, Fukushima Prefecture, Japan 960-21.

Karchi, Zvi Div Vegetable Crops, ARO, Newe Ya'ar Research Ctr, PO Box 90000, Haifa 31900, Israel. Tel: 04-9833186, Fax: 972-3-9665327. Cucurbit breeding, cucurbit physiology.

Kato, Kenji Fac. Agriculture, Okayama Univ., 1-1-1 Tsushima Naka, Okayama, 700 Japan. Tel: 81-86-251-8323, Fax: 81-86-254-0714. E-mail: *kenkato@ccews2.cc.okayama-u.ac.jp*. Use of molecular markers for QTL mapping and cultivar identification in melon.

Katzir, Nurit A.R.O., Newe Ya'ar Expt. Station, P.O. Box 9000, Haifa 31900, Israel.

King, Stephen R. Petoseed Research, 37437 State Hwy. 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax: (916) 668-0219. E-mail: *srking2570@aol.com*. Melon breeding.

Kirkbride, Joseph H. Jr. USDA-ARS, System. Bot. & Mycol. Lab, Bldg 265, BARC- East, Beltsville, MD 20705. Tel: (301) 504-9447. E-mail: *jkirkbride@asrr.arsusda.gov*.

Klapwijk, Ad De Ruiter Zonen CV, Postbus 4, 2665 AZ Bleiswijk, The Netherlands. Tel: 01892-16555, Fax: 01892-17890.

Knerr, Larry D. Shamrock Seed Company, P.O. Box 1223, San Juan Bautista, CA 95045-1223. Tel: (408) 636-0803, Fax: (408) 636-9708. E- mail: *ldknerr@aol.com*. Varietal development of honeydew and cucumber.

Kraakman, Peter Torre Verde 7-2, Aguadulce, Roquetas De Mar, Spain.

Kuginuki, Yasuhisa National Res. Institute Veg/Orn/Tea, Crop Research Station, Ano, Mie 514-23, Japan. Tel: 0592-68-1331, Fax: 0592-68-1339. Breeding for resistance to disease.

Kuhlmann, Hubert Fink GmbH, Benzstrasse 25, D-71083 Herrenberg, Germany. Tel: (0 70 32) 922-0, Fax (0 70 32) 5221.

Kuti, Joseph O. Dept Agron & Res Sci, Hort Crops Lab, Texas A&M University - Kingsville, Kingsville, TX 78363. Tel: (210) 595-3711, Fax: (210) 595-3713. E-mail: *kfjok00@taiu.edu*. Breeding and genetics; host- parasite interrelationships; postharvest physiology.

Kwack, Soo Nyeon Dept Hort Breeding, Mokpo Natl Univ, Dorimri, Chonggyemyun, Muangun, Chonnam 534-729, Korea.

Kwon, Young-Seok Horticultural Experiment Station, 20 Kangdongdong, Kangseoku, Pusan, Rep. Korea 618-300.

Kyle, Molly Cornell Univ, Dept Plant Brdng & Biom, 312 Bradfield Hall, Ithaca, NY 14853-1902. Tel: (607) 255-8147, Fax: (607) 255-6683. E-mail: *mmk9@cornell.edu*. Melon and squash breeding and genetics.

Lecouviour, Michel Clause Semences Professionnelles, 24, boulevard P. Brossolette, 91221 Bretigny-sur-Orge, France.

Lehmann, Louis Carl Dept. Plant Breeding Research, Swedish Univ. Agricultural Sci., S-268 31 Svaloev, Sweden. Tel: 46-418-67200, Fax: 46-418-67081. E-mail: *louis.lehmann@vf.slu.se*.

Lester, Gene USDA/ARS, Subtropical Agric Res Lab, 2301 S. International Blvd., Weslaco, TX 78596. Tel: (210) 565-2647, Fax: (210) 565-6133. E-mail: *glester@pop.tamu.edu*. Stress and pre/postharvest physiology of melons.

