

Cucurbit

Genetics

Cooperative

Report No. 11
July 1988

University of Maryland
Department of Horticulture

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The Cucurbit Genetics Cooperative was organized to develop and advance the genetics of economically important cucurbits. Membership to this Cooperative is voluntary and open to workers who have an interest in cucurbit genetics.

Reports of the Cooperative are issued on annual basis. The reports include articles submitted by members for the use of the members of the Cucurbit Genetics Cooperative. None of the information in the annual report may be used in publications without the consent of the respective authors for a period of five years. After five years, the information may be used in publications without the consent of the authors.

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REPORT OF ELEVENTH ANNUAL MEETING

The 11th Annual Business Meeting was held on November 11, 1987 in conjunction with the 84th Annual Meeting of the American Society for Horticultural Science and the 34th Annual Congress of the Inter-American Society for Tropical Horticulture, at Orlando, Florida. The meeting was called to order by J.D. McCreight, Chairman. Thirty-four members and guests were in attendance.

CGC Report No. 10 (1987) was mailed to members on June 15. This was about one month earlier than No. 9 was mailed in 1986. The cost of printing and mailing CGC 10 was \$1,247.32. Fifty copies each of CGC Reports 5 and 6 were printed at a cost of \$435.47.

Cash reserve at the time of the meeting was \$2,073.13. This will increase greatly in 1988 when approximately half the membership will renew their memberships.

Total membership was 202; 102 from the U.S. and 100 from all other countries. Thirty-four members and guests were in attendance.

Several members reported numerous requests by nonmembers for copies of their CGC Reports, and that university libraries were not aware of the publication or how to obtain it for their collections. They suggested that CGC solicit Land Grant university library memberships in order to preserve their personal copies and generate additional revenue for CGC.

Gene curators reported poor response from members for seeds of genetic stocks.

On behalf of Dick Robinson, Henry Munger discussed botanical nomenclature, specifically unnecessary changes in nomenclature of horticultural by taxonomists. It was suggested that CGC make two proposals to the American Society for Horticultural Science: one, that ASHS adopt the nomenclature proposed by Smith And Welch for horticultural crops (1964. Proceedings of the American Society for Horticultural Science 84:535-548); and two, that ASHS be consulted by the Botanical Congress prior to any formal changes in nomenclature of horticultural crops.

NOTE FROM THE CHAIRMAN

Effective at the 1988 annual business meeting, Timothy J Ng will assume chairmanship of the Coordinating Committee of the CGC. It has been a pleasure to serve as Chairman during the last four years. CGC will continue to grow and through its Report serve a valuable role for cucurbit research.

James D. McCreight

All future CGC correspondence should be sent to the attention of:

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College Park, MD 20742-5611

COMMENTS FROM THE COORDINATING COMMITTEE

The Call for Papers for the 1989 Report will get out in August 1988. Papers should be submitted to the respective Coordinating Committee members by 31 December 1988. The Report will be published by July 1989.

We are eager to hear from the membership regarding the future direction of CGC.

Timothy J Ng was responsible for final editing, printing and mailing of CGC Report No. 11. We look forward to his tenure as Chairman and Editor of CGC.

Coordinating Committee:

G.W. Elmstrom (muskmelon)
W.R. Henderson (watermelon)
J.A. Juvick (Cucurbita spp.)
T.C. Wehner (cucumber)
J.D. McCreight, Chairman

CGC in Europe — CUCURBITACEAE 88

A meeting of CGC for European members was held at the EUCARPIA meeting on Genetics of Breeding of Cucurbitaceae, Centre de Recherches Agronomiques, Montfavet, France on 2 June 1988. Fifty-four members and non-members were in attendance. J.D. McCreight summarized the origins of CGC and its current status, and emphasized that the purpose of CGC was to foster technical communication of cucurbit genetics and breeding research through brief reports and germplasm exchange. Because approximately half the CGC membership is non-US, more active involvement of non-US members is sought. Several suggestions to facilitate this goal were made: (1) include information on cucurbit meetings held around the world, including abstracts, summaries, and person(s) to contact for details; (2) have an option, for an increased membership fee, of sending the CGC Report via air mail to non-US members; and (3) encourage more activity in germplasm exchange, especially genetic markers, linkage stocks maintenance, and the development of monosomics and trisomics.

1988 Watermelon Workshop

The eighth annual meeting of the Watermelon Workshop was held in conjunction with the Southern Association of Agricultural Scientists Annual Meeting at the Hyatt Regency New Orleans on 2 February 1988. The meeting was well attended with 35 to 40 participants.

The following presentations were made at the meeting:

1. Ray Martyn - An initial survey of geographic isolates (races) of the watermelon wilt *Fusarium* in the U.S.
2. Tim Conaty - an electron microscope study of the microsporogenesis and embryo sac formation of a male sterile watermelon.
3. Billy Rhodes - a putative tetrasomic watermelon line.

Gil Lovell and Joe Norton discussed a grant proposal for germplasm maintenance and evaluation. Dr. Norton proposes to increase 450 watermelon PIs over a period of 5 years, determine descriptor for each, and evaluate for resistance to gummy stem blight, anthracnose, and rootknot nematode. The Watermelon Research Group asked the Vine Crops Crop Advisory Committee to recommend the proposal to the U.S.D.A. for funding.

MEETINGS

The Twelfth Annual Meeting of the Cucurbit Genetics Cooperative will be held in conjunction with the annual meetings of the American Society for Horticultural Science at Michigan State University in East Lansing, Michigan, 8-11 August 1988.

Other meetings of interest to CGC members:

<u>Group</u>	<u>Date & Location</u>	<u>Contact Person</u>
National Muskmelon Research Group	29-30 August 1988 Ithaca, New York	Dr. Henry M. Munger 405 Bradfield Hall Cornell University
Squash Breeders	29-30 August 1988 Ithaca, New York	Ithaca, New York 14853 (607) 255-1661
Pickling Cucumber Improvement Committee	10-11 November 1988 Madison, Wisconsin	Dr. Jack Staub, ARS/USDA Department of Horticulture University of Wisconsin
Vine Crops Advisory Committee	9 November 1988 Madison, Wisconsin	Madison, Wisconsin 53706 Phone: (608) 262-0028
Watermelon Research Group	7 February 1989 Nashville, Tennessee	Dr. Gary Elmstrom Univ. Florida - IFAS Agricultural Research & Educ. Center, Leesburg, Box 388 Leesburg, FL 32749-0388 Phone: (904) 787-3423

Development of Callus and Somatic Embryos from Zygotic Embryos of Cucumber (*Cucumis sativus* L.)

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In the cross *Cucumis sativus* x *C. melo* seeded fruits often develop, but the embryos cease growth at the globular-shaped stage (2). Embryo rescue procedures, suitable for embryos from self pollinations, failed for these hybrid embryos (3). This may in part be caused by the difference in the base chromosome number, i.e. $x=7$ in *C. sativus* and $x=12$ in *C. melo*. To overcome this barrier, we are planning to induce callus formation from the hybrid embryos in order to induce chromosome elimination and rearrangements. This might result in cells with an adapted karyotype, from which plants might be regenerated. The present study was undertaken to establish procedures of callus formation and plant regeneration especially from young embryos. Embryos from selfed *C. sativus* were used as a model system.

For supply of the embryos, we used *C. sativus* var. *hardwickii* IVT Gene bank no. (Gbn) 1811A, which was grown in an insect-proof glasshouse with temperature set at 25°C day / 18°C night. Immature seeds were taken out of fruits 7 to 24 days after pollination, and embryos 0.07 to 4.8 mm long from globular-shaped to cotyledonary stages (Table 1) were isolated and incubated on nutrient medium. For each embryo stage at least four different fruits were used. We cultured the globular and heart-shaped embryos enveloped in the embryo sac. A modified Murashige-Skoog medium was used containing as macro salts (in mg/l) NH_4NO_3 330, KNO_3 950, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 350, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 165, KH_2PO_4 85, normal micro salts and organic components, 250 mg/l Edamin, 3% (w/v) sucrose, and 0.55% (w/v) Oxoid agarose. For growth regulators, 4 μM BA and 4 μM 2,4-D were added, since that combination was reported to induce callus formation and embryogenesis on cucumber leaf explants (1). The medium was filter-sterilized, except for the agarose which was autoclaved. The pH was adjusted to 5.8. Six to nine embryos were incubated per 60 mm petri plate. The cultures were kept in the dark at 27°C. Calli obtained from the embryos were transferred to fresh medium after 6 weeks and thereafter subcultured every 4 weeks. Somatic embryos were transferred to a plant development medium, Murashige-Skoog with 0.5 μM K and 0.7% (w/v) Difco Bacto agar.

Table 1 presents the results after three weeks of culture. More than 90% of the globular-shaped embryos did not respond at all (NR) during culture. With older embryos four types of development have been identified: secretion of an exudate (EX), regeneration of somatic embryos directly from the cotyledons (SE), formation of beige callus (BC), and normal growth of the embryos (NG). A drop of a kind of translucent exudate, 5 to 8 mm in diameter, was generally secreted by the heart-shaped embryos, if they were responsive. It contained numerous single cells, which divided and regularly produced cell aggregates and embryo-like structures, 1 to 4 per drop. Direct somatic embryo formation, 1 to 3 embryos per cotyledon, occurred in the heart-shaped and early cotyledonary stage, but the frequencies were rather low. More commonly, in these stages, a watery, beige callus was formed which mostly was accompanied by a spongy, white callus. The two types of calli markedly differed in regeneration capacity. The white callus failed to grow when subcultured, whereas the beige callus, after several subcultures, occasionally formed protuberances of a bright-yellow callus which was embryogenic. Normal development of the embryos *in vitro* was observed in

cotyledonary stage embryos. With prolonged culture, however, these embryos also started to form callus, but this callus was greyer and hardly formed an embryogenic, yellow callus upon subculture.

Results of plant development from the somatic embryos obtained, were rather disappointing so far. Frequency of shoot growth was only 3% and most plants grew abnormally. Since only a few plants were successfully transplanted in soil, more research is required to improve the frequency of recovery of whole plants from somatic embryos.

Notwithstanding the low frequency of plant development from the somatic embryos, the study showed the ability of cucumber zygotic embryos to form undifferentiated tissue and to regenerate plantlets from it. The procedure established might be useful for callus formation and regeneration from hybrid embryos of the cross *C. sativus* x *C. melo*. These embryos will be cultured in further experiments.

Table 1. Response of cucumber embryos from self-pollinated *C. sativus* var. *hardwickii* Gbn 1811A of various stages on Murashige-Skoog medium with 4 μ M BA and 4 μ M 2,4-D².

Stage ^Y	Embryos		Response in culture (%)				
	Size (mm)	No.	NR	EX	SE	BC	NG
Globular	0.07-0.15	76	91	8	0	1	0
Early heart-shaped	0.15-0.3	102	25	53	9	13	0
Late heart-shaped	0.3-0.6	63	2	21	8	63	6
Early cotyledonary	0.6-1.2	84	5	7	15	43	30
Mid cotyledonary	1.2-2.4	77	0	0	0	18	82
Late cotyledonary	2.4-4.8	42	0	0	0	7	93

²Data were taken after 3 weeks of culture. NR: no response, EX: production of a drop of exudate with embryogenic cells, SE: regeneration of somatic embryos directly from the cotyledons, BC: production of beige callus, NG: normal embryo growth.

^YGlobular and heart-shaped embryos were incubated along with the surrounding embryo sac

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2. Niemirowicz-Szczytt, K. and B. Kubicki. 1979. Cross fertilization between cultivated species of genera *Cucumis* L. and *Cucurbita* L. *Genetica Polonica* 20: 117-24.
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Embryogenesis from Cotyledon-Derived Callus of *Cucumis sativus* L.

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Early research on tissue culture of cucurbits dealt with the formation of somatic embryos in culture (2, 4). However, only recently have plants been regenerated through embryogenesis (1, 3, 5). Researchers have had the most success with cucumber cotyledon tissue. The objective of this study was to determine the best medium to use for embryo induction and subsequent plant regeneration from cotyledons.

Seeds from 2 cucumber cultivars (Straight Eight and Gy 14) were sterilized in 50% clorox for 30 minutes and rinsed 5 times in sterile distilled water before germinating at 30°C on water agar in a dark chamber. Five-day-old cotyledon explants were cut into six 2 x 2 mm pieces after the cotyledon margins were removed. Five explants were placed on 100 x 15 mm petri plates of Murashige-Skoog (MS) medium containing 0.8% tissue culture agar, 3% sucrose, and one of 25 combinations of 2,4-D and kinetin concentrations in a 5 x 5 factorial design. Growth regulator concentrations were 0.0, 0.25, 0.50, 1.0, and 2.0 mg/l. The plates were kept in the dark at 22°C for 6 weeks. Each piece of 6-week-old callus was then cut in half and transferred to MS medium containing either 0 or 0.5 mg/l of kinetin. Cultures were maintained in the light for 3 weeks and then transferred to MS medium with no growth regulators and 1% agar at 3 week intervals until plants were fully developed.

In this experiment, globular and heart-shaped structures began forming around the edges of the cotyledon tissue after approximately 3 weeks. Embryogenic callus was smooth, yellow to yellow-orange in color, and was easily removed from the surface of the explant. Callus that was hard and nodular, or white and friable did not form embryos. When the embryogenic tissue was removed from 2,4-D and placed in the light, the embryos differentiated into normal bipolar embryos as well as abnormal embryonic structures. Some of the abnormal structures included multiple or fused embryos, embryos without underdeveloped or callused roots, and embryos with callused or malformed leaves.

Embryos developed at varying frequencies on all media containing 2,4-D (Table 1). The highest number of embryos was obtained on a medium with 2.0 mg/l 2,4-D and 0.5 mg/l kinetin. A high frequency was also obtained on a medium containing 1 mg/l 2,4-D and 0.5 mg/l kinetin. Embryos regenerated on the latter medium produced a higher percentage of plants than the former medium. There were no significant differences for the number of embryos and plantlets produced on secondary media with or without kinetin.

The problem of abnormal embryo production and embryo dedifferentiation remains to be solved. A possible solution includes using charcoal in the secondary medium, which may absorb some of the excess 2,4-D that remains when the embryos are transferred from the primary medium. Another solution may be removing embryogenic tissue from the primary media sooner than 6 weeks.

Table 1. Embryo and plantlet regeneration from cotyledon explants of 2 cucumber cultivars cultured on combinations of 2,4-D and kinetin and subcultured onto a medium with or without kinetin².

2,4-D conc. mg/l	Kinetin conc. mg/l	Secondary medium with kinetin		Secondary medium without kinetin	
		No. embryos per plate	No. plants per embryo	No. embryos per plate	No. plants per embryo
0.25	0.00	0.6	0.1	0.0	-
	0.25	1.0	0.0	1.6	0.0
	0.50	0.8	0.0	0.3	1.0
	1.00	1.0	0.1	2.3	0.0
	2.00	2.1	0.0	1.1	0.0
0.50	0.00	0.4	0.0	2.4	0.1
	0.25	2.5	0.0	5.3	0.2
	0.50	0.2	0.0	2.6	0.0
	1.00	1.6	0.0	3.2	0.0
	2.00	3.3	-	0.7	-
1.00	0.00	0.0	-	0.0	-
	0.25	3.3	0.2	4.8	0.1
	0.50	5.1	0.2	10.9	0.3
	1.00	2.9	0.1	2.3	0.0
	2.00	3.7	0.1	1.6	0.0
2.00	0.00	5.4	0.0	1.9	0.0
	0.25	1.6	0.1	1.4	0.1
	0.50	10.8	0.2	15.3	0.1
	1.00	9.6	0.1	6.7	0.0
	2.00	3.1	0.1	3.8	0.2
LSD (5%)		8.5	0.3	10.2	0.2

²Data for treatments 0.0 mg/l 2,4-D not shown. No embryos were produced on those media. Data are means over 2 lines and 4 replications with 2 petri plates per replication.

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1. Cade, R. M., T. C. Wehner, and F.A. Blazich. 1987. Organogenesis and embryogenesis from cucumber (*Cucumis sativus* L.) cotyledon derived callus. HortScience 22(5):154.
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In Vitro Regeneration and Flowering of Cucumber Cultivars and Lines Cultured from Excised Seed

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Department of Horticulture, University of Illinois, Urbana, IL 61801.

The ability to obtain whole plants from a single seed may be important when there is a limited number of seeds. Recently, Cade, et al. (1) reported shoot regeneration from cotyledons of Cucumis sativus L. after subculturing and/or transferring to another type of medium and keeping the explants in the dark. Lange and Juvik (2) have also reported regeneration from matured seed cotyledons of several Cucurbita species. In this paper we report preliminary observations regarding shoot regeneration from excised cucumber (Cucumis sativus L.) seed tissues.

Seeds of 18 cucumber cultivars and breeding lines (Table 1) from four various seed companies were decoated and excised into pieces consisting of an embryonic axis and 2 cotyledons. The pieces from an individual seed were tied separately in a cheesecloth bag and surface-sterilized for 10 min in 10% (v/v) Clorox bleach (0.525% sodium hypochlorite) to which a pinch of Alconox® powder had been added as a surfactant. The pieces were later rinsed 5 times with sterilized water. The individual bags were untied and the seed pieces were aseptically transferred to 25 x 150 mm culture tubes (one seed piece per tube) containing 10 ml of modified Murashige and Skoog (MS) (4) high-salt medium supplemented with 6-benzylaminopurine (BAP) (2 mg/l) and alpha-naphthaleneacetic acid (NAA) (0.1 mg/l). The pH of the medium was adjusted to 5.7 prior to autoclaving (15 psi, 15 min) and 7% (w/v) Difco Bacto-agar was added for solidification. The experimental treatment consisted of 15 seeds divided into 2 cotyledons and an embryonic axis for each cultivar. The explants were cultured for 6 weeks under 16/8 hour light/dark photoperiod ($40 \text{ Em}^{-2}\text{s}^{-1}$) and approximately 25°C. The cultures were examined regularly.

Within 3 to 5 days after culturing, the embryonic axes germinated in vitro. When the radicle touched the medium, it developed into a thickened root-like callus covered structure. Subsequent roots were normal in size. Shoots developed 3 to 4 weeks later. The most shoots (100%) were obtained from 'Burpless Hybrid', the least from 'West Indian Gherkin' (Table 1). No flowers were observed.

The pattern of development of cotyledon explants was generally similar in all cultivars. Within 3 days of culturing, the cotyledons turned green and expanded rapidly. By the third week of culturing, cotyledons began to form callus near, but usually below, the cut surface. About 5 weeks after culturing, 7 cultivars developed into embryoid-like structures in the callus, some of which developed into plantlets (Table 1). Shoots were tiny and rosette-like in cultivars 'Spacemaster' and 'Marketmore 76'. Male flowers developed on shoots of 'Burpless Hybrid', VGP 5058, 'Spacemaster' and 'Marketmore 76'. Some of the shoots roots spontaneously. 'Burpless Hybrid' and VGP 5058 shoots were transplanted into pots and later transferred to the greenhouse where flowering and fruiting continued.

Cucumber regeneration from excised seeds appear to differ among cultivars and type of explant used. 'Burpless Hybrid' regenerated better than other

cultivars from both cotyledon and embryonic axis explants. 'West Indian Gherkin' (Cucumis anguria) was worst. The ability to regenerate in vitro may be governed by several factors, especially genotype. Our tissue culture medium did not support plant regeneration for all cucumber cultivars. Thus, it might be essential to develop a suitable type of medium for each cultivar. Lange and Juvik (2) made similar observations fro several Cucubita species.

The embryonic axis regenerated shoots faster and better than the cotyledons, but did not flower. Cotyledons are storage organs. Embryoid-like structures consistently formed proximal to the cut surface of isolated cotyledons. This suggests a gradient in growth-promoting factors within the cotyledons, and/or translocation of the factors towards the embryonic axis. The absence of flowering on embryonic axis-derived shoots suggests the stimulus for flower formation on preformed embryonic axes is different than that for adventitious shoots (3).

Rajasekeran et al. (5) reported both male and female flowers in vitro on cultured hypocotyl segments of cucumber cultivar 'Superpickle'. They used MS medium supplemented with benzyladenine (BA) (0.5 or 1.0 mM) and 2,4-d (1.5 or 5.0 mM) and 20 weeks of subculturing, transferring to a medium without growth regulators. In this study we observed no female flowers.

In order to verify the factors regulating flowering, a further study is required. The efficacy of the system as an alternative to conventional cucumber regeneration techniques should also be exploited.

The authors wish to express appreciation to T. Sakata and Company and David Groff of Asgrow Seed Company for some of the cultivar seed samples used n this experiment.

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Table 1. *In vitro* regeneration from embryonic axes and cotyledons of cucumber (*Cucumis sativus* L.) breeding lines and cultivars².

Cultivar or breeding line	Seed Source	No. of cultures with shoots		No. of cultures with flowers
		Embryonic axis	Cotyle- dons	
Burpee Hyb. II	Burpee	9	0	0
Burpee Pickler	Burpee	10	3	0
Burpless Hyb.	T. Sakata	15	13	8
Flurry (VGY 5922)	Asgrow	7	0	0
High Mark II (WTR 615)	Asgrow	9	0	0
Marketmore 76	Asgrow	10	2	1
MS 613 (VGN 211)	Asgrow	12	0	1
MS 617 (VGD 252)	Asgrow	13	0	0
Poinsett 76 (VGS 160)	Asgrow	12	0	0
Spacemaster	Asgrow	14	5	3
Sprint 40	Asgrow	9	0	0
Straight 8	Burpee	12	3	0
Sumter (VGH 807)	Asgrow	9	0	0
VGH 7073	Asgrow	8	0	0
VGP 240	Asgrow	8	0	0
VGP 5049	Asgrow	13	6	0
VGP 5058	Asgrow	13	6	2
West Indian Gherkin	Hollar	4	0	0

²Numbers are from a total of 30 cultures evaluated, except for embryonic axis which had 15 cultures.

A Revision on Controlled Pollination of Cucumber

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Lower and Edwards (1) in discussing pollination technique for cucumber state that "pistillate flowers are receptive in the morning or up to mid-day on the day they open". This is exactly the statement I made for many years, because in New York State, we have had little success in making cucumber pollinations after noon on the day the pistillate flowers opened. However, my thinking on this point has changed as a result of experience in the tropics and, subsequently, in the greenhouse at Ithaca.

When pollinating cucumbers in the Philippines, I assumed it would be even more important to do the work early in the morning, in view of the higher temperatures. Then, in finding flowers for pollination the next day, it became apparent that there was considerable bee activity in the afternoon, which suggested that pollination might be accomplished later in the day. Following this lead, self-pollinations were made in the late afternoon and many of them proved successful. However, data on the success rate were not taken since there was seldom reason to do pollinations in the afternoon.

Applying this in the greenhouse at Ithaca, we have found afternoon pollinations to be as successful as those made in the morning. More importantly, we have gone one step farther to pollinate pistillate flowers on the day following anthesis and found that they are still receptive. This information has been useful when a closely spaced, pruned plant does not have a staminate flower open on the same day as a pistillate one. On one group of cucumbers in the fall of 1987, we pollinated about half the pistillate flowers on the day they opened and the other half a day after opening. There was essentially no difference in set or in amount of self-pollinated seed per fruit.

I can only guess at the reason for the difference between the field and the greenhouse at Ithaca, and the field in the Philippines versus Ithaca. Perhaps it is related to chilling injury from low night temperatures in the Ithaca field. It would be interesting to know whether researchers pollinating cucumbers in the Southern United States have had experiences similar to ours in the Philippines.

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Survey of Cucumber Breeding Methods in the U.S.A.

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In 1987, I surveyed public (state university and U. S. Dept. Agric.) and private (seed or processing companies) plant breeders assigned to the improvement of cucumber. The survey included questions on breeding objectives, selection methods, type of germplasm released, and traits of interest in the breeding program. See (1) for definitions of breeding methods and germplasm types. The traits of interest was an expanded version of a survey run in 1985. The information is summarized here in order that plant breeders can determine what resources need to be developed in the future for germplasm collections, and what research is needed to support efforts to improve cucumbers for the consumer.

Of the surveys sent out, 6 public and 11 private cucumber breeders responded. The public breeders were from New York, North Carolina, South Carolina, Texas, and Wisconsin. The private breeders represented Abbott & Cobb, Asgrow Seed, Campbell Inst. Res. Tech., Ferry-Morse, Harris-Moran, Musser Seed, Nickerson-Zwaan, Northrup King, PetoSeed, and SunSeeds.

As expected, most cucumber breeders were using the pedigree and backcross methods (Table 1). Inbred-backcross and single-seed descent were also used in breeding programs. Methods such as recurrent selection and pure-line family (bulk breeding) were rarely used, especially among private breeders. Breeding objectives for public researchers were to develop inbred lines (germplasm or cultivar) or hybrid cultivars, and for private breeders were to develop hybrid or inbred cultivars. There was some confusion about the definition of inbred vs. open-pollinated cultivars, since inbred cultivars are sib-mated in isolation to produce certified seed, often referred to as open pollination. Generally, open-pollinated cultivars are developed by mass selection from an open-pollinated population, and have an inbreeding coefficient (F) near 0 (vs. F near 1 for inbred cultivars).