Lin, Depei Sichuan Academic Agric. Science, Hortseed Center, Chengdu 61006, People's Rep. China. Tel: (028) 4436732, Fax: (028) 4442025.

Love, Stephen Loyd Aberdeen R&E Center, P.O. Box AA, Aberdeen, ID 83210. Tel: (208) 397-4181, Fax: (208) 397-4311. E-mail: *slove@uidaho.edu*. Small scale private watermelon breeding with emphasis on adaptation to cold climates.

Lower, Richard L. Coll. Agriculture, Univ. Wisconsin, 1450 Linden Drive, Room 240, Madison, WI 53706. Tel: (608) 262-2349, Fax: (608) 262-4556. E-mail: *richard.lower@ccmail.adp.wisc.edu*. Effects of plant type genes on yield, sex-expression, growth parameters, pest resistance & adaptability.

Loy, J. Brent Plant Biology Dept., Nesmith Hall, University of New Hampshire, Durham, NH 03824. Tel: (603) 862-3216, Fax: (603) 862-4757. E- mail: *jbloy@christa.unh.edu*. Squash, melon, pumpkin. Genetics, breeding, plasticulture, mulch, rowcovers.

Lydon, Lewis R.B. Arthur Yates & Co. Pty. Limited, Research Farm, Burroway Road, Narromine, N.S.W. 2821, Australia. Tel: (068) 89-1144.

M.M.T. Hoogenboom, Reg. & Doc. Section, NAKG, Sotaweg 22, P.O. Box 27, 2370 AA Roelofarendsveen, The Netherlands. Tel: + 31 171319102, Fax + 31 171316256.

Maluf, Wilson Roberto Dept. de Agricultura/UFLA, Caixa Postal 37, 37200-000 Lavras-MG, Brazil. Tel: (035) 829-1326, Fax: (035) 829-1301. E-mail: *esal@eu.ansp.br*. Cucumbers, melons, squashes.

Maneesinthu, Likhit Thep Watana Seeds Co., Ltd., 293-293/1-2, Surwongse Road, Bangkok 10500, Thailand. Tel: 66-2-2376540, Fax: 66-2- 2376543. Breeding and seed production of cucumber, watermelon, melon and pumpkin.

Markiewicz-Ladd, Krystyna Polonica International, P.O. Box 2305, Gilroy, CA 95021. Tel: (408) 842-1022, Fax: (408) 675-0103. Melons - breeding, new germplasm, postharvest physiology, biotechnology, cultural practices, new diseases.

Martyn, Ray D. Dept. Plant Path. & Microbiology, Texas A&M University, College Station, TX 77845. Tel: (409) 845-7311, Fax: (409) 845-6483. E-mail: *martyn@ppserver.tamu.edu*. Soilborne diseases of watermelon and melon, particularly the Fusarium wilts and vine declines.

Matsuura, Seiji Kiyohara Breeding Sta., Tohoku Seed Co., 1625 Nishihara, Himuro, Utsunomiya, Japan. Tel: 0286-34-5428, Fax: 0286-35- 6544.

Maynard, Donald N. Univ Florida-IFAS, Gulf Coast R&E Ctr, 5007 60th Street East, Bradenton, FL 34203. Tel: (813) 751-7636, Fax: (813) 751- 7639. Tropical *moschata* improvement; watermelon variety evaluation and production practices.

McClurg, Charles A. University of Maryland, Department of Horticulture, College Park, MD 20742-5611. Tel: (301) 405-4342, Fax: (301) 314-9308. E-mail: *cm19@umail.umd.edu*. Production and culture of cucurbit crops.

McCreight, J.D. USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905. Tel: (408) 755-2864, Fax: (408) 753-2866. E-mail: *jmccreig@asrr.arsusda.gov*. Melon breeding and genetics.

McGrath, Desmond John Dept. Primary Ind., Hortic. Res. Sta., P.O. Box 538, Bowen, Queensland 4805, Australia. Tel: + 61-77-852255, Fax + 61-77-852427. Disease resistance in *Cucumis melo*, particularly gummy stem blight.