Most public cucumber breeders in the U.S.A. were involved in the improvement of pickling types or on more basic research in genetics and population improvement (Table 2). Private breeders emphasized pickling and slicing types about equally. Secondary emphasis included work on Middle-Eastern slicers (so called Beit Alpha types), and a small emphasis on trellis (Japanese burpless) slicers.

Most programs emphasized disease resistance, fruit yield, and fruit quality as primary considerations in selection, with earliness and sex expression nearly as important (Table 2). The traits that cucumber breeders wanted evaluated most in the U. S. germplasm collection were resistance to gummy stem blight (caused by *Didymella bryoniae*), root-knot nematode (caused by *Meloidogyne* spp.), *Alternaria cucumerina*, and fruit rot (caused by *Rhizoctonia solani*). Heat tolerance, combining ability for yield, and seedcell size were the only traits in the top 10 that did not involve disease resistance (Table 3). Most of the traits of high interest are being evaluated currently.

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Table 1. Survey responses of 6 public and 11 private cucumber breeders working in the U.S.A. in 1987 for importance of breeding methods and germplasm types^z.

Question /Answer	Ranking of responses	
	Public	Private
Average ranking of breeding methods:		
Pedigree (cross, select, self)	1.2	1.4
Backcross (cross, select, backcross)	1.3	1.6
Inbred-backcross (cross, BC, self, sel.)	2.0	2.0
Single-seed descent (cross, self, self, sel.)	2.3	2.4
Recurrent selection (RS) - half-sib	2.7	3.3
- full sib	3.2	3.1
- S1 line	2.5	3.3
- S2 line	3.0	3.5
Mass selection (single-plant RS)	2.8	3.1
Pure-line family (cross, bulk, self, sel.)	3.3	3.3
Reciprocal recurrent selection	3.5	3.6
Objective for release of germplasm types:		
Inbred line (germplasm)	2.0	2.8
Population (germplasm)	2.7	3.6
Hybrid cultivar (2-4 inbreds crossed)	2.0	1.1
Inbred or line cultivar (from selfing)	2.5	2.6
Open-pollinated cultivar (from mass sel.)	3.5	3.3
Multiline cultivar (mixture of isolines)	3.5	3.7
Synthetic cultivar (>3 inbreds intercrossed)	3.8	3.6

^zImportance was rated 1 to 4 (1=frequent, 2=occasional, 3=rare, 4=never).

Table 2. Survey responses of 6 public and 11 private cucumber breeders working in the U.S.A. in 1987².

Question /Answer	Number of responses		
	Public	Private	Total
What are your primary research/breeding objectives?			
Breeding pickles	3	7	10
Breeding slicers	1	6	7
Breeding Middle-Eastern slicers	0	1	1
Population development	1	0	1
Genetics research	1	0	1
What are your secondary research/breeding objectives?			
Breeding pickles	3	2	5
Breeding slicers	1	2	3
Breeding other slicers	0	4	4
Population development	1	0	1
Genetics research	1	0	1
What are your minor research/breeding objectives?			
Breeding slicers	2	1	3
Breeding other types	1	4	5
Quality research	1	0	1
What traits are of primary concern?			
Disease resistance	5	9	14
Yield	3	11	14
Fruit quality	4	7	11
Earliness/Sex expression	3	5	8
What breeding method do you use most?			
Pedigree (cross, select, self)	5	7	12
Backcross (cross, select, backcross)	4	6	10
Inbred-backcross (cross, BC, self, sel.)	2	5	7
Single-seed descent (cross, self, self, sel.)	3	3	6
Recurrent selection (RS) - half-sib	1	0	1
- full sib	0	1	1
- S1 line	0	1	1
- S2 line	0	0	0
Mass selection (single-plant RS)	0	1	1
Pure-line family (cross, bulk, self, sel.)	0	1	1
Reciprocal recurrent selection	0	0	0

²Number of answers are greater than number of people answering where several first-choice responses were given.

Table 3. Traits in the plant introduction collection of cucumber that cucumber breeders would like evaluated (listed in order from a 1987 survey).

Rank in survey		Trait
1987	1985	
1	7	Gummy stem blight resistance*
2	5	Root knot nematode resistance*
3	15	Alternaria leaf blight resistance
4	3	Rhizoctonia fruit rot resistance*
5	12	Heat tolerance
6	2	Anthraxnose resistance*
7	26	Pythium cottony leak resistance
8	1	Fruits/plant (combining ability in gynococious hybrid)*
9	8	Downy mildew resistance*
10	6	Fruit seedcell size
11	14	Cold shock resistance
12	11	Pickleworm resistance*
13	17	CMV resistance
14	18	WMV1 resistance
15	19	WMV2 resistance
16	16	ZYMV resistance
17	23	Drought resistance
18	25	Earliness (no. oversized fruits in single-harvest trial)*
19	20	Angular leafspot resistance
20	22	Cold germination
21	21	Target leafspot resistance
22	13	Salt tolerance*
23	4	Fruit shape (appearance, not length/diameter ratio)
24	9	Powdery mildew resistance
25	10	Fruits/plant (actual)
26	28	Days to first flower
27	24	Branching habit
28	27	Cucumber beetle resistance
29	29	Daylength response
30	-	Unique character (character not previously documented)*
31	33	Scab resistance
32	-	Spider mite resistance
33	31	Fusarium wilt resistance
34	32	Bacterial wilt resistance
35	30	Verticillium wilt resistance
36	-	Nutritional value
37	-	Parthenocarpic tendency
38	-	Cold vigor
39	-	Brinestock quality
40	-	Sex expression
41	-	Cold vigor
-	-	Beet Pseudo-yellows virus (cucumber yellows)
-	-	Rhizoctonia seedling damping-off resistance
-	-	Air pollution resistance

*Trait currently being evaluated, or evaluation completed.

-Not on trait list for survey.

Cluster Analysis of Environments for the U.S.A. Southern Cooperative Slicing Cucumber Trials

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Plant breeders interested in developing slicing cucumbers for the southern U.S.A. often use the southern cooperative trials that cover many locations and seasons in that region. Trials use locations from Virginia (northeast corner of the region) to Texas (southwest corner). The cultivars and breeding lines (cultigens) evaluated over the years performed differently from one location to the next, but it appeared that some locations were similar. In order to determine which locations were most similar, a cluster analysis was run using data from trial summaries from 1979 to 1982.

Methods. Data was taken from the U.S.A. Southern Cooperative slicing cucumber trials for 1979 through 1982. The number of locations ranged from 8 to 11 each year, and the number of cultigens tested ranged from 8 to 12. Data from the trials was subjected to the averaging method of cluster analysis using PROC CLUSTER from the Statistical Analysis System (SAS Institute, Cary, N.C.). The character used from the trial was yield, measured as weight of marketable fruits (cwt/A) from multiple-harvest of small, replicated plots.

The locations and cultigens differed from year to year as different cooperators helped run trials and as new cultivars were entered into trials, and old cultivars were discontinued. Therefore, it was not possible to run a combined cluster analysis over all years.

Results. Clustering of locations using yield of different cultigens showed different patterns over the years (Table 1). In 1979, the Charleston, SC spring trial was most different from the other 10 environments. Clinton, NC spring and Leesburg, FL fall were most similar for yield of the cultigens tested. In 1980, the environments could be divided into 2 groups of 5 each. The groups corresponded roughly to eastern and western parts of the Southern Cooperative. The exception was that Charleston, SC and Sanford, FL appeared in both groups, with the fall season of each in the western group and the spring season of each in the eastern group.

The 1981 trials also could be divided into 2 groups. Once again, the spring and fall seasons at Leesburg, FL and Charleston, SC fell into different groups. The Port Sulphur, LA summer and fall trials were similar to each other and to the Leesburg, FL fall trial. In 1982, the environments could be divided into 3 groups with Cullman, AL and Clinton, NC in the first, Charleston, SC spring in the second, and the other 5 environments in the third.

Lack of funds for running trials have resulted in cooperators dropping out of the Southern Cooperative. Cluster analysis shows how the number can be cut back while still maintaining the representation for each group. For example, the 11 environments in 1979 could be cut back to 6 if necessary by using one environment from each cluster (Table 1, 1979, environments shown in bold face).

Table 1. Cluster analysis (average method) of locations in the U. S. A. Southern Cooperative slicing cucumber trials from 1979 through 1982 using marketable yield from multiple harvest of small plots².

<u>Year/</u>	<u>Location</u>	<u>Season</u>	<u>Cluster</u>
<u>1979</u>			
	Alma, AR	Spring	_____
	Beaumont, MS	Spring	_____
	Port Sulphur, LA	Fall	_____ _____
	Clinton, NC	Spring	_____
	Leesburg, FL	Fall	_____ _____
	Charleston, SC	Fall	_____
	Port Sulphur, LA	Summer	_____ _____
	Bixby, OK	Spring	_____ _____
	Painter, VA	Spring	_____ _____
	Sanford, FL	Fall	_____ _____ _____
	Charleston, SC	Spring	_____ _____
<u>1980</u>			
	Bixby, OK	Spring	_____
	Charleston, SC	Fall	_____
	Sanford, FL	Fall	_____ _____ _____
	Leesburg, FL	Fall	_____ _____
	Port Sulphur, LA	Summer	_____ _____
	Charleston, SC	Spring	_____ _____
	Clinton, NC	Spring	_____ _____ _____
	Painter, VA	Spring	_____ _____
	Sanford, FL	Spring	_____ _____
	Cullman, AL	Spring	_____ _____
<u>1981</u>			
	Beaumont, MS	Spring	_____
	Leesburg, FL	Spring	_____ _____
	Charleston, SC	Fall	_____ _____
	Bixby, OK	Spring	_____ _____ _____
	Clinton, NC	Spring	_____ _____ _____
	Painter, VA	Spring	_____ _____ _____
	Charleston, SC	Spring	_____ _____ _____
	Cullman, AL	Spring	_____ _____ _____
	Leesburg, FL	Fall	_____ _____ _____
	Port Sulphur, LA	Fall	_____ _____
	Port Sulphur, LA	Summer	_____ _____
<u>1982</u>			
	Bixby, OK	Spring	_____
	Charleston, SC	Fall	_____ _____
	Painter, VA	Spring	_____ _____ _____
	Leesburg, FL	Fall	_____ _____ _____
	Port Sulphur, LA	Summer	_____ _____ _____
	Charleston, SC	Spring	_____ _____ _____
	Clinton, NC	Spring	_____ _____ _____
	Cullman, AL	Spring	_____ _____ _____

²Number of cultigens tested ranged from 8 to 12 per year.

Number of Seeds per Mature Fruit for Different Types of Cucumber

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In a plant breeding program, it is useful to know the number of seeds per fruit to expect when plants are harvested at the mature fruit stage. As a rule of thumb, we generally count on 100 seeds per fruit for the American pickling and slicing types. Seed yield per fruit increases as the plants are given more room to grow in the field, or as larger pots are used in the greenhouse (1).

In order to determine the seed yield from different plant and fruit types, we measured 8 fruits from each of 15 populations developed in the North Carolina breeding program. The populations included pickling, slicing, Middle-Eastern (Beit Alpha), and Japanese trellis (burpless) fruit types. Plant types included tall-indeterminate, multi-branched hardwickii (*Cucumis sativus* var. *hardwickii*), and dwarf-determinate.

The number of seeds per fruit varied from 24 to 423 in the populations evaluated. The elite pickle population from North Carolina had few seeds per fruit, reflecting the emphasis on slow seed development and small seedcell size. The elite pickle population from Wisconsin had the most seeds per fruit, averaging 330. In most cases, slicing cucumbers had more seeds per fruit than pickling cucumbers with similar backgrounds. For example, the wide base slicer population had 191 seeds per fruit vs. 161 for the wide base pickle population.

It appears that one could expect 150 seeds per fruit from field-grown plants, except in those populations selected for small seedcell, where 100 seeds per fruit would be reasonable. However, as many as 15% of the fruits might be deficient in seed number.

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Table 1. Number of seeds per fruit from different cucumber types².

Popula- tion	Cucumber type	No. seeds per mature fruit		
		High	Low	Mean
Pickling cucumbers				
NCWBP	Wide base	273	24	161
NCMBP	Medium base	236	73	151
NCEP1	Elite 1	220	39	104
WIEP1	Elite Wisconsin-U.S.D.A.	423	258	330
NCEDP	Elite determinate	388	119	217
Slicing cucumbers				
NCWBS	Wide base	234	130	191
NCMBS	Medium base	223	118	183
NCES1	Elite 1	262	235	248
NCEDS	Elite determinate	185	117	147
Other type cucumbers				
NCH1	Hardwickii-pickling type	354	260	279
NCR1	Relish (large fruits)	309	77	177
NCBR1	Belly rot resistant	348	295	310
NCBA1	Beit Alpha	222	116	185
NCJT1	Japanese trellis	235	102	147

²Data from 8 fruits per population.

Development of Tropical Gynoecious Lines in Cucumber

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Gynoecious sex expression has been responsible for phenomenal development and quicker exploitation of hybrid vigor in cucumber which has attained a high degree of perfection in U. S. A., Canada, Japan, and Europe. Maintenance of the gynoecious lines has been possible through exogenously applied GA₃ (Peterson and Anhder, 1960) and silver nitrate (Beyer, 1976; Kalloo and Franken, 1978; More and Munger, 1986). Munger (1979) reported that the gynoecious lines Gy 14, SR551F, Gy 3, Gy 57 and Tablegreen 68 are the most suitable to produce F₁ hybrids in slicing and pickling cucumbers in temperate regions. Unfortunately, those lines have been found to be unstable for gynoecy under high temperature and long photoperiodic conditions prevailing in tropical production areas. Hence, there is a need for development of gynoecious lines suited to tropical production conditions.

Crosses were made between temperate gynoecious lines (Gy 14, SR551F, Gy 3, Tablegreen 68 x Gy 3 F₂, Wisconsin 2757) and tropical monoecious lines (Poona Khira, RKS296, RKS300). Selection was applied in the segregating generations for recombinants having true-breeding gynoecious sex, good horticultural characters, and vigor germination and emergence under tropical conditions. Gynoecious segregates were maintained by application of silver-nitrate (250 ppm, twice). Several tropical gynoecious lines have thus been isolated and are now in F₄ or F₅ generation. Four of these are described here.

Four lines: 87-304-6, 87-316, 87-319-12 and 87-338-15 (Table 1) were found to be true-breeding gynoecious lines during both the summer and rainy seasons of 1987. The first line produced cylindrical, light-green fruits of medium size having sparse black spines, while the latter three produced cylindrical, short to medium fruits of pale yellow color having brownish sparse spines. The node number of first pistillate flower of all lines ranged from 3.00 to 6.75. They did not produce a single staminate flower in the absence of AgNO₃ spray. When they were sprayed with AgNO₃ (300 ppm, twice) the average node number of first staminate and pistillate flowers ranged from 1.09 to 2.89 and 4.29 to 9.29 respectively. These observations indicate that the lines have strong gynoecious sex expression, and they could be easily maintained by AgNO₃, under tropical field conditions.

Before these are released for use in hybrid production, performance *per se* and combining ability will be evaluated.

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Table 1. Node number of first flower in four tropical gynoecious lines with or without two silver nitrate applications^z.

Gynoecious line and pedigree	Rainy season	Summer season, 1987 300 ppm AgNO ₃ twice	
	First pistillate node ^y	First staminate node	First pistillate node
87-304-6 WI 2757 x RKS 300 F ₅	3.00	1.09	4.64
87-316 (Tablegreen 68 x Gy 3 F ₂) x Poona Khira) x Poona Khira BC ₂ S ₃	5.15	2.25	6.14
87-319-12 (Tablegreen 68 x Gy 3 F ₂) x Poona Khira) F ₄	6.75	2.89	8.67
87-338-15 SR551F x Poona Khira F ₅	5.25	2.64	9.29

^zSeeds of Tablegreen 68 x Gy 3 and SR551F were obtained from Dr. H. M. Munger, Cornell University, U. S. A. Seeds of WI 2757 were obtained from Dr. C. E. Peterson, University of Wisconsin, U. S. A.

^yNo staminate flower was produced in absence of AgNO₃ spray.

Association of fasciation with opposite leaf arrangement

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Fasciated cucumber plants develop a very broad main stem, with increased numbers of leaves, tendrils, and flowers per node. Yorty (2) suggested that two recessive genes were required to produce fasciation. An alternative mode of inheritance is a single recessive gene with incomplete penetrance and variable expression. In agreement with this hypothesis, hybrids of normal and fasciated cucumber plants are always nonfasciated, and F_2 generations have varying proportions of fasciated plants in different populations, with the degree of fasciation varying in different plants from barely perceptible to grotesque stems six inches wide. Yorty (2) also obtained evidence of incomplete penetrance for fasciation.

Environment influences the proportion of fasciated plants. Yorty (2) reported that few plants become fasciated with long days and/or high temperatures. In agreement with this, a higher proportion of fasciated plants occur in the winter greenhouse at Geneva, NY than in the field during the summer. Seed treatment with radiation increased the number of fasciated plants.

Another possible case of a single recessive gene with variable penetrance in cucumber is *opp*, for opposite leaf arrangement (1). In the parental and segregating generations, opposite leaf arrangement was always associated with fasciation. All fasciated plants had opposite leaves at some of the nodes of the main stem, before the stem became fasciated and the number of tendrils and flowers increased. Penetrance was higher for opposite leaves than for fasciated stem, since not all *opp* plants become fasciated in the field.

Fasciated plants appear normal as seedlings, but as they develop, fasciation becomes more extreme. When fasciation first becomes apparent, a plant often has two instead of the normal one tendril and leaf at a node, and twice the usual number of staminate flowers. Later in development, there will be a higher multiple of the normal number of leaves, tendrils, and flowers of each sex per node as the plant becomes progressively more fasciated.

Opposite leaf arrangement is also unstable during the course of development. Opposite-leafed plants usually have two leaves per node in the seedling stage, but later always revert to a single leaf per node in alternate arrangement unless leaf number is increased by fasciation.

The association of fasciation and leaf arrangement could be due to linkage, but it is suggested that it is more likely due to pleiotropy. It may be that the same hormonal change during ontogeny is responsible for the change from opposite to normal leaf arrangement and from normal to fasciated stem.

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Preinoculation peroxidase activity in cucumber leaves not associated with race 2 anthracnose resistance

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Anthracnose, caused by the fungus *Colletotrichum lagenarium* (Pass.) Ell. and Halst., is one of the major foliar diseases of cucumbers. An inexpensive screening aid for anthracnose resistance would be useful to plant breeders, since it would permit testing in the seedling stage instead of running field tests. Biochemical assays are potential screening aids.

Preinoculation levels of peroxidase activity in cucumber leaves were investigated for their possible association with race 2 anthracnose (2) resistance. Peroxidase activity has been implicated in disease resistance in plants (1). Three experiments were conducted.

Methods. In experiment 1, the resistant line 'ARF79-95', the susceptible cultivar 'Model', the reciprocal F1 progeny, and the F2 population were grown in the field in a completely random design replicated in 4 locations. The youngest fully-expanded leaf was harvested at the 10-leaf stage. The plants were then inoculated with anthracnose race 2 (10,000 spores/ml) and rated for disease reaction for 5 weeks. The soluble, ionically-bound, and covalently-bound peroxidases were extracted from the leaf samples (5) and their activities determined with the guaiacol assay (4).

In experiment 2, soluble peroxidase activity of 7 different genotypes as well as individuals from reciprocal F2 populations of a cross between 'AR 79-95' and 'Model' were determined using the o-tolidene assay (3). Leaf samples were taken from plants in the 2-leaf stage.

In experiment 3, the isozymes of the soluble peroxidases extracted from the leaves in experiment 2 were separated using flatbed isoelectric focusing. A constant level of peroxidase activity was applied in each lane.

Results. All 3 peroxidase fractions gave similar results for least squares means (Table 1) and the analysis of variance (Table 2) for soluble peroxidase activity of parents and progeny. Preinoculation levels of peroxidase activity varied with location and with location by genotype interaction.

Variations in genotype were primarily maternal. In the F2, there was no consistent relationship between peroxidase activity and disease rating from location to location. Likewise, the use of the substrate o-tolidene in experiments 2 and 3 did not reveal any relationship between soluble peroxidase activity or activity of individual isozymes and disease ratings of parents or progeny.

In conclusion, preinoculation levels of peroxidase activity, as assayed with 2 nonspecific substrates, showed no relationship to race 2 anthracnose resistance and were not useful as a screening aid.

Table 1. Least squares means of locations and genotypes for soluble peroxidase activity (AU/g fresh weight leaf)^z.

<u>Location number</u>	<u>Least squares mean</u>	<u>Genotype</u>	<u>Least squares mean</u>
1	59.8 a	Model	58.1 a
2	58.2 a	Model x AR79-95	56.1 a
3	38.4 b	AR79-95 x Model	49.6 b
4	57.3 a	AR79-95	49.9 b

^zMeans within each column separated by LSD at 5%.

Table 2. Analysis of variance of soluble peroxidase activity (AU/g fresh weight leaf) for the parents and reciprocal F1 progeny of the cross 'Model' x 'AR79-95'.

<u>Source of variation</u>	<u>df</u>	<u>Sums of squares</u>	<u>F ratio</u>
Location (L)	3	2330	21.8**
Genotype (G)	3	417	3.9*
L x G	9	3700	11.5**
Error	15	534	
Total	30	6982	
CV (%)	11		

*,**Indicates significance at the 5 and 1% levels, respectively.

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Improving the Level of Powdery Mildew Resistance in Cucumber

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In attempting to raise the level of powdery mildew resistance (PMR) in 'Poinsett' cucumber, we have made use of several parents with some unexpected results. Initially crosses were made with 'Spartan Salad' 77-717 whose F1's showed somewhat less mildew than their susceptible parents (3). We have subsequently found it possible but less easy than suggested in an earlier report to maintain resistance through successive backcrosses without selfing when the recurrent parent is susceptible (1). However, it is relatively easy to increase the level of resistance by such a backcross program if the recurrent parent has a low level of PMR, probably having the one recessive gene which seems to be present in all resistant varieties. This note is to report that two additional and unrelated sources of high resistance can be used in the same way.

'Poinsett' and improved lines derived from repeated backcrosses to it develop considerable powdery mildew in the greenhouse at Ithaca. Consequently, we crossed 'Poinsett 83' (resistant to CMW) with 'Spartan Salad' 77-717, and with the line we carry as 'Yomaki' (essentially PI 288238). The latter is listed as coming from Egypt but is originally from Japan with some selection for PMR by Dr. Warid A. Warid during a brief stay in Egypt. The intention was to compare continuous backcrossing from 'Spartan Salad' crosses with alternate backcrossing and selfing from the 'Yomaki' crosses. After about 3 backcrosses we tried selecting in the backcross F1's of both series and found resistance maintained equally well.

We have had a similar experience where 'Poinsett 83' was crossed originally with 'Wisconsin 2757' and selection made for target leafspot (*Corynespora asiicola*) resistance (2). After 2 backcrosses, we began selecting for PMR also and found that an intermediate level could be maintained in the F1's of backcrosses without selfing. Higher resistance, equivalent to the 'Wisconsin 2757' parent, reappeared in the F2 after the last backcross, and such segregates (when self-pollinated) have nearly always produced F3's with uniformly high resistance.

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Interaction of Cucurbitacin Genes

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The *Bt* gene of *Cucumis sativus* L. has been reported to produce bitter fruits (1). We found that the bitterness is due to a quantitative, but not a qualitative change in cucurbitacin content. Fruits of *Bt* plants are high in cucurbitacin C (Cuc C), the same cucurbitacin compound present in normal cucumbers (Table 1). Plants heterozygous for *Bt* had fruits with an intermediate content of Cuc C.

The F₂ progeny of 'Eversweet' x PI 173889 was analyzed for Cuc C content in cotyledons by thin layer chromatography (2) to study the interaction of *Bt* and *bi*. If *Bt* were epistatic to *bi*, a ratio of 15 with to 1 without Cuc C would be expected, while a 3:1 ratio would be expected if *bi* is epistatic to *Bt*. The observed segregation of 48 with Cuc C to 16 free of Cuc C conformed precisely to that expected on the basis of epistasis of *bi*. Bimodal segregation occurred among the F₂ plants having Cuc C, with peaks at 0.25 and 0.40 mg Cuc C/g. Dividing the F₂ population into three groups (0.31-1.00, 0.10-0.30, and 0 mg Cuc C/g) resulted in a ratio of 34:14:16, in close agreement (p = 0.7-0.8) with the 9:3:4 ratio expected.

Additional evidence on the epistasis of *bi* to *Bt* was obtained when fruits of 'Spartan Salad' (+/+ *bi/bi*) x PI 173889 (*Bt/Bt* +/+) was analyzed for Cuc C. The ratio of 31 bitter (>0.01 mg Cuc c/g): 11 normal (0.01 Cuc C): 23 nonbitter (no Cuc C) agreed (p = 0.1-0.2) with the 9:3:4 ratio expected on the basis of epistasis of *bi*, and differed (p = < .001) with the 12:3:1 ratio expected if *Bt* were epistatic.

Serious problems have been encountered with some commercial seed lots of summer squash due to the presence of a gene for bitter and highly toxic cucurbitacin compounds that is believed to have resulted from cross-pollination with an ornamental gourd (3). The bitterness of the fruits of some ornamental gourds (*C. pepo* var. *ovifera*) and the closely related *C. texana* is due to a single dominant gene. Since this gene is analogous to the *Bt* gene of cucumber, a nonbitter gene in squash that is similar to the *bi* gene of cucumber would have value; it might protect a squash cultivar, through epistasis, from producing bitter fruits after gene introgression from an outcross.

A single gene, *cu*, of *Cucurbita pepo* is known to govern cucurbitacin B content of cotyledons (4). We confirmed this, and found that *cu* determines cotyledon content of cucurbitacins D, E, and I as well as cucurbitacin B. Classification for *cu* can be made by tasting cotyledons. Cultivars such as 'Scallop' and 'Straightneck' that are recessive for *cu* have nonbitter cotyledons, whereas 'Zucchini' and other cultivars dominant for *cu* have bitter cotyledons. In this respect, the *cu* gene of *Cucurbita pepo* is similar to the *bi* gene of *Cucumis sativus*, since both produce a phenotype of nonbitter cotyledons. They differ, however, in gene action. Cucumber gene *bi* completely blocked cucurbitacin biosynthesis, while squash gene *cu* reduced but did not eliminate cucurbitacin formation. Another fundamental difference between *Cucurbita* gene *cu* and *Cucumis* gene *bi* is that *cu* is not epistatic to the dominant gene in the species for bitter fruits. The F₂ of *C. pepo* cv. Early Prolific Straightneck x *C. texana* segregated 3 bitter fruits: 1 nonbitter, not in the 9:7 ratio that would be expected if *cu* were epistatic.

Table 1. Cucurbitacin content of cucumber fruits of different genotypes.

<u>Line</u>	<u>Genotype</u>	<u>Phenotype</u>	<u>Cuc C content mg/g fr. wt.</u>
Spartan Salad	+/+ <i>bi/bi</i>	nonbitter	0.00
Wisconsin SMR 18	+/+ +/+	normal	0.01
PI 173889	<i>Bt/Bt</i> +/+	very bitter	0.58
(Spartan Salad X PI 173889) F ₁	<i>Bt/+ bi/+</i>	bitter	0.26

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Evaluation of Fruit Quality in *Cucumis sativus* var. *hardwickii* (R.) Alef.-
Derived Lines

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Breeding strategies designed to incorporate the sequential fruiting ability of *Cucumis sativus* var. *hardwickii* (R.) Alef. (hereafter referred to as *hardwickii*) into commercially acceptable cultivars (var. *sativus* L.; hereafter referred to as *sativus*) have been a major objective of the U.S.D.A./A.R.S. cucumber project (3). We have developed several high-yielding inbred lines derived from *hardwickii* which could be used in breeding programs to increase the yield of commercial cultivars (4).

We have used the *hardwickii* lines PI 183967 and PI 218889 in pedigree breeding to improve these derived lines for disease resistance. The lines were then random-mated and the resulting population subjected to 2 cycles of recurrent selection for fruit length and fruit number (4). Although the lines approached acceptable horticultural type in some respects, they had short fruits with unacceptably large seedcells.

University of Wisconsin (UW) researchers used 3 cycles of S1 progeny selection to increase fruit number per plant in a population derived from a cross between a gynocious inbred *sativus* line, 'Gy 14', and a *hardwickii* line derived from PI 183967 (LJ 90430). Inbred line development using 2 cycles of family selection was then conducted, selecting for number of fruits per plant using once-over harvest (1, 2).

Since *hardwickii* confers poor interior fruit quality in *hardwickii* x *sativus* matings and inbred lines derived from these matings also had unacceptable fruit quality, this study was designed to determine whether *hardwickii*-derived lines could be used in combination with *sativus* inbred lines to produce F1 hybrids with acceptable fruit quality. If fruits of F1 hybrids have acceptable quality, then perhaps they could be commercially useful for once-over mechanical harvest.

Nine genetically distinct cucumber populations, which had been maintained by self-pollination for several generations, were evaluated. Three indeterminate U.S.D.A. processing cucumber breeding lines were selected for crossing with 2 *sativus* lines and 4 lines with *hardwickii* in their pedigrees. The U.S.D.A. gynocious breeding lines, WI 1701, WI 2712, and WI 2963 were used as females. The inbred WI 2963 resulted from self-pollination of an F1 progeny from a cross between *hardwickii* PI 212289 and WI 1606, an inbred processing cucumber line. Two *sativus* inbred lines (WI 1983 and 13M) along with 4 lines derived from initial *hardwickii* x *sativus* matings [WI 5098 (USDA), WI 5551 (USDA), 2H1853 (UW), and 4H261 (UW)] were used as males.

To obtain information about F1 fruit quality, a North Carolina Design II mating scheme was used to produce 18 F1 families (3x6). The parents and F1 progenies plus 2 check cultivars, 'Calypso' and 'Fremont', were grown in the field at the UW Experimental Farm, Hancock, WI. The experimental design in each of 2 planting environments was a split-plot treatment arrangement in a randomized complete block design with 2 replications. Single-row plots of 30 plants on 1.5 m row centers were used in each replication. All plots were over-seeded and thinned to 30 plants/plot or about 29,000 plants/ha at a 23 cm plant spacing and about 58,000 plants/ha at a 11.5 cm plant spacing, thus providing plot lengths of 7 m and 3.5 m, respectively, for each planting density tested.

Data on 3 fruit quality traits were obtained using fruits from the third harvest that were fermented in unpurged brine tanks. Fruit firmness was measured through the pericarp of brined fruits at the stem and shoulder using a Magness-Taylor fruit pressure tester with an 8 mm tip. Measurements were taken on 10 fruits (27 to 38 mm diameter) randomly chosen from one replication at the third harvest. The ratio of seedcell diameter to fruit diameter (interior ratio) was calculated from the middle cross section of 15 brined fruits (27 to 51 mm diameter) also chosen from one replication at third harvest. Twelve experienced processors judged the overall quality of the brined fruit based on internal and external color, internal texture, and shape. Samples were rated using the following scale: 10-9=excellent, 8-7=good, 6-5=fair, 4-3=poor, 2=barely acceptable, 1=not acceptable.

The mean interior (seed cavity diameter/fruit diameter) ratio of nearly-homozygous parental lines and their F1 progenies are given in Table 1. Smaller seed cavities were recorded in *sativus* lines when compared to *hardwickii* lines or *hardwickii* x *sativus* (HxS) F1 progenies. Fruit firmness values of *sativus* parental lines were generally higher than those of *hardwickii* parental lines or HxS progenies (Table 2). Curiously, firmness of fruits in the progeny of 13M matings using *sativus* parents (SxS) was lower than parental lines themselves. Fruit firmness in some HxS progeny (WI 1701 X WI 5098, WI 1701 X WI 2H1853, WI 1983 X WI 2963) approached that of the *sativus* parent. Generally, overall quality ratings of *hardwickii*-derived lines and HxS F1 progeny were lower than the *sativus* parents (Table 3). Notable exceptions were WI 1983 X WI 2963, WI 13M X WI 2963, and WI 2712 X 4H261 which demonstrated quality approaching that of the *sativus* parents.

Data from this study indicated that the *hardwickii*-derived lines conferred poor fruit quality on their HxS F1 progeny. Nevertheless, there were F1 hybrids which approached the fruit quality of their *sativus* parents, suggesting that certain *hardwickii*-derived lines may be useful. Undoubtedly, fruit quality characters are quantitatively inherited. The inbred-backcross breeding method is ideal for incorporating quantitative characters from unadapted germplasm into elite lines. Backcrossing to the commercially adapted parent increases the probability of recovering the characters which are horticulturally essential, and provides an opportunity to evaluate the inbred-backcross lines under replication, which enhances the effectiveness of selection for quantitative traits with low heritability. *Hardwickii*-derived lines, such as the ones in this study, will be improved using this method.

Table 1. Mean interior ratio (seedcell diameter/fruit diameter) of nearly-homozygous cucumber lines and their F1 progenies.

Parents	13M	WI 1983	WI 5098*	WI 5551*	2H1853*	4H261*	Mean
WI 1701	0.62	0.54	0.59	0.59	0.61	0.57	0.52
WI 2712	0.60	0.58	0.59	0.58	0.54	0.63	0.55
WI 2963*	0.64	0.56	0.63	0.59	0.55	0.54	0.62
Mean	0.58	0.56	0.57	0.58	0.57	0.60	

LSD(5%) = 0.03

Calypso = 0.54

Fremont = 0.49

²Means from middle cross section of 15 brined fruits (27 to 51 mm diameter).

*Contains *Cucumis sativus* var. *hardwickii* germplasm.

Table 2. Mean interior (seed cavity diameter/fruit diameter) ratio of nearly-homozygous cucumber lines and their F₁ progenies.

Parents	13M	WI 1983	WI 5098*	WI 5551*	2H1853*	4H261*	Mean
WI 1701	23.0	25.8	22.5	19.3	23.5	19.1	25.4
WI 2712	21.8	21.8	22.0	21.1	19.3	19.0	22.7
WI 2963*	20.3	23.3	19.3	21.4	22.0	22.8	18.3
Mean	24.1	23.4	21.3	21.3	21.3	20.7	22.1

LSD(5%) = 1.9

Calypso = 23.4

Fremont = 23.7

²Means from middle cross section of 10 brined fruits (27 to 38 mm diameter).

*Contains *Cucumis sativus* var. *hardwickii* germplasm.

Table 3. Mean interior (seed cavity diameter/fruit diameter) ratio of nearly-homozygous cucumber lines and their F1 progenies.

Parents	13M	WI 1983	WI 5098*	WI 5551*	2H1853*	4H261*	Mean
WI 1701	6.8	7.8	6.3	5.8	5.4	5.5	7.5
WI 2712	6.2	7.3	4.9	6.0	6.9	6.2	4.8
WI 2963*	6.8	6.5	5.1	4.8	4.4	3.7	6.2
Mean	5.3	6.9	4.7	5.0	4.6	2.4	

LSD(5%) = 1.1

Calypso = 8.3

Fremont = 6.8

*Contains *Cucumis sativus* var. *hardwickii* germplasm.

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Lack of chilling resistance in *Cucumis sativus* var. *hardwickii* (R.) Alef.

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Improvement of cucumber (*Cucumis sativus* var. *sativus* L.; hereafter referred to as *sativus*) for low temperature germination and emergence ability has been the focus of several research programs in the U. S. A. (1, 3, 4). In contrast, screening and selection of cultivars for resistance to chilling injury after emergence has not been reported in the U. S. A. However, selection for growth of slicing cucumbers under conditions of cool temperatures (20°C day and 15°C night) and low light has been successful in Europe (2).

C. sativus var. *hardwickii* (R.) Alef. (hereafter referred to as *hardwickii*) is a progenitor or wild relative of *sativus* and was originally collected in the foothills of the Himalaya mountains. It is a potential source for increased yield in *sativus*. Because of its origin, *hardwickii* may be a good source for chilling resistance. Therefore, three experiments were designed in order to measure: 1) The effects of leaf handling on the measurement of photosynthesis rate and stomatal conductance and; 2) The ability of *hardwickii* and *sativus* to recover from exposure to chilling.

Experiment 1. *Sativus* (WI 1606; gynoeocious) and *hardwickii* (PI 215589; monoecious) plants were grown in 100-mm-diameter pots containing Promix in two controlled-environment chambers at 30°C under a 16 hour photoperiod (at 300 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) in a randomized complete block design with 3 replications. At the 6-leaf stage, plants in both chambers were subjected to 5°C for 24 hours (at 300 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) and returned to 30°C. The photosynthesis rate (PR) and stomatal conductance (SC) of the 2nd through 4th leaves (leaf 0 was the cotyledon) of plants in one chamber were measured using a portable photosynthesis system (LI-6000 Li-Cor Inc., Lincoln, Nebraska) 2, 3, and 6 days after exposure. Plants in the second chamber were measured on day 6 after exposure. Analyses of variances were performed on measurements taken on day 6 after exposure.

Experiment 2. *Sativus* and *hardwickii* plants were grown as described in experiment 1. At the 6 leaf stage, the PR and SC of plants in one chamber was measured as previously described and then plants were exposed to 5°C for 24 hours, returned to 30°C and measured again after 2 days. In a second chamber, plants were measured only once 2 days after chilling exposure. Analysis of variance was performed on measurements taken 2 days after exposure.

Experiment 3. *Sativus* and *hardwickii* plants were grown as previously described. At the 6-leaf stage, plants in one chamber were exposed to 5°C for 24 hours, returned to 30°C, and measured for PR and SC 6 days after chilling as described above. Plants in a second chamber were grown continuously at 30°C, and PR and SC measurements were taken immediately following those which had received chilling exposure. Plants were harvested and measured for leaf area, stem and leaf dry weight, and numbers of lateral branches and leaves. Analysis of variance was performed on PR and SC measurements taken 6 days after exposure and on morphometric characters.

Experiment 1 assessed the effect of handling and measurement of plants after exposure to 5°C. No significant difference in PR and SC could be detected among plants measured several times and those measured only once suggesting that handling does not affect subsequent measurements of these plants (Figure 1). Significant differences in PR were recorded between *sativus* and *hardwickii*, but not for SC. Regardless of treatment, the *sativus* inbred always had a higher PR when compared to *hardwickii*. However, only when plants were measured 3 times did the *sativus* inbred have higher SC rates.

Experiment 2 assessed the effect of handling and measurement before and after chilling. Although there was no significant difference in the PR and SC rates of plants due to handling and measurement, the PR and SC rates of the *sativus* inbred was always higher than *hardwickii* (Figure 1). A closer inspection of the reaction of individual leaves suggested that when plants were measured before chilling, the results were inconsistent. At some leaf positions this response was elicited, while in others it was not. These inconsistent responses among leaves were not observed when plants were measured only once.

Experiments 1 and 2 indicated that handling and measurement before or after chilling exposure did not significantly affect PR or SC and, therefore, the interpretation of effects for these response variables. Experiment 3 indicated that, although chilling significantly lowers PH and increases SC (Figure 2), *sativus* consistently had higher PR and SC rates when compared to *hardwickii* regardless of treatment. A reduction in leaf area (54%), stem dry weight (52%), leaf dry weight (61%), lateral branch number (76%), and leaf number (59%) was recorded in plants exposed to chilling temperatures (data not shown).

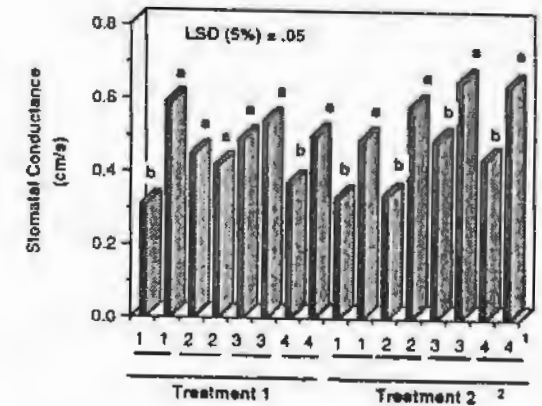
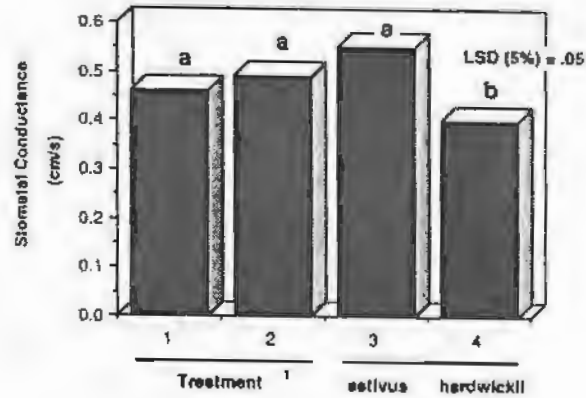
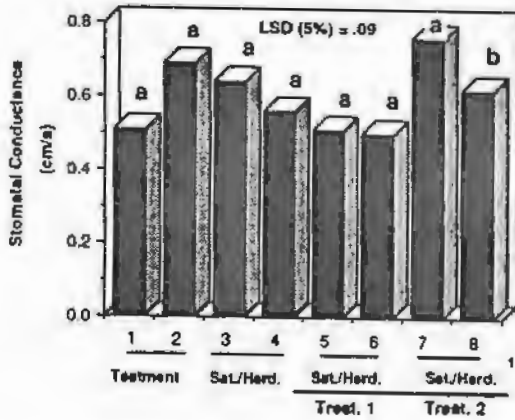
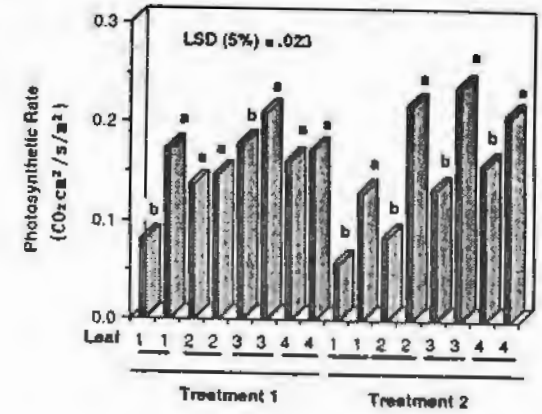
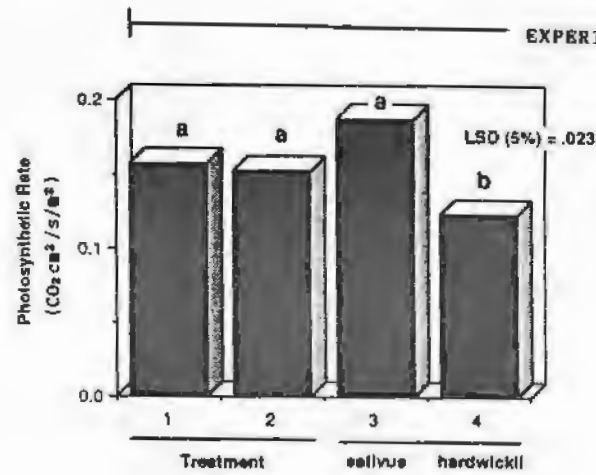
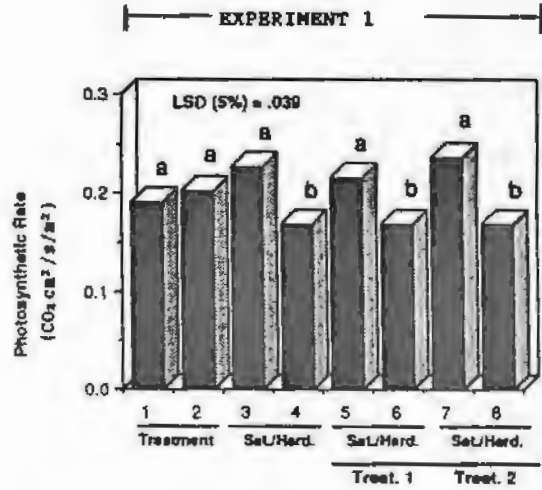
The fact that *hardwickii* stomates are apparently open to a lesser degree (lower SC rates) when compared to *sativus* may provide an explanation to the increased chilling injury of *hardwickii*. An hypothesis that explains the data is that *hardwickii* and *sativus* transpiration/photosynthesis ratios may be relatively equal, and stomatal response to low temperatures may be slower in *hardwickii* than in *sativus* making it more sensitive to chilling and affecting the recovery time.

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TABLE 1. Response of photosynthetic rate and stomatal conductance in *Cucumis sativus* var. *sativus* L. (WI 1606) and var. *hardwickii* (R.) Alef. (PI 215589) to 5°C in two experiments. Experiment 1 assesses the effect of handling and measurements of plants after chilling period. Experiment 2 compares plants grown under continuous 30°C to those measured before and after exposure to 5°C for 24 hrs and then returned to 30°C.

CGC 11:31 (1988)

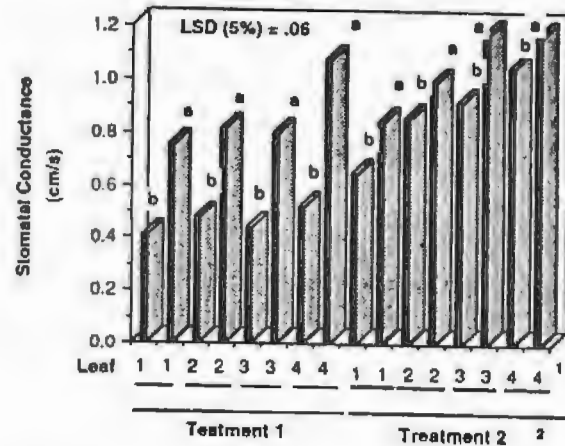
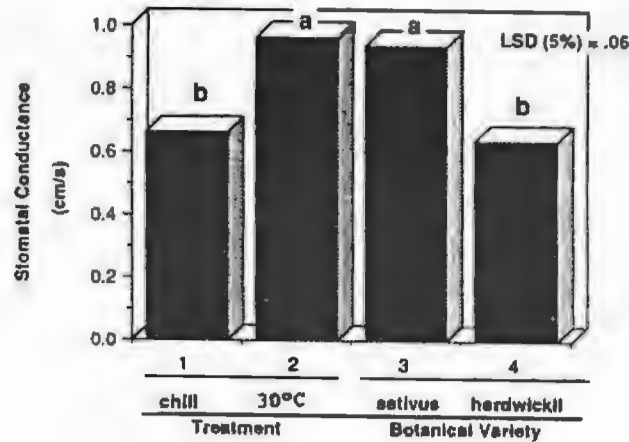
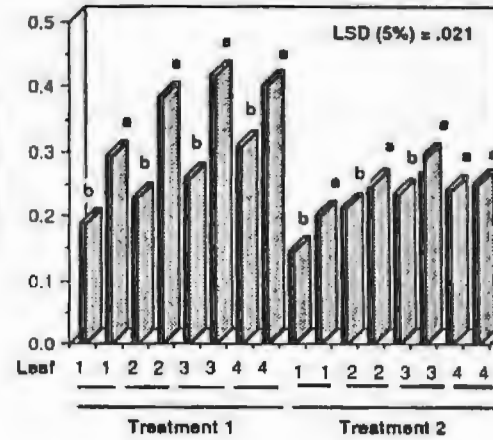
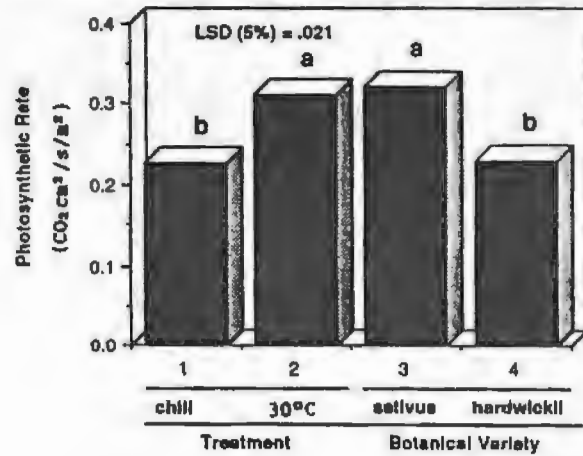


¹Treatment 1=Plants grown at 30°C then chilled (5°C) for 24 hrs, then return to 30 and measurements taken after 6 days. Treatment 2=Same as Treatment 1 except measurements taken 2.3-46 days after chilling (day 6 replicated).

¹Treatment 1=Plants grown at 30°C measurements taken, then chilled (5°C) for 24 hrs, then returned to 30°C & measured after 2 days. Treatment 2 Same as Treatment 1 except no measurements taken before chilling.

¹First bar in each series represents *hardwickii*, second *sativus* for each leaf. ²Treatment measurements taken at random on a particular plant.

Table 2. Response of photosynthetic rate and stomatal conductance in *Cucumis sativus* var. *sativus* L. (WI 1606) and var. *hardwickii* (PI 215589) to chilling (5°C) in Experiment 1.



¹ First bar in each series represents *hardwickii*, second *sativus* for each leaf.

² Treatment 2=Plants grown continuously at 30°C; Treatment 1=Plants grown at 30°C, then chilled (5°C) for 24 hrs, then returned to 30°C and measured after 6 days.

Plant Regeneration from Callus of Cucumis melo L.

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Tissue culture is a promising tool for the recovery of important traits such as disease resistance. Selection in vitro would allow for rapid screening of large populations of cells if suitable selection agents are available and if cell culture techniques exist for the regeneration of plants from single cells or clumps of cells. Recently, techniques for the regeneration of Cucumis melo L. have been reported (1,2,3,4,5). We report here on the regeneration of plants from calli of three muskmelon cultivars ('Hales Best', 'Perlita', and 'Iroquois') using modifications of the technique originally reported by Moreno et al. (2).