Meadows, Mike Rogers Seed Co., 10290 Greenway Road, Naples, FL 33961.

Merrick, Laura C. Dept. Appl. Ecology & Environ. Sci., 5722 Deering Hall, Univ. Maine, Orono, ME 04469-5722. Tel: (207) 581-2950, Fax (207) 581-2199. E-mail: *merrick@maine.maine.edu*. *Cucurbita* evolution; cucurbit germplasm evaluation and conservation; ethnobotany and evolution.

Milerue, Sompong Peto Thailand, P.O. Box 171, 99 Moo 2, Wiang-Y, Mae Gorn, A Muang Chiang Rai 57000, Thailand.

Milotay, Peter Vegetable Crops Research Institute, P.O. Box 116, Kecskemet, 6000, Hungary.

Miranda, Baldwin Rogers NK Seed Co., 10290 Greenway Road, Naples, FL 33961. Tel: (813) 775-4090, Fax: (813) 774-6852.

Miyoshi, K. Sakata Seed Corp., Kakegawa Breeding Sta, 1743-2 Yoshioka, Kakegawa, Shizuoka 436-01, Japan.

Mochizuki, Tatsuya Kurume Br, Natl Res Inst Veg/Orn/Tea, 1823 Mii- machi, Kurume, Fukuoka 830, Japan.

Mohamed, Yousif Fadlalla Dept. Plant Pathology, Fac. Agric. Sci., University of Gezira, Wad Medani, P.O. Box 20, Sudan.

Moraghan, Brian J. Asgrow Seed Co., P.O. Box 667, Arvin, CA 93203. Tel: (805) 854-2390, Fax: (805) 854-4379. Melon and watermelon breeding and disease resistance.

Morelock, Ted Dept. Horticulture & Forestry, University of Arkansas, Fayetteville, AR 72701. Tel: (501) 575-2603, Fax: (501) 575-8619. E-mail: *tem24811@uafsysb.uark.edu*. Cucumber breeding.

Munger, H.M. Cornell University, 252 Emerson Hall, Ithaca, NY 14853. Tel: (607) 255-1661, Fax (607) 255-6683. Cucurbit breeding and disease resistance.

Murdock, Brent A. 3040 Trail Road, Belton, SC 29627. Watermelon breeding; genetic improvement of neglected tropical vegetables.

Nance, John Willhite Seed, Inc., P.O. Box 23, Poolville, TX 76487. Tel: (817) 599-8656, Fax (817) 599-5843.

Navazio, John P. Garden City Seeds, 1324 Red Crow Road, Victor, MT 59875. Tel: (406) 961-4837, Fax (406) 961-4877. Breeding for increased carotenes in cucumber and squash.

Nea, Larry PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax (916) 668-0219. Cucumbers, melons, squash, watermelon.

Nechama, Shulamit Breeding Department, Mivhor Farm, Post Sde Gat 79570, Israel.

Ng, Timothy J Department of Horticulture, University of Maryland, College Park, MD 20742-5611. Tel: (301) 405-4345, Fax (301) 314-9308. E-mail: *tn5@umail.umd.edu*. Melon breeding and genetics; postharvest physiology; seed germination.

Niemirowicz-Szczytt, Katarzyna Warsaw Ag Univ, Dept Gen & Plt Brdng, ul. Nowoursynowska 166, 02-766 Warsaw, Poland. Tel: 43 09 82, Fax (48-22) 471562. Cucumber, melon, winter and summer squash, watermelon - genetics, breeding, tissue culture, biotechnology.

Nuez, Fernando Cat.de Genetica, ETS Ingen. Agron., Univ. Politecnica, Camino de Vera, 14, 46020 Valencia, Spain.

Nugent, Perry USDA-ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29414. Tel: (803) 556-0840. Melon and watermelon inheritance studies, pest resistance, stress resistance, and fruit quality.

Om, Young-Hyun Alpine Agriculture Exp. Station, Heongkye-Ri, Doam- Myun, Pyeongchang-Gun, Kangwon-Do 232-950, Republic of Korea.