Surface disinfection was accomplished by removing the seedcoat and immersing the embryo in a continuously stirred 10% Clorox solution with 0.1% Tween 20 for 20 minutes. After three rinses in sterile distilled water, the cotyledons were removed from the embryonic axes and plated directly on callus induction medium, which consisted of a basal medium of MS salts, 3% sucrose, $100 \text{ mg} \cdot \text{l}^{-1}$ myo-inositol, $1 \text{ mg} \cdot \text{l}^{-1}$ thiamine-HCL, and 0.8% agar supplemented with $6.0 \text{ mg} \cdot \text{l}^{-1}$ kinetin and $1.5 \text{ mg} \cdot \text{l}^{-1}$ indoleacetic acid (IAA). The pH was adjusted to 5.7 with NaOH and HCL prior to autoclaving for 20 minutes at 121°C , 124 kPa for 15 minutes. Cultures were grown either in the dark or under 16 hr of fluorescent lights ($\sim 50 \mu\text{Em}^{-2}\text{s}^{-1}$) at 25°C .

Cotyledon cultures placed in the light expanded and turned green in approximately five days and formed green nodular callus within two weeks. Visible shoots emerged in three weeks. At the end of four weeks, shoots were excised and placed on rooting medium. Rooting medium consisted of basal medium supplemented with $0.001 \text{ mg} \cdot \text{l}^{-1}$ 1-naphthaleneacetic acid (NAA). The green nodular callus was maintained by subculturing every four weeks on basal medium supplemented with $0.1 \text{ mg} \cdot \text{l}^{-1}$ benzyladenine, with emerging shoots removed at the end of each subculture period. Table 1 lists the number of plants established at the end of three subcultures for each genotype. Of the 32 plants recovered only one exhibited a mutant phenotype, with a blind apical meristem and no apparent lateral meristems. The other plants flowered normally.

Cotyledon cultures placed in the dark formed a creamy white, friable callus within two weeks. Only one shoot primordia formed among the 120 cotyledons in the dark. This shoot did not develop when subsequently placed in the light. The dark-grown callus was subcultured and transferred to the light at the end of four weeks. The calli developed small green zones throughout the callus clumps. Subculture of the green zones of calli resulted in uniformly green callus, but no shoot formation occurred during five months of subculturing. Transfer of this callus to basal medium supplemented with IAA (0, 0.15, 0.75, or $1.5 \text{ mg} \cdot \text{l}^{-1}$) and kinetin (6.0, 9.0, or $12.0 \text{ mg} \cdot \text{l}^{-1}$) in a factorial combination resulted in altered callus growth rates, with the greatest on the original medium of $6.0 \text{ mg} \cdot \text{l}^{-1}$ kinetin and $1.5 \text{ mg} \cdot \text{l}^{-1}$ IAA and the least on the

media lacking IAA. However, no change in callus morphology was evident on any of the media tested.

In an effort to increase the number of shoots formed, cotyledon and hypocotyl segments from axenically grown seedlings were placed on basal medium supplemented with kinetin (0.1, 0.5, 1.0, or 2.5 mg·l⁻¹) and NAA (0.1, 0.5, or 1.0 mg·l⁻¹) in a factorial combination. Of the 180 cotyledon segments, 150 formed roots with only one forming a single shoot (0.5 mg·l⁻¹ NAA and 0.5 mg·l⁻¹ kinetin). In contrast, hypocotyl segments formed roots in only 31 of the 180 cultures with no shoots formed in any culture.

Thus far these experiments demonstrate that 'Hales Best' has a greater morphogenetic potential than 'Iroquois' or 'Perlita', and that the number of subcultures may affect morphogenetic potential. Current experiments are focused on alternative growth regulator combinations and concentrations, both to improve the number of shoots formed in culture and to recover shoots from long-term subcultured callus.

Table 1. Number of regenerated plants by cultivar and subculture number.

Cultivar	Subculture no.			Total
	1	2	3	
Hales Best	8	4	11	23
Iroquois	5	1	1	7
Perlita	2	0	0	2

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Isolation of Cells and Protoplasts from Muskmelon Leaves

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Techniques exist for the isolation of cells and protoplasts from a wide range of plant species (3), but few reports describe methods for isolating mesophyll cells and protoplasts from cucurbits (2,4). Moreno et al. (4) produced cells and protoplasts of muskmelon by inducing callus, producing cell suspensions from the callus, and isolating protoplasts from the suspension cell cultures (4). In this study, we report conditions for obtaining protoplasts and cells directly from muskmelon leaves.

Mesophyll Cell Isolation: The muskmelon breeding lines MD 8518 and MD 2042 were grown under greenhouse conditions. Young leaves (approx 4 cm diam and 0.5 g FW) were detached and surface sterilized with 95% ethanol for 15 sec, washed three times in sterile distilled water and aseptically cut into 1.0 mm strips. The strips were transferred into petri dishes (35 x 10 mm) containing 5 ml of 2%, 5% or 10% (w/v) macerase (Calbiochem) and 0.7 M-mannitol in modified Murashige and Skoog (MS) mineral solution (5) at pH 5.7. The dishes were incubated overnight at 25-30°C with constant orbital agitation. The number of isolated cells per dish were counted with an inverted microscope and expressed as a percentage of total observable cells. There were four replicates of each genotype-enzyme combination.

Protoplast Isolation and Culture: Leaf strips were obtained as described above. In one experiment, strips were directly suspended in solutions containing 2% or 5% macerase, 5% cellulysin (Calbiochem) and 0.65 M-mannitol in MS mineral solution at pH 5.7. In another experiment, strips were suspended in solutions containing 2% or 5% macerase, 5% cellulysin, 0.5% (w/v) pectolyase (Sigma) and 0.65 M-mannitol in MS solution at pH 5.7. All treatments were incubated at 25-30°C with constant agitation for 24 hr. Isolated protoplasts was quantified using an inverted microscope, then filtered through sterilized stainless steel mesh (61 μ) and centrifuged three times before resuspension in 0.65 M-mannitol in MS solution. Four protoplast counts per replicate were made using a hemacytometer and protoplast viability was assessed using the procedures of Bornman and Bornman (1). Each genotype-enzyme treatment was replicated three times.

Results and Discussion: The 5% macerase gave a significantly higher percentage of isolated cells than either 2% or 10% macerase (Table 1) but the yield of isolated mesophyll cells was still low (about 8%). As few plant species give cell yields greater than 50% when leaves are treated directly with enzymes (3), high cell yields are probably better obtained through production of cell suspensions from callus cultures. For protoplast isolations, the solution containing 2% macerase, 5% cellulysin and 0.5% pectolyase gave the highest yield (Table 2). Protoplast viability was also highest with this enzyme combination, and viability declined with increasing macerase levels. Although protoplast yields obtained in this study are generally lower than those obtained from other plant species, they are comparable to or greater than those obtained by others working with cucurbits (2,4). These methods should be useful to those interested in isolating cells and protoplasts from muskmelon leaf tissue.

Table 1. Effect of macerases concentrations on production of Intact mesophyll cells from two muskmelon genotypes.

Genotype	Enzyme treatment	% Cells Isolated
MD 2024	2% macerase	2.3
	5% macerase	4.6
	10% macerase	2.7
MD 8518	2% macerase	3.4
	5% macerase	7.8
	10% macerase	3.5

Table 2. Effect of enzyme combinations on protoplast yield and viability from leaves of two muskmelon genotypes.

Genotype	Enzyme combination			Protoplast yield (10^6 /ml)	Protoplast viability (%)
	macerase	cellulysin	pectolyase		
MD 2024	2%	5%		0.26	53
	5%	5%		0.13	40
	2%	5%	0.5%	1.60	72
	5%	5%	0.5%	0.84	56
MD 8518	2%	5%		3.41	64
	5%	5%		1.09	47
	2%	5%	0.5%	14.62	92
	5%	5%	0.5%	7.42	81

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A Fasciated Mutant in Cucumis melo

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As in Cucumis sativus (1) fasciated plants sometimes occur in C. melo. We have observed such a mutation in the line 'Vilmorin 104' in the 'Cantaloup Charentais' type. The plant grows normally for several nodes, then the main stem gets flat and wider and wider (up to 10-15 cm); no more lateral branches appear. The leaves remain small. The male and female flowers are fertile. This character can be seen easily on the main stem but it can also appear on lateral branches when the main stem has been previously cut. Fasciation seems to be related with opposite leaf arrangement on the upper nodes. No influence of environmental factor has been detected.

The study of F_1 and F_2 progenies shows that the fasciation is controlled by a recessive gene (Table 1). We propose the name "fasciated" and the symbol "fas" for this mutant.

First attempts to localize this mutant have been conducted with a line monoecious (a+), male sterile (ms-4), with dark green color of the fruit skin (w+) and an unknown gene for powdery mildew resistance (Pm). Fasciated segregates independently from these four genes.

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Table 1. Observed phenotypes in F_1 and F_2 progenies between 'Vilmorin 104' (fasciated) and 'Bulgarie 7' (normal).

	Normal	Fasciated	chi square (3:1)	
			value	Probability
Vilmorin 104	0	20		
Bulgarie 7	20	0		
F_1 = Bulgarie 7 x Vilmorin	25	0		
F_2 = $F_1 \oplus$	136	37	1.204	27.2

Table 2. Segregation data observed in F₂ progenies between fasciated (fas) mutant and a, Pm, ms-4 and w.

Genes	normal <u>fas/+</u>	fasciated <u>fas/fas</u>	chi square (9:3 : 3:1)	
			value	probability
a+/- a/a	96 38	31 7	2.388	49.6
Pm/- Pm+/Pm+	100 35	29 9	0.945	81.5
ms-4+/- ms-4/ms-4	102 34	26 11	1.582	66.4
w+/- w/w	87 35	23 6	3.879	27.5

The X-ray Detection of Haploid Embryos Arisen in Muskmelon (Cucumis melo L.) Seeds, and Resulting from a Parthenogenetic Development Induced by Irradiated Pollen

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The utilization of haploids or doubled haploids is very important in the field of plant breeding. Several authors have reviewed the subject; among them, Jensen (3) gave a status on the induction and production of haploids or doubled haploids in crop plants, underlined results enabling relatively stable production of haploids and gave information on chromosome doubling techniques (1).

In muskmelon, spontaneous haploids have only very rarely been observed. Dumas de Vaulx (2) attempted to produce individual haploids by interspecific crossing. Crossing of Cucumis melo L. (2x) X Cucumis ficifolius (4x) following removal of the stigma enabled the pollen tube to reach the enabled embryo sac. In order to induce the development of the fruit, a second fertilization by Cucumis melo was performed. A few haploid embryos have been thus obtained, but the rate was always low and highly dependent on the genotype. More recently, the seed companies Clause and Limagrain decided, in collaboration with INRA in the frame of a "Group of Economic Interests" to finance a new project. The work was carried out by A. Sauton in 1985 and 1986 and the results published in 1987 (3).

The anther and ovule cultures have never enabled a complete embryogenesis. The results obtained by utilizing irradiated pollen seem to offer greater possibilities of success compared to previous attempts. The method of parthenogenesis induced in situ has been worked out by Raquin (4) on petunia. It consists of inducing the development of haploid embryos by making a deposit of pollen which has been denatured by gamma rays (Co 60). Despite treatment, the germinative abilities and the fertilizing power are preserved, whereas the chromosomic stock is destroyed. The deposit of irradiated pollen is made on the day of anthesis of the female flowers. Usually, the treated flowers grow normally and fruits can be picked three weeks after hand pollination. It is essential to transfer the rare haploid embryos that have been induced to an in vitro culture medium; otherwise they dry up and degenerate rapidly.

The classical method of embryo detection consists of opening each seed by hand. This operation is a very long and very delicate one, requiring many hours of work for a very small number of saved haploid embryos. So, we had the idea of utilizing an X-ray process, a technique which is already widespread for analyzing the quality of dry seeds (1), in order to detect the haploid embryos. We have found that the medical X-ray machine which gave positive results was the one used for mammography, as the other types of machines did not give a clear film.

Preparation of the seeds: X-rays are only efficient to discriminate four- to seven-week old embryos in the seeds. At a younger stage, embryos can not be

detected. On the other hand, beyond seven weeks, the embryos' germination dwindles considerably and there are great losses of material.

For X-ray detection of young embryos, an adequate desiccation of the seed is needed. If the seeds contain too much water, they appear opaque on the film and it is impossible to observe their internal part. On the other hand, they must not be too dry otherwise the embryo may be desiccated and destroyed.

The preparation requires a thorough washing in order to eliminate all the pulp and a drying in a net for about 15 hours at 4°C.

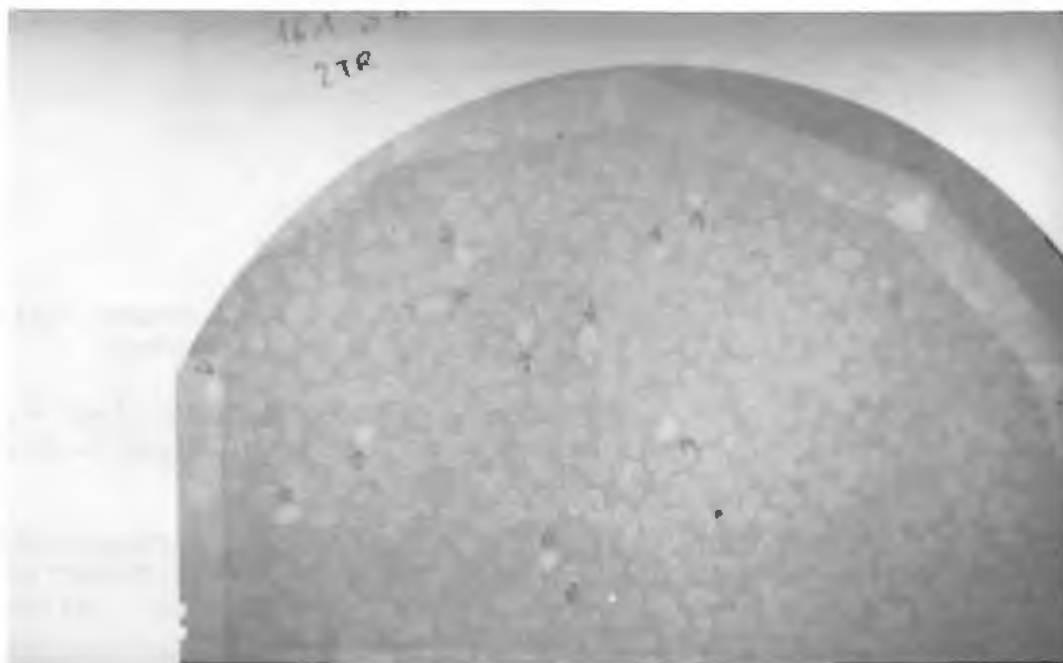
The layout of the seeds on their base: The seeds are laid on an extruded polystyrene plate (5mm thickness) covered by a sheet of transparent adhesive plastic. They are spread out flat and touching each other on the X-ray surface.



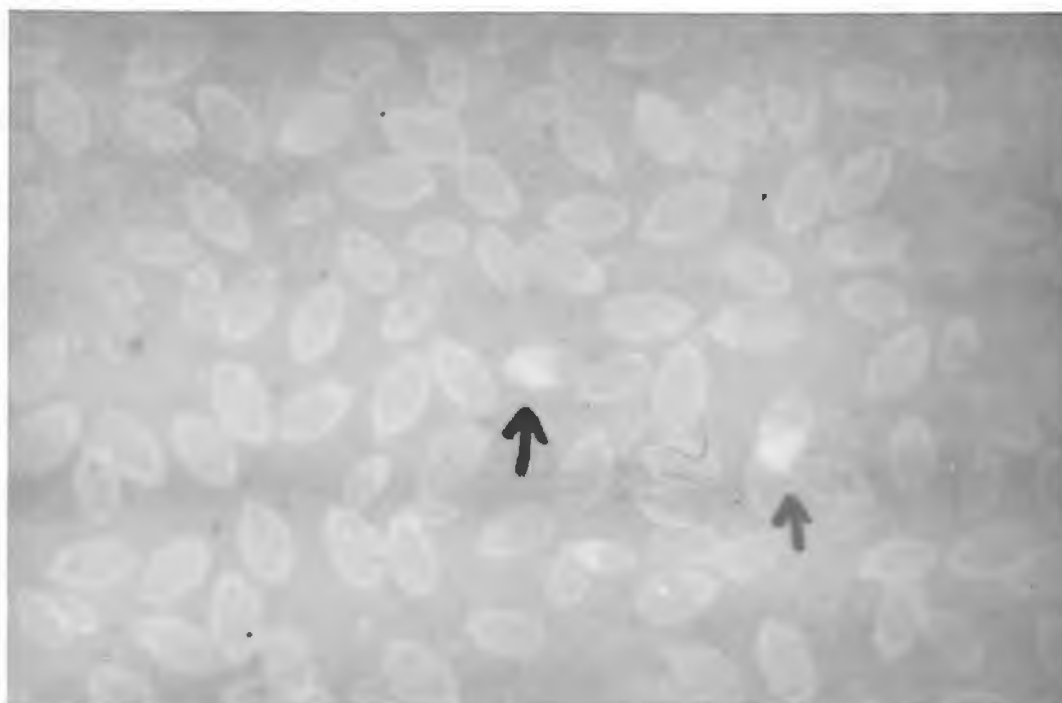
The X-ray operation: The characteristics of the X-ray are:

Exposure time: 1 second
Penetration: 18 kvolts
Flow: 35 to 70 mA according to the size of the seeds

The mammography machine must be focused so as to be just over the seeds. By proceeding in this way, we have been able to rapidly detect (6) the seeds which actually contain a haploid embryo.



The X-ray film enables a considerable saving of time. It is thus possible to treat a much greater number of fruit and therefore envisage a higher production of haploid embryos. On the other hand, the X-ray film enables the determination of the location, as well as the shape and the size of the embryo.



Consequently, opening of the seeds is a less delicate operation.

Apart from the extension to the Cucurbit family, this technique for detecting haploid embryos can also find an interesting application in identifying the rare diploid embryos derived from interspecific crossings which require an in vitro culture for further development.

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A simple procedure and the genetic potential for rooting of stem cuttings in muskmelon.

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Annual crops in which fruits are the important economic product offer particular challenges to the plant breeder for selection and generation advance. At the point that fruit evaluations have been completed and superior plants selected for advancement, plant condition and/or duration of growing season may not permit additional controlled pollinations and seed collection. Where disease reaction is one objective of the program, the introduction of disease into the field planting may disrupt normal expression and genetic distribution of horticultural traits. It would be an advantage to conduct multiple evaluations on the plants without jeopardizing either the plant or the environment. This report presents our results of runner tip propagation of muskmelon to provide plants for evaluation of horticultural traits as well as virus testing and selected seed production.

Initially, we were interested in propagation of zucchini yellow mosaic virus (ZYMV)-resistant selections of PI 414723 in the greenhouse for seed increase and crossing studies (3). Vigorous tips with 4 to 5 nodes were prepared for rooting by removal of leaves from the two subterminal nodes and immersing the cuttings into perlite in 5 x 5 x 10 cm plastic rose pots. These cuttings were then maintained under high relative humidity and intermittent mist until roots had developed, generally at the wound tip or buried nodes. Rooting occurred rapidly in this material. Rooted cuttings were successfully transplanted to soil and could be carried to maturity without difficulty. This procedure compares favorably with the method described in cucumber (4) and is considerably less complicated than the aerated nutrient solution technique described in muskmelon by Foster (1, 2).

We applied this simple perlite rooting technique to several generations derived from a cross of ZYMV-resistant PI 414723 by commercial cv. 'Topmark.' Runner tips were collected from 48-day-old field-grown plants. Flats containing perlite-filled rose pots were carried into the field, and cuttings were inserted directly into the dampened perlite. Cuttings were transported frequently to the greenhouse to prevent excessive wilting. The pots were placed onto a bench enclosed on all sides with plastic sheeting. Overhead mist emitters positioned approximately 1 m above the bench were set to mist 5 seconds each 15 minutes, initially, but were adjusted to each 30 minutes after 2 days. A second group of cuttings from only the parental lines (PI and Topmark) was collected after 67 days of growth.

Results of root development on cuttings from the various breeding populations (Table 1) indicate a high rate of success. The PI parent and generations with a high proportion of PI germplasm rooted more readily than did Topmark and its backcross generation. At 21 days, all populations except Topmark recorded a high percentage of rooting. It is interesting to note the percentage of viable growing tips available in rooted cuttings from the various populations. The growing points on certain cuttings failed to continue growth and eventually died. These cuttings, although they rooted satisfactorily, failed to establish a viable plant. Failure of growing points to survive may have been due to desiccation either during the period after harvest of the cutting until placement under the mist system or during the interval under the mist before roots formed to provide adequate moisture to the growing point. Again, populations derived from the PI parent provided the greater potential for viable plants from the rooted cuttings.

Rootings on cuttings from the two parent materials taken at 67 day growth was quantified by numerical ratings for the expression of various traits (Table 2). In each category, rooting of the PI parent was superior to the cv. Topmark. Examples of these differences are evident for rooting characteristics of cuttings of the two genotypes in Figure 1.

These data demonstrate the existence of genetic differences for rooting potential between the two parents and a strong pattern of inheritance from PI 414723 to the filial and backcross progenies.

In the perlite rooting medium, no soft rot or other microbial damage to cuttings was observed. Wound surfaces quickly formed callus with eventual initiation of roots from these callus cells. Therefore, we feel there is no necessity to treat cuttings after harvest with antimicrobial materials. This no doubt relates to the very porous nature of the perlite rooting medium. Similarly, we experienced no difficulty in transferring cuttings from perlite into greenhouse soil after rooting occurred. Cuttings transferred to 12.5 cm square plastic pots continued growth without disruption, and resultant plants could easily be maintained to flowering and fruit maturity in the greenhouse.

In a limited study, indolbutyric acid (IBA) either as an 100 ppm solution or as 0.5% dry powder in talc increased rooting frequency and root length on stem tip cuttings compared to controls. An additional advantage of IBA treatment was the formation of roots from cells in the internodal regions as well as from nodes and wound callus.

This propagation procedure has enabled us to reproduce the plants from the field into a duplicate set of greenhouse plants. The field plants were evaluated for horticultural genetic traits and for fruit harvests, while the greenhouse plants were inoculated with ZYMV to provide inheritance data and breeding progress for this virus. Virus-resistant or symptomless plants in the greenhouse, which derived from field plants exhibiting favorable horticultural traits, were used for selected pollinations to provide selfed and crossed seed for next generation evaluations.

Table 1. Rooting of muskmelon stem tips from 48-day-old field-grown plants.

Population	Cuttings rooted after ___ days ^z			Percent cuttings transplanted (21 days)	Percent cuttings with viable growing tips
	7	14	21		
Topmark	1	12	27	66	43
PI 414723	17	33	35	94	92
F ₁	11	31	35	97	96
F ₂	15	31	35	98	91
BC _{TM}	10	29	34	96	78
BC _{PI}	16	32	34	96	96

^z Data adjusted to 36 plant populations. Actual populations were: parents, 36; F₁, 72; F₂, 144; BC's, 72.

Table 2. Rooting of muskmelon stem tips from 67-day-old field-grown plants.

Population	Rooting position ^z	Root frequency ^y	Avg. root length (cm)	Avg. top growth ^x	Survival potential %
Topmark	T	3.3	6.5	2.5	72
PI 414723	T,N	3.7	8.5	3.4	92

^z T = wound tip; N = node

^y Root frequency - average value calculated from: 1 = single root; 2 = double root; 3 = few roots; 4 = multiple roots.

^x Top growth - average value calculated from ratings: 1 = none; 2 = slight; 3 = moderate; 4 = extensive growth.

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Figure 1: Topmark and PI 414723 cuttings rooted 21 days in perlite under intermittent mist.

Reaction of Muskmelon Genotypes to Races 1 and 2 of Sphaerotheca fuliginea in Israel.

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Races 1 and 2 of Sphaerotheca fuliginea (Schlecht. ex. Fr.) Poll. cause powdery mildew on cucurbits in Israel (5). Race 3 was not reported in the country. Race 1 predominates in the winter in greenhouse-grown cucumbers and muskmelons, whereas both races 1 and 2 are found in the summer in the open field. (Y. Cohen, unpublished). Race 2 was considered less aggressive on muskmelon than race 1 in Israel. (Karchi, in (3) with no experimental evidence.) Our preliminary observations with mixed (1:1 ratio) race inoculations in greenhouse-grown muskmelon revealed predominance of race 2 one month after inoculation.

In this paper we report on the pathogenicity of races 1 and 2 to cultivars, breeding lines and F1 hybrids of Cucumis melo L. Plants were grown in 0.5L pots in the greenhouse. When developed two fully expanded true leaves they were inoculated with either race in walk-in growth chambers (23 C, 12h photoperiod). Race 1 was maintained on 'Ananas-Yokneam' whereas race 2 was maintained on 'PMR-45' plants. Disease records were taken 14 days after inoculation. Each inoculation test was done three times. Results summarized in Table 1 show that 23 out of the 43 genotypes tested were resistant to race 1 and 11 genotypes were resistant to both races 1 and 2. No genotype was resistant to race 2 and susceptible to race 1. Most genotypes resistant to both races are breeding lines or plant introduction entrees, indicating the difficulty in incorporating race 2 resistance into commercial cultivars. Indeed, race 1 resistance genes are dominant and available in 'PMR 45' (Pm-1), PI 124111 (Pm-3) and PI 124112 (Pm-4), whereas race 2 resistance genes available in PI 124111 (1) and PI 124112 are partially dominant (Y. Cohen, H. Eyal, and D. Deningsbach, unpublished). The dominant gene B in PI 414723 against race 2 was effective in Monfavet, France 1983 but not in 1984 (3).