Omara, Sadig Khidir Dept. Horticulture, Fac. Agric. Sci., University of Gezira, Wad Medani, P.O. Box 20, Sudan.

Ortega, Sergio Garza Univ. Sonora, Dept. Agric. y Ganad., A.P. Postal 305, Hermosillo, Sonora, Mexico. Breeding of Cucurbita spp.; testing of new muskmelon lines.

Ouyang, Wei PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax (916) 668-0219. Squash & cucumber breeding.

Owens, Ken PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax (916) 668-0219. Cucumber breeding.

Pablo Gomez Riera, Director, INTA La Consulta, Casilla de Correo 8 - (5567), La Consulta, Mendoza, Argentina.

Palmer, Louis Pioneer Vegetable Genetics, 4331 Cockroach Bay Road, Ruskin, FL 33570-2612. Tel: (813) 645-3946, Fax (813) 645-4900. Breeding and genetics.

Palomares, Gloria Dept Biotecnologia, Univ Politecnica, Camino de Vera, s/n., E-46022 Valencia, Spain. Tel: 34 (6) 387 74 20, Fax 34 (6) 387 74 29. E-mail: *secret@btc.upv.es*.

Paris, Harry Div. Vegetable Crops, A.R.O., Newe Ya'ar Expt Sta, PO Box 31900, Haifa 31900, Israel. Tel: 972-4-894516, Fax 972-4-836936. Breeding and genetics of squash, pumpkin, melon and watermelon.

Perl-Treves, Rafael Dept. Life Science, Bar-Ilan University, Ramat- Gan, Israel 52900. E-mail: *perl@brosh.cc.biu.ac.il*.

Peter, K.V. Natl. Research Ctr for Spices, ICAR, Post Bag No. 1701, Marikunnu P.O., Calicut - 673 012, Kerala, India.

Picard, Florence Vilmorin, La Menitre, 49250 Beaufort-en-Vallee, France.

Pierce, Lawrence 800 Seely Ave., Aromas, CA 95004-9612.

Pierce, Vickie 800 Seely Ave., Aromas, CA 95004-9612.

Pitrat, Michel I.N.R.A., BP 94, 84143 Montfavet cedex, France. Tel: (33) 90 31 63 00, Fax (33) 90 31 63 98. E-mail: *Michel.Pitrat@avignon.inra.fr*. Melon, disease resistance, mutants, genetic map.

Poostchi, Iraj 97 St. Marks Road, Henley-on-Thames RG9 1LP, England.

Price, E. Glen American Sunmelon Research Center, P.O. Box 153, Hinton, OK 73047. Tel: (405) 542-3456, Fax (405) 542-3457. Seedless watermelon; polyploidy, genetics, breeding, cytogenetics.

Provvidenti, Rosario Cornell Univ., Dept. Plant Pathology, NY State Agric. Experiment Sta., Geneva, NY 14456-0462. Tel: (315) 787-2316, Fax (315) 787-2389. E-mail: *rp13@cornell.edu*. Breeding & genetics of resistance to viral diseases of cucumber, squash, melon, watermelon & other cucurbits.

Punja, Zamir K. Dept. Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada. E-mail: *punja@sfu.ca*.

Quemada, Hector Asgrow Seed Company, 2605 East Kilgore Road, Kalamazoo, MI 49002-1782. Tel: (616) 337-9400, Fax (616) 337-9418. Biotechnology, breeding, pathology.

Ray, Dennis Department of Plant Sciences, University of Arizona, Tucson, AZ 85721. Tel: (602) 621-7612, Fax: (602) 621-7186. E-mail: *dtray@ccit.arizona.edu*. Genetics and cytogenetics of *Cucumis melo* and *Citrullus* spp.

Reuling, G. Nunhems Zaden B.V., P.O. Box 4005, 6080 AA Haelen, The Netherlands. Tel: 04759-9222, Fax: 04759-9223/5104.

Rhodes, Billy B. Clemson Univ./Horticulture, Poole Agricultural Center, Clemson, SC 29634-0375. Tel: (803) 656-0410, Fax: (803) 656- 4960. E-mail: *bill_rhodes@quickmail.clemson.edu*. Watermelon genetics, breeding, micropropagation, disease resistance, male sterility, triploids.

Robinson, R.W. Dept. Hort. Sci., New York State AES, Hedrick Hall, Geneva, NY 14456-0462. Tel: (315) 787-2237, Fax: (315) 787-2397. E-mail: *rwrl@cornell.edu*. Breeding and genetics of cucurbits.

Robledo, C. Asgrow - France, Centre de Recherches, Mas d'Aptel, 30510 Generac, France. Tel: 66 01 89 07, Fax: 66 01 31 68. Melon breeding.

Roig, Luis A. Departamental Biotechnology, ETS Ingen. Politec., Camino de Vera 14, 46022 - Valencia, Spain. Tel: 34(6) 3877424, Fax: 34(6) 3877429.

Rumsey, Anthony E. New World Seeds Pty Ltd., P.O. Box 18, Dural 2158, 22-24 Crosslands Road, Galston, N.S.W., Australia.

Scheirer, Douglas M. The Nestle Food Co./Baking/Libby, 216 N. Morton Ave., P.O. Box 198, Morton, IL 61550. Tel: (309) 263-2133. Breeding, cultural practices, etc., associated with processing pumpkin (*Cucurbita moschata*) Dickinson.

Schroeder, Robert Harold Harris Moran Seed Co., R.R. 1, Box 1243, Davis, CA 95616. Tel: (916) 756-1382, Fax: (916) 756-1016. Incorporating disease resistance into useful commercial cultivars.

Schultheis, Jonathan R. Dept. Horticulture, 264 Kilgore Hall, North Carolina St. University, Raleigh, NC 27695-7609. Tel: (919) 515-3131, Fax: (919) 515-7747. E-mail: *jonathan_schultheis@ncsu.edu*.

Semillas Fito, S.A., c/. Selva de Mar, 111, 08019 Barcelona, Spain. Tel: (93) 307 62 12, Fax: (93) 307 03 64.

Shifriss, Oved 21 Walter Avenue, Highland Park, NJ 08904. Tel: (908) 246-0028. Regulation of the *B* genes in *Cucurbita*.

Shiga, Toshio Plant Biotech. Ctr., Sakata Seed Corp., 358 Uchikoshi, Sodegaura, Chiba, 299-02, Japan. Tel: 0438-75-2369, Fax: 0438-75-2594. Cell biology.

Simon, Philipp W. 5125 Lake Mendota Drive, Madison, WI 53705. Tel: (608) 264-5406, Fax: (608) 262-

4743. E-mail: *simon@macc.wisc.edu*. Breeding and genetics.

Sipeyre, Bruno Mas de Rouzel, Chemin des Canaux, 30900 Nimes, France. Tel: 66.84.21.32, Fax: 66.38.09.42.

Skirvin, Robert M. Univ. Illinois, Dept. Horticulture, 258 PABL, 1201 Gregory Dr., Urbana, IL 61801. Tel: (217) 333-1530, Fax (217) 333- 4777. E-mail: *skirvin@ux1.cso.uiuc.edu*. Micropropagation; somaclonal variation.

Snyder, James W. 1231 Kirkwood Drive, Vineland, NJ 08360. Tel: (609) 794-3880.

Staub, Jack E. USDA-ARS, Dept. Horticulture, Univ. Wisconsin, Madison, WI 53706-1590. Tel: (608) 262-0028, Fax: (608) 262-4743. E- mail: *jestaub@facstaff.wisc.edu*. Cucumber breeding & genetics, physiology, biochemical genetic markers, evolution, environmental stress.

Stephenson, Andrew G. 208 Mueller Lab, Penn State University, University Park, PA 16802. Tel: (814) 863-1553, Fax: (814) 865-9131. E-mail: *as4@psuvm.psu.edu*.