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Table 1. Pathogenicity of Sphaerotheca fuliginea race 1 and race 2 to genotypes of Cucumis melo var. reticulatus

<u>Genotype</u>	<u>Severity of powdery mildew</u>		<u>Remarks</u>
	<u>Race 1</u>	<u>Race 2</u>	
1. Ananas-Yokneam	+++	+++	
2. Hemed	++	++	
3. Ein-Dor	-	+*	*severe in stems
4. Sharon F1	+*	+*	*severe in stems
5. Galia F ₁	+	+++	
6. Makdimon F ₁	+*	+HS	*in stems only
7. Golden Perfection	+++	+++	
8. Hale's Best 36	+++	++	
9. Hale's Best Jumbo	+*	++	*severe in stems
✓10. Top Mark	+++	+++	
✓11. PMR 45	-	++	
12. Perlita 45	-	nt	
13. Smith's Perfect	+++	+++	
14. Cum Laude	++	+++	
15. Delectious 51	+++	+++	
16. Honey Dew Green Flesh	+++	+++	
17. Planter's Jumbo	+++	++*	*on stem mainly
18. Gulfcoast	-	nt	
19. Green Ice	++	nt	
20. Hiline	-	nt	
21. Gulf Stream	-	+	
22. Amarello	+++	+++	
23. Sierra Gold	+ Sq.	+++	
24. Edisto 47	-	+	
25. Rio Gold	-	-	
26. Tam Uvalde	-	+	
27. GA 47	-	+*HS	*moderate on stems
28. Cinco	-*	+	*moderate on stems
29. Chilton	-	+	
30. Mission F ₁	-	+	
31. Seminole	-	+HS	
32. Charity Ball	-	-	
33. Emerald Pearl	+++	+++	
34. Doublon	-	nt	
35. Charantais - T	+++	+++ (correction made by authors)	
✓36. PMR 5	-	-	
37. PMR 6	-	-	
38. MR - 1	-	-	
39. PI 124111	-	-	
40. PI 124111F	-	-	
41. PI 124112	-	-	
42. Male-Sterile 1	-	-	
43. Dulce	-	-	

Scale: - no apparent disease development; + one or two colonies per leaf with sparse sporulation; + up to 5 colonies per leaf with abundant sporulation; ++ up to 10 colonies per leaf with abundant sporulation; +++ 20 or more colonies per leaf with abundant sporulation.

HS - hypersensitive response; Sg - segregates.

Sources: Hazera, Israel, genotypes No.: 1,2,3,4,5,6,
Petoseed, : 11, 12
Asgrow : 20, 22, 30,
Hollar : 8, 9, 13, 17, 18, 23, 26, 29
USDA : 7, 10, 14, 15, 16, 19, 22, 25, 27, 28,
31, 36, 37, 38, 39, 41, 42, 43
Sakata, Japan: 32, 33
INRA, France : 34, 35

Resistance to Aphis gossypii in Spanish Melon (Cucumis melo)

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Resistance to the melon aphid Aphis gossypii has been described in muskmelon lines from India and from Far East (2, 3).

A single dominant gene symbolized by Vat (Virus Aphid transmission resistance) (1, 5) controls the resistance to colonization of melon by A. gossypii by non-acceptance and antibiosis mechanisms and resistance to viruses transmission by this aphid (4).

We report here the discovery of a resistance to the melon aphid in some cultivars of melon from Spain. The inheritance of this resistance is analyzed.

The study of a collection of 72 melon landraces from the Spanish Horticultural Germplasm Bank has shown the existence of resistance to A. gossypii in 3 accessions. 'Ariso' from Ibiza is early maturing with orange flesh; 'Invernizo' from Malaga is very late, in the 'Tendral' type (dark green skin, white flesh, oval, fruit weight about 3.5 kg); 'Escrito' from Murcia is early with green-yellow skin, orange flesh, oval, fruit weight is about 2 kg.

The resistant plants from the spanish lots were selfed and their progenies tested and crossed with 'Védraçais' (susceptible) and 'Margot' (resistant 'Cantaloup Charentais' lines with the Vat gene). The plant resistance was tested by non-acceptance assay (2).

According to the results in F_1 and F_2 progenies for 'Ariso' x 'Védraçais' (Table 1), a single dominant gene controls this resistance. Results of the 'Ariso' x 'Margot' progenies indicate that resistance in 'Ariso' is controlled by the same gene (Vat) than in 'Margot'.

In 'Invernizo', resistance is not very clear cut and there are often more adult aphids remaining on the plants and more larvae than in 'Margot'. But in the F_2 'Invernizo' x 'Margot' we found no really susceptible plants (as 'Védraçais'). We conclude that 'Invernizo' carries the Vat gene but that the expression of the gene is not very good in this cultivar type. And so in the F_2 'Invernizo' x 'Védraçais' we may have underestimated the number of resistant plants which gives a ratio resistant : susceptible different from 3:1.

Resistance to viruses transmission by A. gossypii has been studied. 'Ariso', 'Invernizo' and 'Escrito' are all resistant to Watermelon Mosaic Virus 2 and Zucchini Yellow Mosaic Virus transmission.

Table 1. Results for the behavior to Aphis gossypii after non acceptance tests in 3 different spanish muskmelons cultivars and the F₁ and F₂ with 'Védrantais' (aphid susceptible) and 'Margot' (aphid resistant).

	Resistant	Susceptible		chi square	
				value	probability
Ariso	148	0			
F ₁ Ariso x Védrantais	20	0			
F ₂ (Ariso x Védrantais) ⊕	234	66	3:1	1.44	23%
F ₁ Ariso x Margot	36	0			
F ₂ (Ariso x Margot) ⊕	201	0	15:1	13.4	<0.1%
Invernizo	30	0			
F ₁ Invernizo x Védrantais	10	0			
F ₂ (Invernizo x Védrantais) ⊕	193	110	3:1	20.65	<0.1%
F ₁ Invernizo x Margot	20	0			
F ₂ (Invernizo x Margot) ⊕	280	0	15:1	13.87	<0.1%
Escrito	139	0			

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Resistance to Yellowing Disease in Wild Relatives of Muskmelon

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Cultivation of greenhouse muskmelon on the south east coast of Spain is being seriously affected by a yellowing disease which might be related to others described in Japan (6) and France (5). The disease noticed in Spain causes the leaves to turn yellow except in the veins, coming either from an interveinal chlorotic spotting or from a golden yellow basal stain. There is a closed relationship between the whitefly (Trialeurodes vaporariorum West-Wood) and both the intensity and early appearance of the symptoms of the yellowing disease of muskmelon (2).

Adequate levels of resistance to the yellowing disease of muskmelon have not been found in Spanish land races. Although 'Nagagata Kim Makuwa', PI 161375 and PI 157084 have shown some tolerance (3), all of them display systemic symptoms. Therefore the susceptibility to yellowing disease has been evaluated in four accessions of Cucumis ficifolius, three of C. anguria var. longipes, two of C. zeyheri and of C. myriocarpus and one accession of C. metuliferus, Citrullus colocynthis and Cucurbita martinezii under conditions of natural infection.

The test was made in the greenhouse under massive whitefly infestation. More than 1000 plants of Cucumis melo were grown in the same greenhouse and all of them showed high levels of susceptibility. The Cucumis species evaluated have been reported as more susceptible than muskmelon to whitefly by Kowalewski (4).

Citrullus colocynthis, all of the accessions of Cucumis anguria var. longipes and C. zeyheri and one accession of C. myriocarpus showed a high level of resistance to yellowing disease. Cucurbita martinezii, three accessions of Cucumis ficifolius and one accession of C. myriocarpus were highly susceptible, and the first symptoms appeared early. Cucumis metuliferus and one accession of C. ficifolius displayed the first symptoms of the disease later (Table 1).

Several plants of two accessions of Cucumis ficifolius behaved as resistant to yellowing disease and they were more vigorous than the other plants of those accessions. Their fruits did not fully develop and had very few seeds. There were indications that these plants may have been the F₁ progeny of Cucumis ficifolius x C. anguria var. longipes. This fact could indicate that a dominant inheritance pattern confers resistance to yellowing disease in Cucumis anguria var. longipes.

If the observed resistances are confirmed, two ways for gene exchange between these resistant species and Cucumis melo can be used: protoplast fusion or by using both Cucumis africanus and C. metuliferus like a genetic bridge. In fact, Cucumis anguria var. longipes, C. zeyheri, C. myriocarpus and C. africanus have been reported to cross among themselves. All of them are incompatible with Cucumis melo but C. metuliferus can be crossed with both C.

melo and C. africanus (1), although the crosses are quite difficult to make. We are studying the feasibility of this way.

Table 1. Susceptibility to yellowing disease and days elapsed between sowing and first symptoms.

Species	Accession	Susceptibility or resistance	Days between sowing and first symptoms
<u>Cucumis metuliferus</u>		susceptible	120
<u>C. myriocarpus</u>	A-1	susceptible	53
	A-2	resistant	-
<u>C. anguria</u> var. <u>longipes</u>	A-1	resistant	-
	A-2	resistant	-
	A-3	resistant	-
<u>C. zeyheri</u>	A-1	resistant	-
	A-2	resistant	-
<u>C. ficifolius</u>	A-1	susceptible	101
	A-2	susceptible	53
	A-3	susceptible	65
	A-4	susceptible	53
<u>Cucurbita martinezii</u>		susceptible	53
<u>Citrullus colocynthis</u>		resistant	-

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Collecting Cucumis melo L. in Spain

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During 1984 and 1985 a collection of Cucumis melo L. was carried out in Spain. The project, designed for collecting several vegetable crop species germplasm, was partially supported by I.B.P.G.R./F.A.O. The accessions of muskmelon collected, were indicated in the C.G.C. Report 9(1986):10-11. Since then, we have collected new accessions of different crop species. Among them, 130 accessions are of muskmelon from the following sampling areas: Murcia (MU), Andalucía (AN), Valencia (V), Castilla-La Mancha (CM), Extremadura (E), Cataluña (C), Baleares (B), Castilla-León (CL), Aragón (A).

We have grouped the accessions according to several fruit characteristics observed. The abbreviations for the observations are: C=Cracked fruits; W=Wrinkled; E-"Escriturado" (In Spain the work "escrito" is used to describe the set of lines or marks that generally appear on the fruit skin and which look like letters or features made by a pen or some other very thin cutting object).

GROUP 1: "PIEL DE SAPO" .-Predominant skin colour at maturity: green. Secondary skin colour: yellow or dark green. Design produced by secondary skin colour: speckled and spotted. Fruit skin texture: firm. Fruit shape: elliptical. Observations: MU-C-24, CM-C-51, E-C-37 and C-C-21 show some of E; V-C-127 shows C.

<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>
MU-C-7	Toatana	AN-C-116	Gabia	CM-C-51	Illescas
MU-C-11	Balsicas	AN-C-152	Cartama	E-C-36	La Haba
MU-C-18	Torre Pacheco	V-C-9	Venta Del Moro	E-C-37	La Haba
MU-C-24	Corvera	V-C-125	Novelda	E-C-39	La Codosera
MU-C-26	Balsa-Pintada	V-C-127	Petrel	C-C-21	Campillonc
MU-C-42	Lorca	CM-C-13	Villatoya	C-C-25	Ripoll
MU-C-43	Lorca	CM-C-15	Filanco	C-C-38	Artesa de Segre
MU-C-45	Mazarrón	CM-C-20	Tolosa	CL-C-4	Carracadelo
AN-C-85	Sabiote				

The local names are: AN-C-58= escrito; AN-C-116= alagartado; V-C-9, CM-C-20= piñoncillo; E-C-36= cocio; E-C-37= verdejo; E-C-39= verde pinto; C-C-21= sucre; All of the other accessions= piel de sapo or pinta sapo.

GROUP 2: "AMARILLO" . - Skin colour at maturity: yellow. Fruit skin texture: superficial wrinkled (except MU-C-23); some of the accessions show E; V-C-119 shows C. Flesh colour: white. Flesh texture: firm. Fruit shape: globular.

<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>
MU-C-10	Balsicas	MU-C-32	Cuevas del	ANC-122	Jauja
MU-C-12	Balsicas		Reyllo	AN-C-151	Santo Tome
MU-C-17	Torre Pacheco	MU-C-35	Cartagena	V-C-116	Guardamar
MU-C-23	Los Marinez	MU-C-37	Torreaguera	V-C-119	Cox
MU-C-27	Lobosillo	MU-C-40	Totana	V-C-136	Masamagrell
MU-C-28	Lobosillo	AN-C-88	Sabiote	E-C-38	Don Benito
MU-C-31	La Aljorra	AN-C-108	Alcaudete	E-C-42	Don Benito
MU-C-33	Palas				

The local names are: MU-C-17, MU-C-23, MU-C-28, MU-C-40 and V-C-116= amarillo oro; MU-C-27, MU-C-35 and E-C-42= amarillo canario; MU-C-37= amarillo tardio; V-C-19= amarillo alicantino; all of the other accessions=amarillo.

GROUP 3 : "TENDRAL".- skin colour at maturity: green or dark green. Fruit skin texture: intermediate or deep wrinkled; MU-C-13, MU-C-47, V-C-109, V-C-115, V-C-122, CM-C-52 and E-C-41 show some of E. Flesh colour: white Flesh texture: firm. With difficult fruit abscision. Fruit shape: oblate, except B-C-2 which has globular fruits

<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>
MU--13	San Pedro	AN-C-101	Alcalá la Real	V-C-121	Crevillente
MU-C-15	Dolores de Pacheco	AN-C-117	Santa Fe	V-C-122	Elche
MU-C-16	Torre Pacheco	AN-C-140	Cartagena	CM-C-52	Daimiel
MU-C-20	La Puebla	AN-C-156	Lucena del Puerto	CM-C-55	Arcicollar
MU-C-25	Valladolises	V-C-109	Pilar de la	E-C-41	Santa Amalia
MU-C-47	Aquillas		Horadada	C-C-33	Montiró
AN-C-86	Sabiote	V-C-115	San Fulgencio	B-C-2	Favaritx

The local names are: MU-C-16, B-C-2 = de todo el año; AN-C-86, AN-C-117, AN-C-156 = verrugoso; AN-C-140 = de invierno negro; V-C-115 = verde; V-C-121 = negro de Elche; CM-C-55 = negro escriturado; E-C-41 = verdejo; C-C-33 = valencià; all of the other accessions = tendral.

GROUP 4 : "ROCHET" . - Skin colour at maturity; green. Fruit skin texture: superficial wrinkled; "escrito" with variable intensity; AN-C-157 shows C. Flesh colour: white or white-green. Fruit shape: oblate-globular.

<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>
MU-C-6	Murcia	AN-C-157	Moguer	V-C-123	Elche
MU-C-14	Dolores de Pacheco	V-C-108	Pilar de la Horadada	CM-C-50	Rielves
MU-C-30	Fuente Alamo	V-C-118	Callosa de Segura	C-C-36	Puigcercos
MU-C-41	Totana			CL-C-2	Toro

The local names are: MU-C-6 = flor de Murcia; AN-C-157, CL-C-2 = escrito; V-C-118 = verde valenciano; CM-C-50 = mochuelo; C-C-36 = valenciano; all of the other accessions = rochet.

GROUP 5 : "BLANCO" . - Predominant skin colour at maturity: white, Secondary skin colour: green. Design produced by secondary skin colour: spotted (poligonal shape). Flesh colour: white ot white-green. Flesh texture: firm. Fruit skin texture: superficially wrinkled; some of "escriturado". Fruit shape: globular.

<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>	<u>Label</u>	<u>Locality</u>
AN-C-90	Canena	AN-C-93	Canena	A-C-19	Sadaba

The local names are: AN-C-90 = tempranillo; N-C-93, A-C-19 = blanco.

GROUP 6 : OTHER TYPES . - The abbreviations used for the observations are: Skin colour G = green, Y = yellow, H = white; design produced by secondary skin colour S = spotted, K = speckled; fruit skin texture N = netted, R = ribbed, E = "escriturado" (SE = some of "escriturado", IE = intermediate), C = cracked, W = wrinkled (SW = superficial, IW = intermediate). D = dry farming.

<u>Label</u>	<u>Locality</u>	<u>Observ.</u>	<u>Label</u>	<u>Locality</u>	<u>Observ.</u>
MU-C-19	Torre Pacheco	G,E,IW	E-C-35	La Haba	Y-G,IW
AN-C-109	Santa Ana	H,R	E-C-40	Olivenza	Y,W
AN-C-124	Jauja	Y,S,R,C,	AN-C-150	Sanot Tomé	Y,S,N
AN-C-131	Arcos de la Frontera	G,K(white),IW	E-C-46	Quintana de la Serena	G,W
V-C-24	Lliber	G,SE	C-C-37	Pons	G,S,IW
V-C-126	Elda	Y,IW	C-C-39	Artesa de Segre	(pale),S,W
CM-C-26	Tarazona de la Mancha	G.S.W.D	B-C-1	Ciudadela	G,N,IW
CM-C-40	Ribagorda	G,N,IE,SW,C			

The local names are: MU-C-19 = canario; AN-C-109 = coca; AN-C-131, CM-C-26, B-C-1 = de olor; AN-C-124 = de tajadas señaladas ("Ogen type"); V-C-24, V-C-126 = tendral; CM-C-40 = piel de sapo; E-C-35, E-C-40 = melona; E-C-46 = banda de Godoy; AN-C-150 = rayado; C-C-37 = escriturado de Montsonis; C-C-39 = cuarenteno.

In addition to these groups we found 35 accessions showing variability of types: 9 from Andalucía, 7 from Valencia, 6 from Castilla-La Mancha, 5 from Murcia, 3 from Cataluña and Extremadura, and 2 from Baleares.

Acknowledgements; We are extremely grateful to the Diputación Provincial de Valencia, Servicio de Extension Agraria and to all those who have collected vegetable crop germplasm: C. Cortés, G. Anastasio, R.V. Molina, P. Corella, M.C. Ayuso, G. Palomares, A. Alonso-Allende.

Isozyme Analysis of Hybrids and Their Parents of Watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai]

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Abstract

Ten F_1 hybrids and their 16 parents of watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai] were used for isozyme analysis of peroxidase (POD) and esterase (EST) of dry seeds, germinating seeds, cotyledons and roots at the cotyledonary stage. The results obtained indicated that zymograms of POD and EST varied at different stages and different parts of the plant. Isozyme variation between hybrids and their parents was found at the seed germination stage and cotyledonary stage of POD, as well as cotyledonary stage of EST. The following cases were observed in our study: F_1 hybrids had the same zymogram as their two parents, or maternal parents, or paternal parents, or had 'hybrid enzyme' or lacked some zymographic bands of their parents. High heterosis was generally found in the hybrids with the same isozyme zymograms of POD as their paternal parents, and EST as their maternal parents.

Evidence for a Tetrasomic Line in Watermelon

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A tetrasomic genotype has $2n + 2$ chromosomes. If the chromosomes are simply duplicates of a given pair of homologues and segregation of this extra pair is normal, genes on these chromosomes should behave as expected for a tetraploid. Phenotypic differences between a tetrasomic and tetraploid should be a distinguishing feature. Also, a tetraploid x diploid cross will produce sterile triploids. However, a tetrasomic x disomic cross will produce trisomic progeny which should be much more fertile than triploids.

A glabrous, male sterile tetraploid watermelon line has been described (1). This line has been hybridized with diploid lines to produce triploid seedless progeny. We reported a related line that generally segregated as expected for a tetraploid line (2). The $++$ gms gms genotype generally produced progeny

segregating 35:1 hairy:glabrous. Crossing the genotypes gms gms gms gms x + + gms gms generally produced progeny segregating 5:1 hairy:glabrous. These crosses distinguished the tetrasomic from the disomic condition. Cytological examination of microsporocytes from male fertile plants from this line suggested high fertility and a haploid chromosome number nearer 1 than 22.

Glabrous plants from this line were crossed with three diploid genotypes: 'Crimson Sweet', LA 390 and SC 7. Germination of the hybrid seed in the greenhouse averaged 95% compared with 51% for the triploid cultivar 'Triple Sweet'. Seed number in F₁ hybrid fruit growing in the field was high. Seed morphology was similar to that of diploid rather than tetraploid seed.

Segregation ratios were re-examined in nearly 100 self- and sib-pollinations among glabrous, male sterile and hairy, male fertile plants. First, the progeny of a single plant was examined. This plant, when selfed, produced progeny segregating 39:7 hairy:glabrous. Forty-five of the plants were selfed and/or sib-pollinated to determine their genotype (Table 1).

Second, plants determined to be + gms gms gms among the F₁ progeny of the single + gms gms gms parent were testcrossed to gms gms gms gms sister plants. Hairy, male fertile plants from these testcrosses were selfed. All progeny from the single parent are grouped in Table 1. The tetrasomic nature of the line is indicated by segregation ratios of 35:1 and 5:1 in the F₂ progeny. Trisomy, rather than triploidy, was indicated by the high germination rate of seed from crosses with the three diploid lines noted earlier and by the presence of several hundred seed per melon produced on the hybrid plants. Progeny from the open-pollinated trisomic plants are being evaluated.

Seed of the putative tetrasomic line, segregating 1:1 for hairy, male fertile: glabrous, male sterile are available from the senior author.

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Table 1. Distribution of genotypes from a single self-pollinated plant determined to be of the genotype \pm gms gms gms.

Putative genotype ^Z	Fraction of 46 F ₁ progeny		F ₂ segregation	
	Theoretical	Actually ^Y	Selfed	sibbed
+ + gms gms (1)	11.5	15	35:1	5:1
+ gms gms gms (2)	23.0	23	3:1	1:1
gms gms gms gms (3)	11.5	7	male sterile	

Crosses	Progeny			
	Hairy	Glabrous	Expected	Chi-Square
(3) x (2) F ₂ selfed	29	18	1:1	2.57
	36	7	3:1	1.74
	40	7	3:1	2.56
(3) x (2) F ₂ selfed	29	21	1:1	1.28
	37	13	3:1	0.03
	37	9	3:1	0.72
	40	10	3:1	0.67
(3) x (2) F ₂ selfed	9	11	1:1	0.05
	33	7	3:1	1.20
	41	5	3:1	4.90
	40	9	3:1	1.15
(3) x (2) F ₂ selfed	11	6	1:1	1.47
	34	11	3:1	0.01

^ZDetermined by self and sib pollinations and Chi-Square analysis.

^YOne hairy, male fertile plant lost after count of F₁ progeny.

Breeding Few-seed/Seedless Watermelon via Chromosome Reciprocal Translocation Induces by Gamma-ray.

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The development of autotriploid watermelon was a great advance in the field of watermelon breeding. However, some disadvantages still existed with this type of seedless watermelon. Partial sterility may be induced in diploid watermelon via chromosome reciprocal translocation. We used gamma-rays to irradiate the seeds of homozygous translocation strains with one translocation ring composed of 4 chromosomes (symbol: ④). Watermelon strains were 'Asahi Yamato', 'Miyaka', and 'Fumin' sent to us by H. Kihara in 1977. In order to further induce multiple reciprocal translocations for developing new few-seed/seedless watermelon strains, the seeds of the above 3 strains were sown for further selfing in 1978. The seeds of each selfed fruit were grown as a single plant line in 1979 for evaluation of their characters. In addition, some crosses between common diploid watermelon cultivars and translocations were carried out to test the seed setting rate of the heterozygous translocation strains. Some of the crosses were 'Sugar Baby' x 'Asahi Yamato AT-1' and 'Akakotama' x 'Asahi Yamato AT-2'. The plump seed setting rate of the F₁ of these crosses were ca. 50%.

In general, the common cultivars of 'Sugar Baby', 'Asahi Yamato' and 'Akakotama' without any translocation produce ca. 500 seeds per fruit, whereas the heterozygous translocation strains contain ca. 200 seeds per fruit. Thus, they must be semi-sterile with one translocation ring at their 1st meiotic division in PMCs. It follows that the initial translocation strains were really homozygous ones with two pairs of chromosomes. In order to increase translocation chromosomes numbers/rings and decrease seed setting rate, in 1981 we used ⁶⁰Co gamma rays to irradiate the seeds of the initial homozygous translocation strain 'Asahi Yamato.' The dose of irradiation was 50Kr and the dose rate was 108r/min. Cytological examination indicated that some extraordinarily complicated aberrations of chromosome divisions occurred, such as chromosome segments, bridges, lagging chromosomes, chromosome adhesion, micronuclei, etc. However, these kinds of abnormal chromosome divisions were of no use to our breeding program. Some new useful translocations were also obtained in the following generations. In 1983, the seeds of these new strains were irradiated again by gamma-ray and induced more translocation strains with different translocation chromosomes/rings and then crossed with each other for syntheses of multiple reciprocal translocation complexes. The translocation in heterozygous strains varied both in chromosome numbers and translocation positions. These can be described as follows: ④ + 9II (ring with 4 chromosomes + 9 bivalents), ④ + ④ + 7II, ④ + ⑥ + 6II, ④ + ⑧ + 5II, ④ + ④ + ⑧ + 3II, ⑥ + 8II, ⑧ + 7II, ⑥ + ⑧ + 4II, ④ + ④ + ⑥ + 4II, ⑩ + ④ + ④ + 2II, ⑫ + 5II and asynapsis, etc. The seed setting rate of some of the different translocation strains and their F₁ hybrids are listed in Table 1.