Stern, Joseph Royal Sluis Inc., 650 Leanna Drive, Arroyo Grande, CA 93420-4400.

Stevens, M. Allen PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax: (916) 668-0219. Direction of research.

Summers, William S. Iowa State University, 106 Horticulture, Ames, IA 50011-1100. Tel: (515) 294-1978, Fax: (515) 294-0730. E-mail: *summers@iastate.edu*. Genetic improvement of watermelon

Tatlioglu, Turan Institut of Applied Genetics, Univ. Hannover, Herrenhauser Str. 2, 3000 Hannover, Germany.

Taurick, Gary Ferry Morse Seed Company, P.O. Box 392, Sun Prairie, WI 53590. Tel: (608) 837-6574, Fax: (608) 837-3758. Population improvement and hybrid development for cucumber and summer squash.

Teppner, Herwig Institute of Botany, Univ. Graz, Holteigasse 6, A- 8010 Graz, Austria. Tel: 316-380-5656, Fax: 316-38-12-21. Systematics, morphology, ecology, crops & medicinal plants (teaching) and small scale breeding.

Thomas, Claude E. USDA-ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29414.

Thompson, Gary Dept. Plant Sciences, University of Arizona, Tucson, AZ 85721. E-mail: *gthompso@ccit.arizona.edu*.

Tolla, Greg Asgrow Seed Company, Rt. 1, Box 1907, Tifton, GA 31794. Tel: (912) 386-8701, Fax: (912) 386-8805. Cucumber breeding and genetics.

Tsaftaris, A.S. Dept. Genetics & Breeding of Plants, Aristotelian Univ. of Thessaloniki, Thessaloniki, 54006, Greece.

Vakalounakis, Demetrios J. Plant Protection Inst., N.A.R.F., P.O. Box 1803, 711 10 Heraklion Crete, Greece. Tel: 081-245858.

van Deursen, S. Sluis & Groot Research, Blaker 7, 2678 LW de Lier, The Netherlands.

van Kooten, Hank Bruinsma Seeds B.V., P.O. Box 93, 2675 ZH Honselersdijk, The Netherlands. Tel: + 31-174-61 50 15, Fax: + 31-174-61 50 20.

van Leeuwen, Loes Sementi Nunhems, Via Ghiarone, 2, 40019 S. Agata Bolognese, Italy.

Vardi, Eyal Hazera Ltd., Mivhor Farm Doar, Sede Gat 79570, Israel.

Vecchio, Franco Pioneer Hi-Bred Italia SpA, via Provinciale 42/44, 43018 Sissa (PR), Italy.

Walters, Terrence The Cucurbit Network, 11901 Old Cutler Road, Miami, FL 33156-4242. Tel: (305) 667-3800, Fax: (305) 661-5984. Communication via *The Cucurbit Network*; the whole family Cucurbitaceae.

Wang, Ming Department of Horticulture, Northwestern Agricultural University, Yangling, Shaanxi 712100, P.R. China.

Wann, E. Van South Central Agric. Res. Lab, USDA-ARS, P.O. Box 159, Lane, OK 74555. Tel: (405) 889-7395, Fax: (405) 889-5783. Stress tolerance in cucumber.

Warid, Warid A. Paseo de las Fuentes No. 18, Col. Valle Verde, 83200 Hermosillo, Sonora, Mexico. Breeding of cucurbits.

Wasilwa, Lusike Dept. Horticulture & Forestry, University of Arkansas, Fayetteville, AR 72701. E-mail: *lwasilwa@comp.uark.edu*.

Wehner, Todd C. Dept. Horticultural Science, Box 7609, North Carolina St. Univ., Raleigh, NC 27695-7609. Tel: (919) 515-5363, Fax: (919) 515-2505. E-mail: *todd_wehner@ncsu.edu*. Pickling/slicing cucumber, watermelon, luffa gourd; selection, disease resistance, yield, genetics & chilling.

Weng, Chris S&G Seeds, No. 2, Chang Hsing 1 St., Tai Tzu Tsuang, Jen Te, Tainan, Taiwan, Republic of China. Tel: (06) 272-6366~ 8, Fax: (06) 272-1386.

Wessel-Beaver, Linda Department of Agronomy & Soils, Coll. Agriculture, Univ. Puerto Rico, Mayaguez, PR 00681. Tel: (809) 832- 4040, Fax: (809) 265-0220. E-mail: *l_beaver@rumac.upr.clu.edu*. Pumpkin & squash breeding; disease resistance; insect resistance.

Wiebe, Wayne PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax: (916) 668-0219. Cucurbit diseases and disease resistance.

Williams, Tom V. Rogers NK Seed Co., 10290 Greenway Road, Naples, FL 33961. Tel: (813) 775-4090, Fax (831) 774-6852. Watermelon breeding.

Wolff, David W. Texas A&M Experiment Station, 2415 East Hwy. 83, Weslaco, TX 78596-8399. Tel: (210) 968-5585, Fax: (210) 968-0641. E- mail: *d-wolff@tamu.edu*. Melon breeding and genetics, molecular markers, QTLs.

Wu, Mingzhu Hort Inst, Xinjiang Acad Agric Sci, Nanchang Road NO. 38, Urumqi, Xinjiang, People's Rep. China. Tel: 0991-4840311-2094.

Wyatt, Colen PetoSluis Co., Inc., 37437 State Highway 16, Woodland, CA 95695. Tel: (916) 666-0931, Fax: (916) 668-0219.

Yamanaka, Hisako Yamato-Noen Co., Ltd., 110, Byodobo-cho, Tenri-City, Nara, Japan 632. Tel: 07436-2-1182.

Yorty, Paul Rogers Seed Co., P.O. Box 104, Twin Falls, ID 83303-0104. Tel: (208) 733-0077. Cucurbit breeding.

Yukura, Yasuo 46-7, 3-Chome, Miyasaka, Setagaya-Ku, Tokyo, Japan.

Zhang, Jiannong Melon Research Institute, Gansu University of Agriculture, Lanzhou, Gansu, 730070, P.R. China.

Zhang, Xingping Hollar Seeds, P.O. Box 106, Rocky Ford, CO 81067. Tel: (719) 254-7411, Fax: (719) 254-3539. E-mail: *hollar@iguana.ruralnet.net*. Watermelon and melon genetics & breeding

Zhao, Yanru 2018 SW 143rd Street, Newberry, FL 32669. Breeding of resistance to WMV and ZYMV in watermelon (*Citrullus lanatus* L.).

Zitter, Thomas A. Cornell Univ., Dept. Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-5908. Tel: (607) 255-7857, Fax: (697) 255-4471. E-mail: *taz1@cornell.edu*. Fungal and viral diseases; disease resistance.

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Graines Gautier
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Humberto Gómez
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Peter Kraakman
Fernando Nuez
Gloria Palomares
Luis A. Roig
Semillas Fito, S.A.

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Ali Elamin El Jack
Yousif Fadlalla Mohamed
Sadig Khidir Omara

Sweden
Louis Carl Lehmann

Thailand
Usa Duangsong
Likhit Maneesinthu
Sompong Milerue

Turkey
Gulat Çaglar

United Arab Emirates
Ahmed A. Al Masoum

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

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.

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.

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.

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.

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.

Cucurbit Genetics Cooperative
Financial Statement
31 December 1995

Balance (31 December 1994)   **$3,725.33**

Receipts:

Dues & CGC back issue orders	$1,704.00	
Interest on savings	$96.14	
Total receipts		$1,800.14

Expenditures:

CGC Report No. 18 (1995)

Printing	$1,740.07	
Mailing	$561.69	
Call for papers (Report No. 19)	$90.00	
Miscellaneous (envelopes, postage, etc.)	$48.24	
Bank fees and adjustment charges	$126.09	
Total expenses		$2566.09

Balance (31 December 1995)   **$2,959.38**