Table 1. Seed setting rate of the parent strains and their F₁ hybrids

Parent translocation strains	Seed setting rate of parent (%)	Cross combinations	Seed setting F ₁
83-10	42.6	83-11 x 83-2	23.8
83-11	62.6	83-12 x 83-8	19.8
83-12	51.1	83-12 x 83-11	24.9
83-13	ca.50	83-12 x 83-6	27.5
83-15	51.2	83-13 x 83-16	23.0
83-16	ca.50	83-16 x 83-10	21.0
83-19	43.0	83-15 x 83-19	30.2

The results showed that the above translocation strains were really different ones with different translocation chromosomes. The pollen of partially sterile strains were abnormal in size, shape, and content as well. Some pollen grains were empty and wrinkled without germinability. Some small white pollen and giant ones were also found in their anthers (Fig. 1).

Morphological effects of chromosome translocations occurred extensively, for instance, a seedling with one or three, even four cotyledons; and a plant densely covered with white hair and the leaves becoming curly resembling a virus infection. In a few plants, female flowers and fruits grew thickly and almost set at every node (Fig. 1); different kinds of rind color variations, e.g. a golden yellow color rind variation occurred in 'Asahi Yamato' translocation strain. In addition, the rind of some fruits become so hard that one melon can support three persons standing on it.

Three multiple translocation strains of 'Asahi Yamato', 'Miyaka' and 'Fumin' were also bred by Northwestern Agricultural University in 1984-1985. Some crosses between the above strains as well as between the 'Fumin' translocation strain and 16 different common watermelon cultivars (such as 'Charleston Gray', 'Crimson Sweet', 'Asahi Yamato', 'Sugar Baby', 'Lovrin 532', etc.) were conducted and 198 fruits were selected individually. The best crosses were 'Fumin' translocation strain crossed with 'Charleston Gray' and 'Crimson Sweet', respectively. The least number of seeds per fruit were only 25 and 52 seeds.

In brief, theoretically and practically, the more chromosome translocations involved, the higher the sterility. In this study, through self-pollination combined with segregation, selection and cross breeding, several new strains of few-seeded watermelon were developed. Their seed setting rate was 50-80% less than that of common watermelons. The extreme type contained only a few seeds. Some of the new strains are very promising for commercial production with high yield, good quality, early maturity and good storage ability.

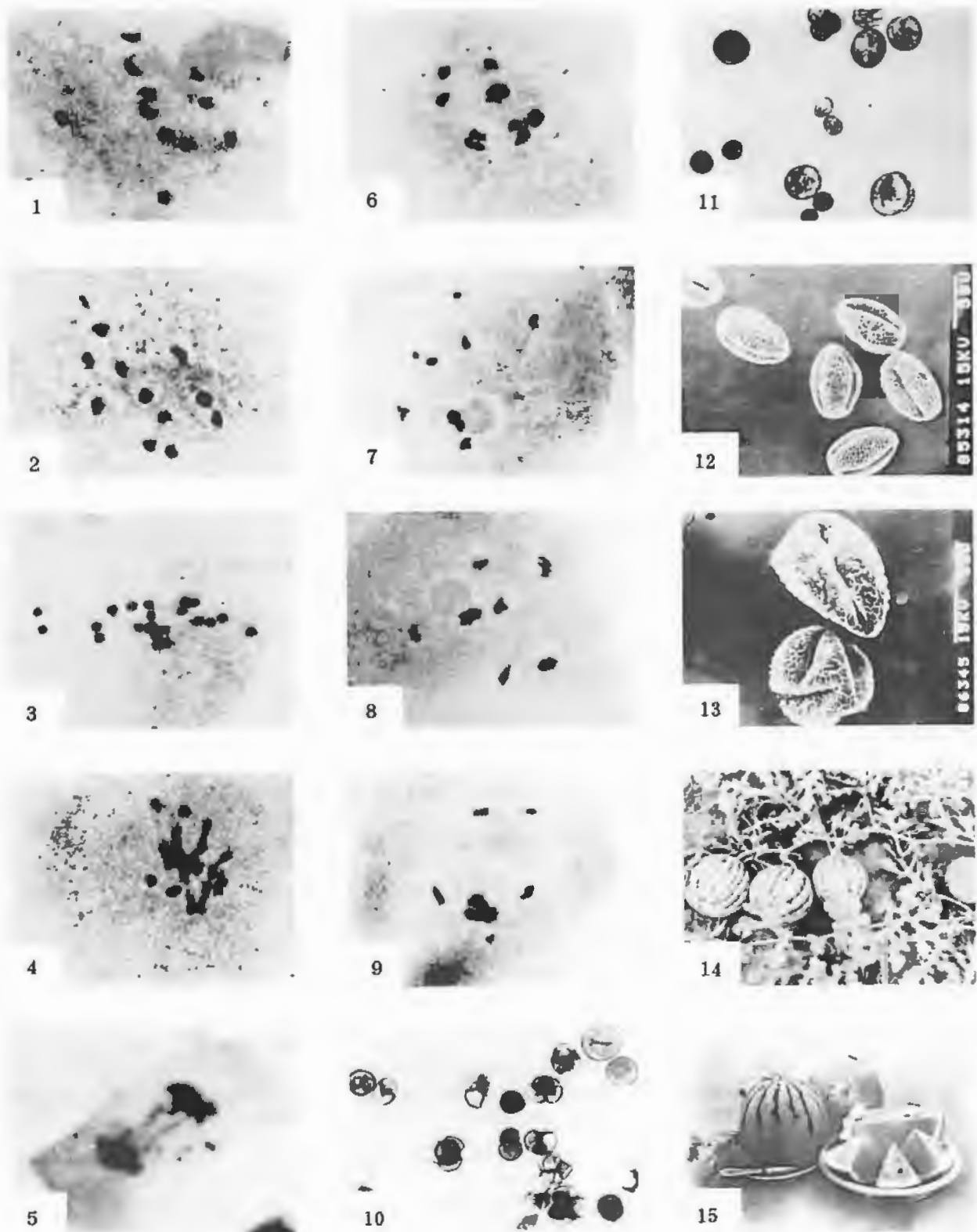
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(Chinese references omitted)

Figure 1. Chromosomes and fruit of few-seed/seedless watermelon via chromosome reciprocal translocations induced by gamma radiation. Plate 1 - normal chromosomes of watermelon: 11 bivalents (x 1000). Plate 2 - 11 bivalents + 1 segment. Plate 3 - asynapsis: 22 monovalents. Plate 4 - abnormal synapsis. Plate 5 - bridge and segments. Plate 6 - ⑥ + 8II (bivalents). Plate 7 - ⑧ + 7II. Plate 8 - ⑥ + ④ + ④ + 4II. Plate 9 - ⑫ + 5II. Plates 10 & 11 - the pollen grains of partial sterile strains showed significantly abnormal in size, shape and content, some of them adhering together. Plate 12 - the scanning electron microphoto of pollen grains: normal fertile pollen grains. Plate 13 - the scanning electron microphoto of pollen grains: sterile pollen grains, empty and wrinkled without germinability. Plate 14 - one of the very special variations: the fruits grew thickly, and all the fruits can grow to maturity for commercial use. Plate 15 - the fruits of few-seed watermelon (heterozygous translocation strains).

Fig. 1. Chromosomes of few-seed / seedless watermelon via chromosome reciprocal translocations induced by gamma-ray.



Single Gene Control of Anthracnose Resistance in *Citrullus*?

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This research was supported by a grant from the USDA CRGO.

Previous reports (1, 3, 6, 7) indicate that resistance to Colletotrichum lagenarium (Pass.) Ell. and Halst. in watermelon is governed by a single dominant gene. These studies differed in classification of resistance, race identification, inoculum levels and susceptibility of greenhouse-grown seedlings.

If a single gene controls resistance to race 2, it should be simple to incorporate the high level of resistance found in resistant into commercially accepted cultivars. Actually, no cultivar has been developed with as much resistance to race 2 anthracnose as the resistant source. A greenhouse study of inheritance of race 2 resistance in documented resistant and susceptible Citrullus genotypes was conducted in 1982. A field study was conducted in 1983. Methods were modified in the second study as indicated.

Methods. An isolate of the fungus was obtained from the EREC and determined to be race 2 by the method of Jenkins et al. (2). Culture and spore production methods were similar to those of Littrell and Epps (4). Randomized blocks of parents and progeny of the host were mist inoculated with spore suspensions: 50,000 spores/ml in the greenhouse and 20,000 spores/ml in the field study. Greenhouse seedlings were inoculated at the 2-4 leaf stage. Field plants were inoculated at fruit set. Greenhouse seedlings were rated 8 days after inoculation on a 0-10 scale (0 = no lesions, 10 = dead) by comparing with a set of 11 plants representing 1 point on the scale. Plants rated 0-6 were considered resistant, 7-10 susceptible. Field plants were rated in two ways five weeks after inoculation: 1 - the oldest branch on each plant was rated for percent defoliation and 2 - percent of remaining leaves showing lesions. A disease index was a composite of both ratings. Plants indexed 0-85 were considered resistant, above 85 susceptible. The average between resistant and susceptible parent means was used as the division between resistant and susceptible plants.

Treatments assigned to each block were F_1 , F_2 and BC_1 generations from a diallel cross among the resistant watermelon plant introduction PI 189225, PI 299379, the susceptible watermelon cv. New Hampshire Midget and a resistant line of Citrullus colocynthis (1) Schrad., designated R309.

Results. The greenhouse seedling study could not distinguish resistant plants from susceptible plants. Anthracnose symptoms developed on even the most resistant lines. This result is consistent with the report of Winstead et al. (7), who reported that PI 189225 was susceptible to anthracnose race 2 and Sowell et al. (5), who reported the PI resistant. Unlike Sowell et al., Winstead et al. depended entirely on greenhouse seedling inoculations to screen for resistance and used an inoculum level of 50,000 spores/ml instead of 20,000 spores/ml.

Resistant and susceptible plants were more easily identified in the field study. Table 1 shows the ratios of resistant and susceptible plants for each of the 3 susceptible x resistant set of parent-progeny populations. Chi-square analysis of F_2 and backcross progenies of the NHM x PI 299379 and NHM x PI 189225 crosses cannot reject the hypothesis that resistance in PI 299379 and 189225 are controlled by a single dominant gene. However, the NHM x R309 progeny does not fit any expected ratio for a 1 or 2 gene dominant, recessive or additive trait.

Frequency distributions of resistance levels in PI 299379 and PI 189225 and their progeny suggest that the inheritance of resistance is more complex than that suggested by Chi-square analysis. The shift of the F_1 populations toward the susceptible parent does indicate complete dominance. Also, the F_2 populations are not divided into discrete classes.

Comparison of means using orthogonal contrasts for each of the 6 sets of parent-progeny populations is found in Table 2. A comparison of the F_1 and F_2 populations with the midparent (average of the 2 parental means) is a test for dominance. Comparison of the F_1 and F_2 with the midparent from PI 299379 x NHM supports the conclusion that resistance is largely due to dominance. However, the failure of the F_1 and backcross generations to show as much resistance as PI 299379, the lack of a bimodal distribution in the F_2 and the failure of the F_2 to produce plants as susceptible as the susceptible parent indicates the presence of other modifying genes. The same conclusion can be drawn from the progeny populations from PI 189225 x NHM.

Comparison of the F_1 and backcross populations from R309 x NHM to the resistant parent indicates resistance is due to complete dominance. However, the F_2 is not significantly different from the midparent and fails to support the conclusion of either 1 or 2 dominant genes. Instead, it is more representative of the distribution expected from several dominant genes acting in concert.

The F_1 population from PI 299379 x PI 189225 (Table 2) is significantly more susceptible than either of the resistant parents, indicating the presence of additive or recessive resistance factors in each line that are not found in the other. The failure of the F_2 population to show distinct segregation for susceptible plants suggests that both lines share a common major dominant gene factor for resistance and that the unshared factors were minor modifiers.

Comparison of progeny means from R309 x PI 189225 indicates that the higher level of resistance displayed by PI 189225 is due to dominance. Thus, it follows that R309 owes its resistance to a set of factors separate from PI 189225. The same conclusion can be reached from the comparison of progeny means from R309 x PI 299379. Small populations, due to poor germination, made statistical analysis of this family difficult. Nevertheless, the involvement of separate genetic factors was apparent from the segregation of the progeny.

In conclusion, two types of inheritance for resistance was identified in the 3 resistant lines. R309 (*C. colocynthis*), demonstrated an intermediate level of resistance attributed to several dominant genes acting in concert. In PI 299379 and PI 189225 resistance is controlled largely by a single dominant gene. In the latter 2 lines, resistance is modified by minor genes.

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Table 1. Reaction of the parents and progeny from each of the 3 susceptible x resistant crosses to inoculation with *G. lagenarium* race 2 in the 1981 field study. The Chi-square analysis tests the hypothesis that resistance is due to a single dominant gene^a.

Population	Total Plants	Resistant	Susceptible	Expected Ratio	χ^2	P
MIM x PI 299379						
MIM	64	1	63	0:04		
PI 299379	5	5	0	5:0		
F ₁	58	50	8	95:0		
F ₂	71	55	16	3:1	2.230	.50-.70
BC to MIM	60	28	40	1:1	2.118	.10-.20
BC to PI 299379	70	61	9	70:0		
MIM x PI 189225						
MIM	72	5	67	0:72		
PI 189225	58	58	0	58:0		
F ₁	40	39	1	60:0		
F ₂	74	60	14	3:1	1.439	.70-.30
BC to MIM	70	33	37	1:1	.229	.50-.70
BC to PI 189225	74	74	0	74:0		
MIM x R309						
MIM	49	13	36	0:49		
R309	9	9	0	9:0		
F ₁	45	44	1	45:0		
F ₂	69	38	31	3:1	19.174	.01
BC to MIM	34	22	12	1:1	2.961	.05-.10
BC to R309	70	9	1	10:0		

^aAll plants were indexed on a 0-200 scale with 0 being immune and 200 being dead. Plants with an index from 0-85 were considered resistant, those above 85 susceptible.

Table 2. Comparison of the mean disease index of parents and progeny from each of 6 susceptible x resistant or resistant x resistant crosses in the 1981 field study^a.

Population	Mean	Comparison ^b	Significance
MIM x PI 299379			
MIM	154.35	MIM vs PI 299379	**
PI 299379	76.37	PI 299379 vs P ₁	**
P ₁	72.78	PI 299379 vs BC to PI 299379	**
P ₂	70.31	Midparent vs P ₁	**
BC to PI 299379	58.45	Midparent vs P ₂	**
Midparent	60.68		
MIM x PI 189225			
MIM	142.93	MIM vs PI 189225	**
PI 189225	25.46	PI 189225 vs P ₁	**
P ₁	49.11	PI 189225 vs BC to PI 189225	**
P ₂	37.92	Midparent vs P ₁	**
BC to PI 189225	27.66	Midparent vs P ₂	**
Midparent	64.30		
MIM x R309			
MIM	115.24	MIM vs R309	**
R309	46.70	R309 vs P ₁	**
P ₁	45.86	R309 vs BC to R309	**
P ₂	87.37	Midparent vs P ₁	**
BC to R309	74.82	Midparent vs P ₂	**
Midparent	81.97		
PI 299379 x PI 189225			
PI 299379	76.34	PI 299379 vs PI 189225	**
PI 189225	32.10	PI 299379 vs P ₁	**
P ₁	42.38	PI 189225 vs P ₁	**
R309 x PI 189225			
R309	51.32	R309 vs PI 189225	**
PI 189225	22.99	PI 189225 vs P ₁	**
P ₁	34.02	PI 189225 vs BC to PI 189225	**
P ₂	43.78	R309 vs P ₁	**
BC to PI 189225	32.82		
R309 x PI 299379			
R309	55.80	R309 vs PI 299379	**
PI 299379	23.33	PI 299379 vs P ₁	**
P ₁	44.41	PI 299379 vs BC to PI 299379	**
P ₂	63.02	R309 vs P ₁	**
BC to PI 299379	39.24		

^aPlants were indexed on a 0 to 200 scale with 0 being immune and 200 being dead.
^bNon-significant (ns) or significant of 5% (*) or 1% (**) levels. Comparisons were made using orthogonal contrasts.

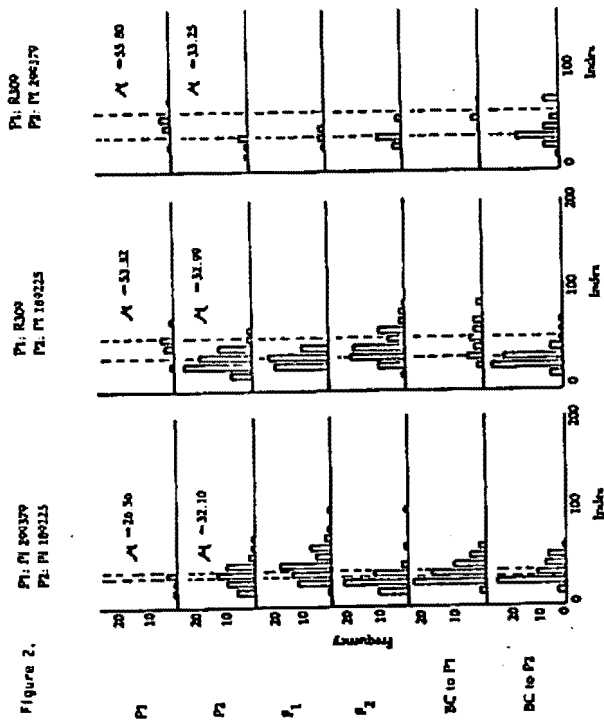


Figure 2.

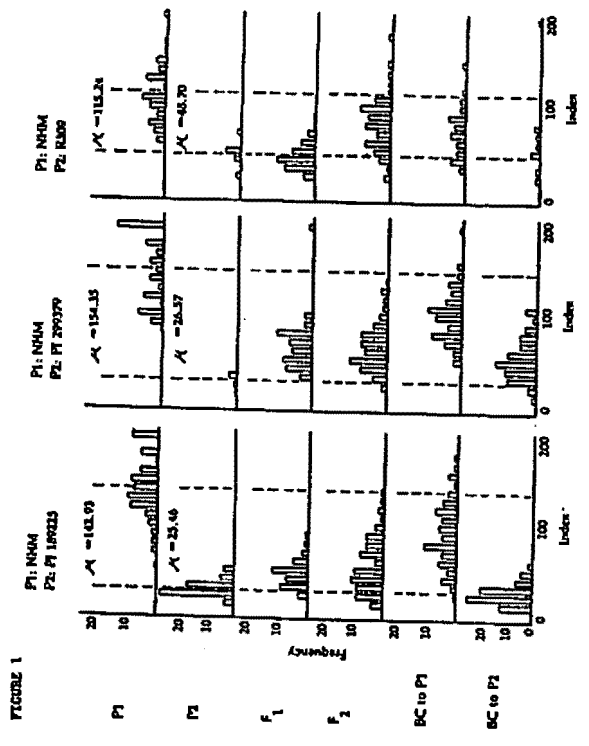


FIGURE 1

Studies on Watermelon Germplasm Sources Resistant to Fusarium Wilt Disease at the Seedling Stage.

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Abstract

Three seedling inoculation methods (soil inoculation, radical inoculation and the root dip inoculation) were compared to identify and screen for Fusarium wilt resistance (FWR). The results showed that the root dip inoculation method is a fast, correct and material saving method suitable for seedling screening of watermelon for FWR. The optimum spore concentration for inoculation was 5×10^3 spores per millilitre and the suitable root dipping time was three minutes. Resistance to Fusarium wilt of 79 watermelon varieties from different origins was studied using the seedling root dip inoculation technique. Watermelon varieties from different sources and different ecotypes were found to differ in resistant to Fusarium wilt. Watermelon varieties of African ecotype, West European ecotype and partial American ecotype were resistant type ones; the bulk of the watermelon varieties of the Xingliang ecotype, Russian ecotype, East Asia ecotype and HuaBei ecotype were susceptible-types ones.

Based on the above results, the cross section of the main roots of seedlings of 3 watermelon varieties differing in disease resistance were observed with the Scanning Electronic Microscope. Isozyme analysis of peroxidases (POD) and esterases (EST) in different organs of 11 watermelon varieties differing in disease resistance were compared and analyzed at different stages by means of Polyacrylamide Gel Electrophoresis. Activity of peroxidase, polyphenol oxidase and vitamin C oxidase in 13 watermelon varieties was determined. Seventeen kinds of amino acid, 4 kinds of organic acids and total organic acid content in 6 watermelon varieties in the root system were determined as well. The relationship between the above results and resistance to Fusarium wilt in the different varieties was studied and analyzed. The results showed that the distribution of vessels, the number of central vessels of the root system and the thickness of the cell wall of the xylem determined FWR of the watermelon varieties. There was no significant correlation between the activity of polyphenol oxidase and peroxidase and resistance, but there was a remarkable correlation between activity of vitamin C oxidase and resistance. Thus, activity of vitamin C oxidase can be used as a biochemical parameter for screening watermelon varieties for resistance to Fusarium wilt. There was no significant correlation between content of malic acid, oxalic acid, total content of organic acid and FWR, but there was a significant correlation between both acetic acid and citric acid and FWR. Thus, they also can be used as parameters to identify FWR of watermelon varieties. There were some differences between POD and EST isozyme spectrum in stems and leaves and the varieties differing in FWR. Therefore, the isozyme method can be used to screen watermelon varieties for FWR. Fusarium wilt resistant watermelon varieties have a higher content of glycine, serine, alanine, threonine, proline and arginine than Fusarium wilt susceptible watermelon varieties which in turn have a higher content of leucine, methionine and tyrosine.

Evaluation and Utilization of the Valuable African Watermelon Germplasm

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Abstract

Ten African watermelon [Citrullus lanatus (Thunb.) Matsum. and Nakai] (AW) were first introduced from Botswana, Africa, to China in 1984. Their morphological, biological and agronomic characters, and disease resistance have been studied for three years. Albumin components and iso-esterase of AW seeds were assayed by using electrophoresis. The content of crude protein and fat were tested as well. The AW accessions are obviously different from those of commercial watermelon cultivars (CW) in many characters, and a great diversity was found among them. Inoculation with 5×10^8 spores/ml of Fusarium wilt (Fusarium oxysporum f. sp. niveum) at the seedling stage indicated that the AW accessions were highly resistant to Fusarium wilt and field testing indicated resistance to other diseases. The AW have a great yield potential. The fruits of AW have an amazingly long storage life which can reach as long as half a year or one year and even two years. In addition, high heterosis was shown in the hybrids between AW and CW cultivars. Although the albumin components of AW and CW were quite similar, the iso-esterase of dry seeds was obviously different. The crude protein content of AW seeds ranged from 33.9 to 43.6 percent, while the crude fat was between 35.7 - 46.8 percent. The oleic acid (C₁₈) and linoleic acid (C₁₈) are 12.9 - 23.9 and 59.5 - 68.2 percent of the total fat content, respectively. Thus it is a valuable plant oil with high quality. In view of the above rare characters, the newly introduced AW accessions can be widely used for the following purposes: a) stock food; b) sources for refining plant oil and protein; c) raw materials for processing candied and canned watermelon fruit; and d) resistance stock for grafting of watermelon. Also, the AW accessions can be used as a very useful germplasm resource in watermelon breeding, especially for disease resistance, high yielding and long storage life.

Inheritance of Bush Habit in Cucurbita pepo L.

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Bush growth habit in squash is a very important character for dense planting, especially under the plastic house cultivation. To breed bush-type pepo cultivars for the cultivation, it is an essential step to investigate the inheritance of the bush habit.

Several researchers reported the inheritance of bush-type in C. pepo (1,3,4) and C. maxima (1,2), which include some controversial results. It was generally recognized that the bush character is almost completely dominant during the early growth stage, and recessive or incompletely dominant during the late growth stage.

In Cucurbita pepo L., the bush-type variety 'Zucchini' was crossed with the vine-type 'PI 285611'. The inheritance of bush habit was studied in F_2 and BC_1F_1 populations. The length of main stem was measured in the three different growth stages ; early, middle, and late stages.

The frequency distribution of vine length in each stage is presented in Fig.1. The mean of the F_1 hybrids was almost similar to the bush parent during the early stage, but the bush was not completely dominant to the vine. As the growth continued, the F_1 hybrid population leaned slightly toward the vine, but it was always below the mid-parent value. The segregation ratios of the F_2 and BC_1F_1 populations were 3:1 and 1:1, respectively, with the bush partially dominant to the vine regardless of the growth stages. The segregation ratios were accepted at the 10 to 50 percent level of probability (Table 1). The result obtained almost agreed with Shifriss (4), but was somewhat different from the others (1,2,3).

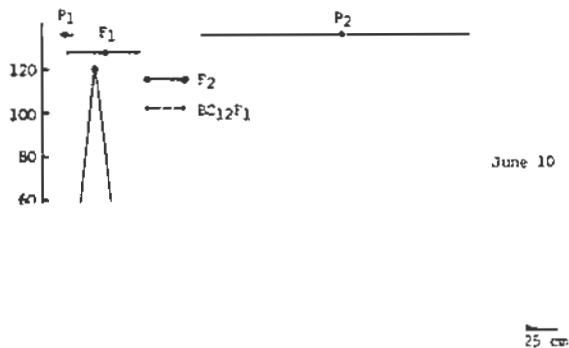
In conclusion, the characteristic feature of the bush gene action was partial dominance. The degree of dominance lessened as the growth proceeded.

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Table 1. Segregation of bush and vine types in the 3 different growth stages in the F_2 and BC_1F_1 populations from the cross 'Zucchini' x PI 285611.

Date	Generation	No. of plants		Expected ratio	χ^2 -value	P
		Bush	Vine			
June 10	F_2	199	79	3:1	1.731	0.10 - 0.25
	BC_1F_1	78	82	1:1	0.1	0.75 - 0.90
July 6	F_2	201	77	3:1	1.079	0.25 - 0.50
	BC_1F_1	77	81	1:1	0.101	0.75 - 0.90
August 5	F_2	197	76	3:1	1.173	0.25 - 0.50
	BC_1F_1	65	77	1:1	1.014	0.25 - 0.50



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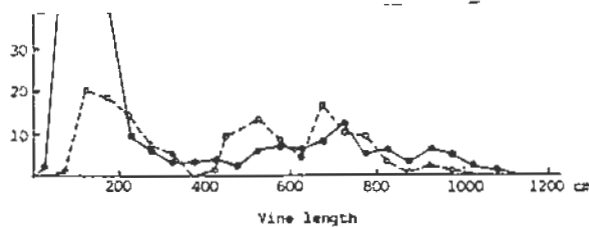


Fig.1. Frequency distribution of vine length at the 3 different growth stages in the F_2 and backcross populations from the cross, Zucchini/PI 285611. The means of parents (P_1 , P_2) and F_1 populations are indicated as black and open circles, respectively.

Improving Seed Yield in Hull-less Seeded Strains of Curcubita pepo

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Research on hull-less seeded strains of pumpkin was initiated at the NH Agr. Exp. Sta. 10 years ago, and during the past 6 years there has been a major effort to develop snack seed strains or strains bred chiefly for seed yield. The approach to increasing seed yield has been as follows: (1) develop bush strains amenable to high density planting, (2) develop strains which produce small fruit with a minimum of fleshy tissue, and (3) select strains which have high seed yield per fruit. There are numerous other important characters such as seed size, seed fill, seed color, ease of seed removal, flower ratios, fruit maturity, and pollen load which have to be dealt with, but I wish to comment only on progress we have made with respect to the three objectives above.

We have developed two F7 bush lines of pumpkin with hull-less seed and fruit size ranging from 0.5 to 2.0 kg, depending upon the spacing and fruit load per plant. Seed yield of one strain, NH14-40-6, was evaluated in 1985 at different planting densities, and seed yield and several components of seed yield were determined in 1986 with two strains, NH14-40-6 and NH55-7-20. To evaluate components of yield, a spacing trial was conducted using what will be referred to as a gradient density planting scheme. Within a multiple density planting block, subplots of 7 plants each (2 guards) were arranged across rows spaced 0.3 m apart. Different density subplots were then spaced at progressively increasing within row spacing from 0.3 to 1.5 m. This produced plant populations from 7,980 to 35,880 plants/ha.

Maximum yields were low in 1986 due to a poor growing season, but the results, nonetheless, illustrate relationships between plant density and different components of yield (Table 1). Seed yield of NH14-40-6 decreased from 58.7 g per plant at the lowest density to 22.2 g per plant at the highest density. In contrast, total seed yield per hectare was lowest (468 kg/ha) at the lowest plant density and was greatest (795 kg/ha) at the highest plant density. The same relationship between planting density and total yield was observed in 1985, only maximum yields were in excess of 1,400 kg/ha at the highest plant density (23,919 plants/ha). Seed yield per plant decreased at higher plant densities primarily because of fewer fruit per plant, and to a much lesser extent because of smaller fruit and concomitant lower seed yields per fruit. We obtained results similar to those shown above the other inbred strain, NH55-7-20, but seed yield per plant was 15 to 20% higher. There was considerable variability in seed yield among replicates in both years; in future gradient density yield trials, data plants per subplot will be increased from 5 to 10.

The proportion of dry matter partitioned into seeds versus flesh in an especially important component of yield. This component is affected by the number and weight of seeds, the proportion of fleshy tissue, and the percent dry matter of the fruit. I have chosen to describe this component by a seed index (SI) which is the ratio of seed dry weight to total dry weight of the fruit. Both of the strains used in the spacing trials exhibited very low seed indices between 23 to 30% (Table 1). Both strains had low flesh dry matter (mean of 4.6 to 6.1%), but neither strain was particularly seedy and both had

relatively thick flesh. Through appropriate crosses early generation lines have now been produced which have SI's between 40 to 57%. We have observed good correspondence between SI's obtained in F2 selections and those observed in F3 progeny, although there was obvious segregation among some lines for seed fecundity, flesh thickness, and flesh dry matter. Fruits within a plant selection were surprisingly uniform for both percent dry matter and seed yields among bush segregants. Breeding plots of these bush lines are now being seeded at a relatively high density (0.3 m within row, 1.8 m between rows), so that interplant competition approaches that likely to be encountered in commercial production.

Table 1. Effect of plant density on yield components in a hull-less seeded strain (NH14-40-6) of pumpkin.¹

Yield Components	Plant density (plants/ha)				
	7,973	10,251	14,351	23,919	35,879
Fruit no./plant	3.5	2.7	2.1	2.1	1.5
Fruit wt. (kg)/plant	1.2	1.2	1.0	0.9	0.8
Seeds (g)/fruit	17.1	18.0	21.0	14.4	14.6
Seeds (g)/plant	58.7	48.8	44.4	29.8	22.2
Seed index (SI) ²	24.7	22.7	28.2	27.1	29.4
Seed wt. (kg/ha)	468	500	637	714	795

¹Three replications, 5 data plants per subplot spacing.

²Seed index (SI) is the ratio of seed dry weight to total fruit dry weight x 100. It is a measure of the efficiency with which dry matter is partitioned into seed within a fruit.

Inheritance of Resistance to Zucchini Yellow Mosaic Virus in the Interspecific Cross Cucurbita maxima x C. ecuadorensis

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Cucurbita ecuadorensis has been reported to be resistant or tolerant to the major cucurbit viruses, namely zucchini yellow mosaic (ZYMV), cucumber mosaic, papaya ringspot, W-strain (= watermelon mosaic 1), squash mosaic, and watermelon mosaic 2 (1, 2, 3). The purpose of this study was to determine the inheritance of resistance to ZYMV in populations derived from the interspecific cross C. maxima cv. Buttercup x C. ecuadorensis.

Plants of both parents, F_1 , F_2 , and of the backcross to Buttercup were mechanically inoculated with the Connecticut strain of ZYMV (2) when the cotyledons were fully expanded. A week later, all plants were reinoculated on the first leaf. This dual inoculation minimized the number of plants escaping infection and increased the reliability of the data. Test plants were held in a greenhouse at 25 C (day) and 20 C (night), with a photoperiod of 14 hours. Specimens of every plant were analyzed for isozymes, using the electrophoretic procedures previously described (5).

Plants of C. ecuadorensis reacted to ZYMV with a few scattered chlorotic spots on the inoculated leaves, but the virus failed to move systemically. Conversely, 'Buttercup' was severely stunted and exhibited prominent foliar yellow mosaic, accompanied by lamina malformation and reduction. F_1 plants also reacted with a persistent mosaic, but symptoms were generally less severe than those of the susceptible parent. In an F_2 population, three classes were discernible: 16 plants were systemically resistant, 19 were severely infected, and 44 showed symptoms of intermediate intensity (for 1:2:1, $P = .54$). Plants of the backcross to the susceptible parent segregated in the ratio of 25 severely infected to 23 moderately infected (for 1:1, $P = .78$). Thus, it appears that resistance to ZYMV in C. ecuadorensis is conferred by a single major gene, to which the symbol zym is assigned.

The varying degrees of symptom expression in heterozygous plants suggested the presence of modifying genes influencing zym. It has been possible through selection and selfing to develop breeding lines from the backcross to C. maxima that have good fertility and a high degree of resistance to ZYMV.

Leaves of C. ecuadorensis are marbled with a white mottle, whereas those of 'Buttercup' are uniform green. Plants of the F_1 and the backcross to C. maxima had leaves similar to those of 'Buttercup'. In an F_2 population, segregation was 14 mottled to 125 uniform green. Inheritance of the mottled pattern differed from the single monogenic dominant (M) segregation previously reported for Cucurbita (4). The intensity of

mottling on different F_2 plants varied considerably and ranged from prominent to barely perceptible, hence, penetrance may have been incomplete. Of 80 F_2 plants inoculated with ZYMV, 2 were mottled and ZYMV-resistant, 5 mottled and intermediate susceptible, and none were mottled and very susceptible. Thus, there was no indication of zym being closely linked to the foliar mottle.

Twenty isozyme loci have been identified as distinguishing C. ecuadorensis from C. maxima (5). During this study, no linkage was detected between zym and Aat-mb, Ast-m2, Aat-p2, Acp-1, Acp-2, Est-1, Gal-1, Gal-2, Gpi-c2, Lap-1, Mdh-c2, Mdh-m2, Pgm-p, Pgm-c2, Per-1, Per-3, Sod-1, Tpi-c2, or Tpi-p2. Relatively few plants were scored for the SKDH phenotype, but it was clear that Skdh-1 was not tightly linked to zym.

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Cucurbita Blossom Aroma and Diabrotica Rootworm Beetle Attraction

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Recent research has shown that many diabroticite species found on cucurbits, including the striped cucumber beetle (SCB) Acalymma vittata, the spotted cucumber beetle (SCR) D. undecimpunctata howardi, the western corn rootworm (WCR) D. virgifera virgifera, and the northern corn rootworm (NCR) D. barberi, are attracted to olfactory cues. The preponderance of described attractants are phenylpropanoids or closely related compounds e.g., eugenol, estragole, and p-methoxycinnamaldehyde (1,2). We examined the attraction of Diabrotica beetles to the odors of Cucurbita blossoms independent of visual and contact cues, such as color, size, shape, and cucurbitacin content. Thirty grams of blossoms from cv. Dickinson Field (C. moschata) and cv. Blue Hubbard (C. maxima) were placed inside paper cartons, the top covered with cheesecloth (preventing contact with the blossoms), and the outside of the trap coated with sticky material. As shown in Table 1, the attraction of WCR and SCB beetles to the isolated Blue Hubbard blossoms, demonstrates that blossom odor alone plays an important role in distribution of these rootworm beetles. The apparent lack of response from SCR adults is probably due to extremely low number of beetles present in the field.

Beetle counts (conducted on July 16, 1987) on blossoms of C. maxima and C. moschata from field plants provided corroborative results. Mean numbers of WCR, SCR, and SCB collected per blossoms (n=31) of C. maxima were 24.5, 0.6, and 2.7, respectively. In contrast mean beetle counts were 4.3, 0.1, and 3.7 on blossoms (n=23) of C. moschata.

Over 40 individual volatile chemicals have been isolated from Cucurbita maxima blossoms and circa 25 of these have been unequivocally identified (3,4). We evaluated the major odor components as attractants for adult Diabrotica spp. using cylindrical sticky traps baited with dental cotton wicks (5) containing from 0.01 mg to 200 mg of volatile compound. A summary of tests conducted during the summers of 1985-1987 are shown in Table 2. The effective attractants show a linear log-dosage response and the attractive compounds have been rated according to their limit of response (LR), i.e. the least amount of compound producing significant attraction over a 24 hour period vis-a-vis unbaited control traps. The results are based on the means of four replicate traps and were significantly different from the control traps at P=0.01 by Duncan's multiple range test.

The majority of the C. maxima blossom volatiles are unattractive when tested singularly. The green volatiles, especially trans-2-hexenol and cis-3-hexenol, are marginally attractive, as are the aromatic compounds, 1,2,4-trimethoxybenzene, phenylethanol, and phenylacetaldehyde for SCR (4). trans-Cinnamaldehyde is highly attractive to SCR and moderately attractive to WCR, but not appreciably attractive to NCS. trans-Cinnamyl alcohol is highly attractive to NCR, although only slightly attractive to SCR and WCR.

Indole is highly attractive to WCR (LR 1 mg), but not appreciably attractive to NCR and SCR (2). The terpenoid beta-ionone is highly attractive to WCR (LR 3 mg), but unattractive to NCR and SCR. Its isomer alpha-ionone is completely unattractive to all three Diabrotica spp. For comparison, the most effective volatile attractants yet identified for the respective species and their LR values are eugenol for NCR (LR 10 mg), estragole for WCR (LR 3 mg), and p-methoxycinnamaldehyde for WCR (LR 0.03 mg) (5).

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Table 1. Counts of western corn rootworm (WCR), southern corn rootworm (SCR), and spotted cucumber beetle on traps containing flower blossoms from an accession of Cucurbita maxima and Cucurbita moschata

Treatment	Trapping period	WCR	SCR	SCB
control	60 min.	6.8 + 7.5	0.5 + 0.6	1.8 + 0.5
<u>C. maxima</u>	60 min.	86.0 + 30.6	3.0 + 1.4	11.8 + 4.5
<u>C. moschata</u>	60 min.	11.3 + 6.2	0.5 + 1.5	2.8 + 1.5

Table 2. Activity of volatile compounds in field traps as attractants for the northern corn rootworm (NCR), southern corn rootworm (SCR), and western corn rootworm (WCR) beetles

<u>Volatile Compound</u>	<u>Attractant Rating* For</u>		
	<u>NCR</u>	<u>SCR</u>	<u>WCR</u>
<u>Green Volatiles</u>			
1-hexanol	0	0	0
1-hexanal	0	0	0
<u>trans-2-hexanol</u>	0	+1	+1
<u>cis-3-hexenol</u>	0	0	+1
<u>trans-2-hexenal</u>	0	0	0
<u>Aromatics</u>			
1,4-dimethoxybenzene	0	0	0
1,2,4-trimethoxybenzene	0	+1	+1
benzyl alcohol	0	0	0
benzaldehyde	0	0	0
phenylethanol	0	+1	0
phenylacetaldehyde	0	+2	0
<u>p-methoxybenzyl alcohol</u>	0	0	0
<u>p-methoxybenzaldehyde</u>	0	0	0
<u>Phenyl propanoids</u>			
indole	0	0	+4
cinnamyl alcohol	+3	+2	+1
cinnamaldehyde	0	+4	+3
<u>Terpenoids</u>			
alpha-ionone	0	0	0
beta-ionone	0	0	+4
nerolidol	0	0	0

LR= Limit of Response = 200 mg(0), 30-100 mg(+1), 10-30 mg(+2), 3-10 mg(+3), and 1-3 mg(+4).

Dry Cucurbitacin-containing Baits for Controlling Adult Western Corn Rootworms, Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae), in Field Corn

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The Diabrotica beetles and their rootworm larvae are probably the most costly insect pests of agriculture in the U.S. The combined attacks of the western corn rootworm (WCR), D. virgifera virgifera (LeConte), the northern corn rootworm (NCR), D. barberi (Smith & Lawrence), and the southern corn rootworm (SCR), D. undecimpunctata howardi (Barber), annually cost the U.S. farmer approximately one billion dollars. In attempts to control larval damage to the root systems of corn, soil insecticides are routinely applied to 50-60% of the corn acreage or as much as 30-40 million acres at costs now averaging \$15-20 per acre. In addition, aerial sprays are also applied to as much as 10 million acres to curb adult beetle damage to corn silks, at a cost of \$4-5 per acre. Heavy infestations of rootworms may cause an overall loss of 10-12% of corn production.

Understanding of host selection by the Diabroticite beetle lacked focus until the discovery that adults of such pests of Cucurbitaceae as the spotted cucumber beetle or SCR and the striped cucumber beetle were compulsive feeders on bitter Cucurbitaceae containing the tetracyclic triterpenoid cucurbitacins (1). The role of the cucurbitacins in promoting arrest and feeding stimulation by Diabroticite beetles was strengthened by Sharma and Hall (2). It is now apparent that the bitter cucurbitacins evolved as allomones to protect the Cucurbitaceae against herbivore attack (3,4,5). However, through coevolution the cucurbitacins have become kairomones for host selection by the Diabrotica. Adults of all the species investigated: D. balteata, NCR, D. cristata, SCR, D. u. undecimpunctata, and WCR are arrested by and feed compulsively on bitter cucurbits (6,7) and these species are responsive to nanogram quantities of pure cucurbitacins B, D, and E (4,5). This affinity for the cucurbitacin kairomones has been utilized to promote host-plant resistance by antixenosis (8) and in the development of insecticide impregnated baits and artificial trap crops for Diabroticite control (4). By devising hybrid cultivars of domestic Cucurbita with wild bitter Cucurbita (e.g., C. maxima x C. andreana and C. pepo x C. texana) high yields of cucurbitacin containing fruits have been produced that have been used successfully in the field control of SCR and WCR beetles (9). Dried, ground bitter Cucurbita fruit containing 0.1-0.3% cucurbitacins have been impregnated with 0.1% carbamate or organophosphate insecticides and when broadcast in corn or cucurbits at 11 to 33 kg per ha, have repeatedly given almost complete control of adult rootworms for periods up to a week (4,10). By incorporating a pesticide into a compulsive feeding stimulant the amount of overall environment contamination is drastically reduced.

In 1987, a large field trial was conducted to determine if cucurbitacin baits could reduce WCR beetle populations sufficiently to reduce egg laying and thus prevent larval damage to a subsequent corn crop. A continuous cornfield, 36 by 130 m, with a plant population of 64250 plants/ha at the Pell Farm of the University of Illinois at Urbana was selected for this study. The field was divided into 8 plots by cutting 2 row alleys between plots. The plots were each 0.06 ha in size (18 by 33 m). Four of the plots were located on the north and the other four on the south. The two plots on the north were treated with cucurbitacin bait and the other two were not treated and served as controls. The same arrangement was made with the plots located on the south portion of the field. The rate of bait used was 33 kg per ha (containing 0.1% isophenphos insecticide by weight). Dried Cucurbita baits were produced from TEX x PEP F₂ fruits grown in Arizona; the bait contained ca. 0.3% total cucurbitacins. Weighed samples of the baits were evenly broadcast by hand over the tops of the corn plants (so that a portion of the bait was retained by leaves and silks) on July 22 and August 3 (a second treatment was needed because adult populations of beetles began to exceed the economic threshold of one beetle per plant). Plant pollination was essentially complete by the initial treatment date. Prior to treating the plots, beetles were collected from the center of each plot to determine the sex ratio of the adults and the reproductive status of the females. Abundance of beetles (numbers per 30 or 40 plants in each plot) was also determined using the visual whole plant count method (11). To determine the effect of the bait on the mortality of the beetles, two metal quadrats (27 by 27 cm) were located on the ground in each of six rows within each plot. The fields were visited daily and the dead beetles in the quadrats were brought to the laboratory to determine sex ratio and mating status of the adults. Adult population density (visual plant count) was determined once every few days. At this time, some beetles were also collected and brought to the laboratory to be examined under a binocular microscope for sex ratio and mating status determination. After harvest on October 8, the plots were sampled for WCR eggs using a gasoline powered trencher (12). Four trenches (each 35 cm deep, 13 cm wide, and 175 cm long) were dug in each plot and two 0.5 l of soil were sampled from the pile of soil produced by digging each trench. These samples were processed using an egg extraction device (13).

Analysis of the data revealed that: (1) beetle abundance declined abruptly after treatment (Table 1; 84.8% versus 37.6% for the treated and untreated plots, respectively). During the period from July 27 to August 1, the number of beetles per plant in the treated and untreated plots increased and exceeded the economic threshold of one beetle per plant, therefore, baits were reapplied on August 3. Again, beetle densities declined sharply in the treated plots. Beetle abundance never exceeded the economic threshold after this application both in the treated and untreated plots probably due to the effectiveness of the bait, climatic conditions, and declining attractiveness of the crop, (2) the highest beetle mortality due to baiting was achieved one day after treatment (Table 2). The magnitude of beetle mortality generally decreased as time progressed. This reduction in mortality might have been due to the reduction in bait efficacy as it aged and to the reduction in beetle populations (resulting from the removal of a large part of the population by the baiting and reduced attractiveness

of the corn crop), (3) the sex ratio (male:female) of the beetles collected prior to and post-treatment was 0.62, whereas this ratio for the dead beetles collected from the quadrats in the baited plots was 1.56. This could indicate that the males were more susceptible to or were more likely to come in contact with the bait than the females, (4) ovarian development of the beetles collected from the untreated plots was higher (reproductively more mature) than those collected from the baited plots for the first 10 days after treatment during which time the bait had its highest efficacy. This suggests that the females in the untreated plots had the opportunity to remain alive and develop further, whereas the existing females in the treated plots were killed by the bait. Ovarian development for the beetles collected from treated and untreated plots became similar after this 10 day period, (5) although the baited plots (mean of 0.19 eggs per 0.5 l of soil) contained fewer WCR eggs than the control plots (mean of 0.25 eggs per 0.5 l of soil), the difference was not significant at the 5% level (t-test). Due to the great variability investigators often encounter with sampling rootworm eggs, root damage ratings of corn grown in the baited and control plots will be examined in the summer of 1988.

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Table 1. Average number of beetles per plant in treated and control plots and percent beetle reduction between two consecutive sampling dates (Pell Farm, Urbana, IL 1987)

Date	No. plants	Bait	Check	% reduction	
				Bait	Check
7/22	160	3.49	3.56	84.8	37.6
7/24	120	0.53	2.22	3.8	41.0
7/27	120	0.51	1.31		
8/1	120	1.32	1.47	87.9	42.2
8/4	120	0.16	0.85	12.5	61.1
8/7	120	0.14	0.33	57.1	66.7
8/11	120	0.06	0.11		
8/17	120	0.12	0.12	100.0	100.0
8/31	120	0.00	0.00		

Table 2. Number of dead male and female beetles collected in quadrats (n = 48) from treated and control plots (Pell Farm, Urbana, IL 1987)

Date	Bait		Control	
	male	female	male	female
7/23	262	113	0	0
7/24	164	119	0	0
7/25	113	96	2	3
7/27	76	60	0	0
7/29	29	14	1	0
8/4	81	52	0	0
8/5	25	23	0	0
8/6	21	21	0	0
8/7	38	27	0	0
8/8	35	25	0	0
8/10	10	7	0	0
8/11	5	5	0	1
8/13	3	3	0	0
8/14	5	0	0	0
8/17	0	0	0	0
Total	867	555	3	4

Taxonomic Rank and Rarity of Cucurbita okeechobeensis

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Cucurbita okeechobeensis (Small) L.H. Bailey is, as the name implies, a gourd first described as endemic to the muck-laden shores and hammocks which once surrounded Lake Okeechobee. It was originally reported to be locally abundant (14), but most of this region has since been cleared for prime farmland development. The gourd though recently relocated after 7 years (12) may now be limited to remnant habitats on islands in the lake. The only evidence that this restricted range may have once been more extensive in Florida is from a journey made by William Bartram up the St. Johns River in 1774. He observed along the river between Lake George and Lake Dexter, around 150 miles north of Lake Okeechobee, "the wild squash climbing over lofty limbs of the trees; their yellow fruit somewhat of the size and figure of a large orange, pendant from the extremities of the limbs over the water" (7). He named the gourd C. peregrina, but gave no diagnosis; therefore, this name is a nomen nudum. Merrill (10) states that Bartram's gourd "seems to be the same as C. okeechobeensis". While the Okeechobee gourd is the only known non-cultivated spontaneous gourd in Florida today, Decker and Newsom (4) suggest, based on a numerical analysis of archaeological seeds, that the spontaneous gourd, C. texana (Scheele) Gray (soon to be treated as an infraspecific taxon of C. pepo) was present in the St. Johns River watershed from 500 B.C. up to Bartram's time. Thus Bartram's gourd was not necessarily C. okeechobeensis and the latter, sensu stricto, appears to have been limited historically to the Lake Okeechobee region.

Cucurbita okeechobeensis has not been given legal protection through the U.S. Endangered Species Act partly because of the uncertainty of its relationships with the cross compatible species, C. martinezii Bailey and C. lundelliana Bailey, two fairly common mesic gourds of Mesoamerica. Robinson and Puchalski (13) suggested C. okeechobeensis and C. martinezii should be considered synonymous (with the former having nomenclatural priority) after comparing their morphology, crossability, responses to disease inoculation, and preliminary isozyme results. Filov (6) listed C. martinezii as a variety of C. okeechobeensis but did not cite the basionym and therefore failed to validly publish this taxonomic change. It is unlikely that legal protection can be considered for the Okeechobee gourd if it is merely synonymous with C. martinezii, without any distinct taxonomic status. Therefore, we attempted to review the literature more comprehensively and further characterize the genetic variation and relationships between these taxa using isozyme analysis.

Considerable differences have been reported between the three compatible taxa in their seed oil and amino acid compositions (3,5) and cucurbitacin content and resistance to insect pests (11,8,9). However these results are not convincing since only one or a few accessions were analyzed per taxon; varying genotype, environmental interactions, and laboratory error need to be statistically addressed.

Using starch gel electrophoresis, Robinson and Puchalski (13) reported that C. okeechobeensis and C. martinezii were identical in their electrophoretic profiles and distinct from the other Cucurbita species. The senior author tested an additional ten isozyme systems, different from the three used by Robinson and Puchalski (13), in two accessions of C. okeechobeensis (of Florida) and ten accessions of C. martinezii (from the Mexican states of Veracruz and Puebla) and three accessions of C. lundelliana. Six to 12 plants were sampled per accession. These were compared with other species of Cucurbita.

A much more complex genetic situation than that reported by Robinson and Puchalski (13) was evident. While C. okeechobeensis showed no allozyme, or allelic, variation in any of the systems tested, presumably due at least in part to founder effects and a small sample size, C. martinezii showed considerable variation within populations including a few heterozygous individuals. Certain of the zymograms of both taxa were not distinguishable from those found in other species, particularly in the closely allied species, C. lundelliana. All three of these taxa shared identical fixed allozymes in two of the enzyme systems, triosephosphate isomerase and isocitrate dehydrogenase, which includes a minimum of six loci, yet were unique from other mesic cucurbit species.

However, for the enzyme phosphoglucosomerase (PGI), a relatively conservative three or four loci system in Cucurbita, there was no variation within the populations and no difference between C. martinezii and C. lundelliana. But significantly, two unique fixed PGI allozymes occur in C. okeechobeensis.

We therefore provisionally propose that there is one gourd species with a cream-colored corolla, C. okeechobeensis (Small) Bailey, but recognize the following two eco-geographic subspecies¹:

- (i) Cucurbita okeechobeensis (J.K. Small) L.H. Bailey ssp. okeechobeensis; Gentes Herbarum 2:179. 1930; =Pepo okeechobeensis J.K. Small, J. N. Y. Bot. Gard. 31:12. 1930; endemic to Annona swamps in Palm Beach County, Florida; with divergence in PGI allozymes, and perhaps a high cucurbitacin content and low seed oil content. Endangered.
- (ii) Cucurbita okeechobeensis (J.K. Small) L.H. Bailey ssp. to encompass what is now known as C. martinezii L.H. Bailey; widespread in eastern Mexico from sea level to about 1500 m. elevation in Veracruz and a few adjacent states including southern Tamaulipas, eastern San Luis Potosí and Puebla, and northern Oaxaca and Chiapas; almost always in the vicinity of streams, and often regarded as a weed in coffee and citrus plantations; with moderate cucurbitacin content; moderate oil seed content; and PGI allozymes undifferentiated from C. lundelliana. Locally common.

C. lundelliana though cross-compatible, appears to represent a sister species of Cucurbita okeechobeensis, endemic to calcareous soils of the Yucatan Peninsula with an orange corolla, a less lignified exocarp, and a different seed morphology including a crenulated seed margin. Both species have a high tolerance for moist conditions and saturated soils.

The divergence of the two subspecies may be related to (a) natural oceanic dispersal of intact fruits of the Florida subspecies from Mexico; (b) a splitting of a more continuous distribution since the post-glacial contraction of the continental shelf, with a relic isolate remaining in Florida; (c) prehistoric cultural diffusion by Carib or Arawak peoples to the Calusa of the Everglades; or (d) companion diffusion with C. moschata (Lam.) Poir. 'Seminole Pumpkin', with which it persisted around Indian villages (14). While the Seminole pumpkin was an important local crop, the unrelated Okeechobee gourd may have been used like the fruit of C. martinezii was, at least until the recent past, as a ball or rattle, a utensil such as a small ceremonial cup, or for its detergent quality (first author's personal observation).

Clearly these plants deserve more detailed biosystematic and genetic studies, for they have already proven themselves valuable to the breeder. Their resistance to cucumber mosaic virus and powdery mildew has practical importance: seed companies will soon be introducing cultivars from germplasm developed at Cornell University via the 3-way cross (C. moschata x C. okeechobeensis ssp. martinezii) x C. pepo (R.W. Robinson, pers. comm.). It would be short-sighted, to say the least, to let the few remaining spontaneous populations around Lake Okeechobee become extinct. At the subspecific rank, the Okeechobee gourd will remain eligible for federal protection in situ.

¹Formal taxonomic changes will be made in a reviewed botanical journal.

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Germplasm Resources of Cucurbita from Spain

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This paper reports on the recent collection of 254 accessions of Cucurbita species in Spain. Partial support for this work has been provided by the I.P.B.G.R./F.A.O. and the Exema Diputacion Provincial of Valencia.

Cucurbita ficifolia

Fifteen accessions of C. ficifolia have been collected in Aragon, Andalucía, Extremadura and Valencia. These accessions are used exclusively for fresh market production. They have been grown with irrigation at altitudes ranging from 12 to 823 m. Fruit of these accessions are predominately large, round and light green colored with dark stripes. Among the accessions there exists substantial variation in fruit size and shape.

Cucurbita maxima

We have collected 37 accessions of C. maxima from Aragon, Andalucía, Cataluña, Castilla La-Mancha and Valencia. With only four exceptions, all these accessions were collected from irrigated plots between 96 and 1303 m. Round and flat fruit with marked ribbing were common. One large fruited accession displayed uniform white color. This accession is primarily cultivated for baked consumption or occasionally as animal forage.

Cucurbita pepo

The largest number of collections, (192) were accessions of Cucurbita pepo. The majority of these were collected in Valencia and the rest in Aragón, Andalucía, Asturias, Cataluña, Castilla La-Mancha, Extremadura and Santander. Except for two of these accessions, all are grown with irrigation at altitudes between 8 and 320 m. The fruit from this group are commonly cooked for the preparation of sausages, black pudding and doughnuts. Some accessions are also used for ornamentation. Fruit shape, color and size varied significantly among the accessions. Two accessions are remarkable for their weights; being either white and yellow with green spots. Ten accessions collected in Ademuz (Valencia) used for ornamental purposes, displayed small fruit size with wide variation in color, shape and warts.

Lagenaria siceraria

Of the 10 accessions collected, 9 were from Valencia and Castilla La-Mancha. Altitudes where the collections were made varied from 13 to 670 m. The gourds of Lagenaria siceraria are generally used as vessels to store and transport water or wine. Large variability in size and neck thickness was found among the accessions collected.

Acknowledgements: We are extremely grateful to the Servicio de Extension Agraria and to all those who have collected vegetable crop germplasm: G. Anastasio, C. Cortés, M. L. Gómez-Guillamón, C. Ferrando, R. V. Molina, M. C. Ayuso, M. S. Catalá, A. Alonso-Allende.

Pathogenicity of Erysiphe cichoracearum to cucurbits

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Powdery mildew is a devastating disease of cucurbits throughout the world. The two largely reported causal agents are Sphaerotheca fuliginea (Schlecht. ex Fr.) Poll. and Erysiphe cichoraceatum DC ex. Merat (5,8). The distribution of different Erysiphaceae (including Leveillula taurica (Lev.) Arn.) on cucurbits in the world was reviewed by Khan (5). He stated that S. fuliginea (=Sf) and E. cichoracearum (=Ec) may singly or together attack Cucumis, Cucurbita and Citrullus. Other workers (see below) reported on cross-susceptibility of cucurbits and species of other families to powdery mildews. Molot and Lecoq (9) reported that both Sf and Ec are economically important on cucurbits in France. Ec from tobacco, Aster sp. and Senecio vulgaris, but not from Lactuca serriola, was pathogenic to Cucurbita pepo (Diamant). Cucumis sativus (Marketer) became infected with Ec from Aster sp. or L. serriola but not from tobacco, whereas C. melo (Verndrais) was resistant to all Ec's. Molot et al. (10) further showed that Ec from other cucurbits or tobacco was pathogenic to squash and cucumber. In Hungary Ec and Sf were found in the field on cucumber, squash, melon and watermelon (12). Ec was able to overwinter on Aster dumosus (11). In Germany all 12 greenhouse cucumber cultivars were highly susceptible to both Ec and Sf (19). Lebeda, in Yugoslavia, failed to transfer Ec from L. serriola to cucumber (7). Stone (20) suggested that Sonchus aspen (Compositae) is a very probable source of reinfection for cucurbits with Ec in the UK. In New Zealand Sf and Ec can each attack Cucurbitaceae, Compositae and Solanaceae (1). In Israel Sf was recognized as the causal agent of powdery mildew in cucurbits (2,3,13,16,18) although earlier reports claimed for Ec as a causal agent (14,17). Eshed and Whal (3) found that powdery mildew from Hibiscus esculentum, Xanthium stramanium and Verbena hybrida was infective to melons. Khan (5) observed both Ec and Sf on cucurbits in India. While Sf attacked most of the cucurbits, Ec was confined to Benincasa hispida and Coccinia cordifolia in the field but produced perithecia on muskmelon in the greenhouse. In the USA since 1900 up to 1963 powdery mildew in cucurbits was assigned to Ec (5). In 1979 Sf was found on C. pepo (6). Kontaxis (6) suggested that the causal agent of powdery mildew in California and possibly in all USA is Sf in spite of the fact that perithecia of Ec were reported on cucurbits before (15). Indeed, perithecia of Sf were reported on various cucurbits in North Carolina in 1986 (4). McCreight et al. (8) observed Sf only on muskmelons in both the USA and France in 1964-67 and 1981-86. In Canada, Ec was reported on cucurbits (5), but cleistothecia of Sf were found in 1983 in Ontario on glasshouse-grown cucumbers (4a).

The present study was aimed at elucidating the pathogenicity of Sf from muskmelon and Ec from tobacco to various cucurbits. Plants were grown in the greenhouse to the 2-3 true leaf stage and inoculated with powdery mildew in growth chambers at 23 C. Results are given in Table 1. They show that Ec was highly infective to Cucurbita pepo, Cucurbita maxima and Lagenaria vulgaris, moderately infective to Citrullus lanatus and Citrullus colocynthis, but not infective to Cucumis sativus or Cucumis melo var. reticulatus. C. melo var. makuwa and C. melo var. acidulus were highly susceptible. Ec from Cichorium pumillum and L. serriola was infective on tobacco and C. pepo (yellow zucchini cv. Goldy) but Ec from Senecio vernalis, S. vulgaris, Erigeron crispus and

watermelon
Crepis sp. was not infective to C. pepo (Goldy) or tobacco. Susceptibility to Sf varied according to plant species, cultivar and fungal race (Table 1). All cultivars of C. lanatus were resistant to Sf race 1 (although became slightly infected on hypocotyls and stems) and susceptible to race 2. All cultivars of C. pepo and C. maxima were susceptible to both races 1 and 2. In Cucumis species reaction to Sf ranged from resistance to susceptibility to either race.

We concluded that Nicotiana tabacum, C. pumillum and L. serriola are potential sources for infection of cucurbits with Ec.

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Table 1. Infectivity of Erysiphe cichoracearum from tobacco (cv. KY-16) to cucurbits.

<u>Species</u>	<u>cultivar</u>	<u>origin</u>	<u>Disease severity</u>		
			<u>Sf race 1</u>	<u>Sf race 2</u>	<u>Ec</u>
<u>Citrullus lanatus</u>	Adom	Israel	-	++	+
	Karmit	Israel	-	++	+
	Talmor	Israel	-	++	+
	Hazera - 2	Israel	-	+++	+
	Dafna	Israel	-	++	-
	Malali	Israel	-	+	+
	Charleston Gray	USA	-	++	-
	Shin-Yamato	Japan	-	-	-

			<u>SF 1</u>	<u>SF-2</u>	<u>EC</u>
<u>Citrullus colocynthis</u>		Israel	-	-	+
<u>Curcubita pepo</u>	Goldy	Israel	+++	+++	+++
	Lavan	Israel	+++	+++	++
	Maayan	Israel	+++	+	+
	Bareketh	Israel	+++	+++	++
	Beiruti	Israel	+++	+++	+
<u>Cucumis sativus</u>	Dalila	Israel	+++	++	-
	Poinsett-76	USA	+	+	-
	Marketer	USA			-
	Aonagao	Japan	++		-
	Sagami-hanjiro	Japan	++		-
	Howay GOGIO	Japan	+		-
	Jomaki	Japan	+		-
<u>Lagenaria vulgaris</u>	-	Israel	+++	++	-
<u>Lagenaria sp.</u>	Williams	USA			+++
<u>Lagenaria siceraria</u>	Oomatu-yungao	Japan			-
<u>Benincasa hispida</u>	Naga-tougan	Japan			+++
<u>Cucurbita maxima</u>		Israel	+++	+++	+++
<u>Luffa cylindrica</u>		Israel		+	-
<u>Luffa acutangula</u>		USA	-	-	-
<u>Cucumis melo</u>					
var. makuwa	Kinpyo	Japan			+++
var. conomon	Honen-ao	Japan	+++		-
var. acidulas	Kinpyo	Japan			+++
var. reticulatus	Sunrise	Japan			-
	Ananas				-
	Yokneam	Israel	+++	+++	-
	Ein-dor	Israel	-	++	-
	Galia	Israel	+	++	-
	Charantais-T	France	+++	++	-
	PMR-45	USA	-	++	-
	PMR-5	USA	-	-	-
	PMR-6	USA	-	-	-
	PI124111	USA	-	-	-
	PI124112	USA	-	-	-
	Seminole	USA	-	+	-

- no disease; + to +++ increasing amount of disease.

Salinity Responses among Wild Cucurbits.

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Melon is a vegetable crop usually grown in regions with potential salinity or drought problems due to its moderate salt tolerance (Maas and Hoffman, 1977). Nevertheless important losses in productivity are undergone year by year. The intraspecific variation has been the source of salt tolerance exploited until now (Shannon et al, 1984; Anastasio et al., 1987). Experimental data support the idea that only a partial degree of salinity tolerance exists in the genetic pool of this species. Therefore the search of new sources of tolerance beyond the species limit, now that the interspecific hybridization is more feasible through biotechnological manipulation, could be a useful tool for the breeding of melon.

Nine accessions of wild Cucurbitaceae: Cucumis zeyheri, C. myriocarpus, C. metuliferus, C. ficifolius L-1 and L-4, C. anguria L-1 and L-2, Cucurbita martinezii and Citrullus colocynthis were studied under two salt conditions of 5 (ST-5) and 35 dS/m (ST-35) and a control experiment. Culture ran from July to December 1987. Trials were in hydroponics with 10 x 0.5 x 0.4 mts. plastic beds and sand as a substrate in a 400 m² plastic covered shelter. Irrigation solutions were built up by adding to the Hoagland solution amounts of marine salt to reach the mentioned EC. Eight plants per accession were allocated in each treatment. All treatments were irrigated with fresh water after transplanting. Saline treatments started two weeks after the transplant.

Every week all plants were surveyed for possible symptoms of salt stress and their state of health was individually listed. At the end of the cultivation period fruits were harvested, counted and weighed. The whole plants were also harvested and dried and the shoots and roots weighed separately on a per plant basis.

Salinity strongly reduces the dry matter in the plants both in the shoot and in the roots (Table 1a). The greater the salinity level applied the lesser the development rate reached by the plants. No plant set any fruit in the ST-35 (Table 1b) although blossoming and first stages of fruit set were noticed in Cucumis myriocarpus, C. ficifolius L-4 and C. zeyheri.

A plant of C. zeyheri displayed an unusual growth in the ST-5 (Table 1a) together with a different pattern of behaviour in contrast to the other plants of the accession. This plant was removed from the data monitored in Table 1a.

Citrullus colocynthis, an accession from the Canary Islands where it grows in environments with salinity and drought problems, had the smallest reduction in dry matter, however it reached a very poor development both in the control and in the ST-5, dying in the ST-35. We think that the culture condition was possibly not the most suitable for a species adapted to drought and therefore the cause of the lack of growth.

Table 1. Relative shoot and root dry weight and fruit yield based upon performance under salt treatment (a)ST 5 dS/m, b)ST 35 dS/m) expressed as the percentage of their growth under nonsaline conditions.

Species	Shoot d.wt.	Root d.wt.	Fruit no.	Fruit avg.wt.
a) ST-5				
<u>Cucumis zeyheri</u>	0.36	0.65	1.94	0.82
<u>Cucumis metuliferus</u>	0.21	0.22	-	-
<u>Cucumis myriocarpus</u>	0.41	0.73	0.48	0.71
<u>Cucumis ficifolius</u> L-1	0.45	0.96	3.77	1.81
<u>Cucumis ficifolius</u> L-4	0.35	0.41	0.41	0.54
<u>Cucumis anguria</u> L-1	0.54	0.92	0.05	0.07
<u>Cucumis anguria</u> L-2	0.38	0.62	0.19	0.22
<u>Cucurbita martinezii</u>	0.18	0.24	-	-
<u>Citrullus colocynthis</u>	0.57	0.57	-	-
b) ST-35				
<u>Cucumis zeyheri</u>	0.12	0.29	-	-
<u>Cucumis metuliferus</u>	0.03	0.28	-	-
<u>Cucumis myriocarpus</u>	0.13	0.66	-	-
<u>Cucumis ficifolius</u> L-1	0.01	0.08	-	-
<u>Cucumis ficifolius</u> L-4	0.14	0.08	-	-
<u>Cucumis anguria</u> L-1	0.03	0.75	-	-
<u>Cucumis anguria</u> L-2	0.10	0.41	-	-
<u>Cucurbita martinezii</u>	0.05	0.39	-	-
<u>Citrullus colocynthis</u>	-	-	-	-

Cucumis ficifolius L-1 and C. anguria L-1 coped fairly well at low salinity ST-5 but collapsed in ST-35.

There has not been found any real source of salt tolerance among the wild species of Cucurbits tested in the trial, although Citrullus colocynthis is a promising species if tested in another environment closer to its natural conditions.

Acknowledgment: This work was financially supported by the research projects CA-84-0917 and PR-83-2971 held by the CAICYT. We thank the helpful collaboration of M. Angeles Sanchis in preparing this paper.

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1987 Germplasm Collections of Cultivated Cucurbits from China and Hong Kong

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During the last week of May and the first week of June 1987, we visited five cities (Beijing, Xi'an, Guilin, Yangshou, and Guangzhou) in the People's Republic of China and Hong Kong. As part of our trip, we gathered data and germplasm from a variety of cultivated cucurbits (Cucurbitaceae). We were unable to make germplasm collections of many common cucurbits, particularly bottle gourds (Lagenaria siceraria (Mol.) Standl.) and loofahs (Luffa acutangula (L.) Roxb. and L. cylindrica (L.) M. J. Roem.), because the fruits were sold in the markets only in the immature state. Even from mature fruits, the amount of seeds collected was small. Therefore, we are currently increasing this germplasm with controlled pollinations. We will be sending the increased seed to the USDA-ARS over the next few years. Some original samples of viable, mature seeds have already been sent to USDA-ARS, Germplasm Introduction Office, Building 001, Room 322, BARC-West, Beltsville, Maryland 20705. These include collection numbers 367, 368, 369, 370, 372, 380, and 382 (Table 1).

Species collected in China and Hong Kong and their codes in Table 1 are: Benincasa hispida (Thunb.) Cogn. (BEN), Citrullus lanatus (Thunb.) Matsum. and Nakai (CIT), Cucumis melo L. (CUC-M), Cucumis sativus L. (CUC-S), Cucurbita maxima Duch. ex Lam. (CUK-A), Cucurbita mixta Pang. (CUK-I), Cucurbita moschata (Duch. ex Lam.) Duch. ex Poir. (CUK-M), Cucurbita pepo L. (CUK-P), Cyclanthera pedata (L.) Schrad. (CYC), and Momordica charantia L. (MOM).

Table 1. Germplasm collections of the Cucurbitaceae from China and Hong Kong.

Collection	Species	Locality	Observations
DDW 367	BEN	Hong Kong	seeds bought from large jar
DDW 377	BEN	Guangzhou	fruit oblong, ca. 30 cm x 15 cm
DDW 365	CIT	Hong Kong	seeds bought from large bin, seeds large and red
DDW 368	CIT	Beijing	rind green, flesh pale pink, seeds small and brown
DDW 369	CIT	Beijing	rind green, flesh pale pink, seeds small and brown
DDW 371	CIT	Xi'an	fruit ca. 18 cm diameter, rind dark green, flesh red, seeds large and black
DDW 370	CUC-M	Xi'an	fruit ca. 12 cm diameter, rind pale green, flesh pale yellow
DDW 372	CUC-M	Guilin	fruit ca. 9 cm diameter, rind pale green, flesh white
DDW 380	CUC-M	Guangzhou	fruit ca. 11 cm diameter, rind light brown and netted, flesh orange
DDW 382	CUC-S	Hong Kong	fruit oblong, ca. 18 cm x 7 cm, rind yellowish brown and cracked, flesh white and juicy
DDW 369.5	CUK	Beijing	bag of roasted seeds bought, represented were CUK-A, CUK-I, CUK-M, and CUK-P
DDW 374	CUK-M	Yangshuo	fruit bell-shaped, rind dark green turning orange, flesh pale yellow, seeds immature
DDW 376	CUK-M	Guangzhou	fruit large and squarish, rind green and orange spotted, flesh orange
DDW 383	CUK-M	Hong Kong	fruit bell-shaped, rind green turning white, flesh deep yellow, seeds immature
DDW 366	CUK-P	Hong Kong	seeds bought from large jar, salted
DDW 379	CYC	Guangzhou	fruit elliptic, ca. 17 cm long, rind green and white spotted, seeds with wavy margins, immature
DDW 363	MOM	Hong Kong	rind green, seeds immature?
DDW 378	MOM	Guangzhou	rind green, seeds light brown

STOCKS AND GERMLASM DESIRED OR FOR EXCHANGE

Dr. Billy B. Rhodes, Clemson University, Edisto Research and Education Center, Blackville, South Carolina 29817, has the following Citrullus lanatus seedstocks available in small quantities:

<u>Gene symbol</u>	<u>Character</u>	<u>Reference</u>
<u>dg</u>	<u>delayed green.</u>	CGC 10:106
<u>I-dg</u>	<u>Inhibitor-delayed green.</u>	CGC 10:107
<u>Sp</u>	<u>Spotted.</u>	CGC 10:106
<u>ts</u>	<u>tomato seed.</u> Inheritance not yet determined. Seeds are less than 4 mm in length.	CGC 3:38
<u>gms</u>	<u>glabrous male sterile.</u>	CGC 10:107

REQUEST FROM THE CUCURBIT GENETICS COOPERATIVE GENE CURATORS

CGC has appointed Curators for the four major cultivated groups: cucumber, muskmelon, watermelon, and Cucurbita spp. A curator for the Other Genera category is needed. Anyone wishing to take on this responsibility should contact the Chairman. Curators are responsible for collecting, maintaining and distributing upon request stocks of the known marker genes.

CGC members are requested to forward samples of currently held gene stocks to the respective curator:

- Cucumber** ----- Todd C. Wehner, Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27695-7609.
- Muskmelon** ----- Edward L. Cox, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, Texas 78596-8399.
- Watermelon** ----- Billy B. Rhodes, Clemson University, Edisto Research and Education Center, Blackville, South Carolina 29817.
- Cucurbita spp.** --- Richard W. Robinson, New York Agricultural Experiment Station, Department of Horticultural Sciences, Hedrick Hall, Geneva, New York 14456.

Gene List for Cucurbita spp.

Lists of known genes for the Cucurbitaceae have been published previously in three installments (4,5,25), and complete, updated gene lists have recently been published for cucumber (CGC 8:86-96), muskmelon (CGC 9:111-120) and watermelon (CGC 10:106-110). In the interest of updating and collecting information on the genetics of Cucurbita in one place, the following is a complete list of known genes. We hope to continue this practice and publish a complete list for the Cucurbita spp. every four years.

<u>Gene Symbol</u> <u>Preferred</u> <u>Synonym</u>	<u>Character</u>	<u>Species</u>	<u>Reference</u>
<u>a</u>	<u>androecious</u> . Produces only male flowers	<u>pepo</u>	19
<u>Aat-mb</u>	<u>Aspartate aminotransferase - microbody isozyme</u>	<u>maxima x ecuadorensis</u>	42
<u>Aat-m</u> (<u>Aat-m1</u>)	<u>Aspartate aminotransferase mitochondria isozyme-1</u>	<u>maxima x ecuadorensis</u>	42
<u>Aat-m2</u>	<u>Aspartate aminotransferase mitochondria isozyme-2</u>	<u>maxima x ecuadorensis</u>	42
<u>Aat-p2</u>	<u>Aspartate aminotransferase plastid isozyme-2</u>	<u>maxima x ecuadorensis</u>	42
<u>Acp</u> (<u>Acp-1</u>)	<u>Acid phosphatase - isozyme-1</u>	<u>maxima x ecuadorensis</u>	42
<u>Acp-2</u>	<u>Acid phosphatase - isozyme-2</u>	<u>maxima x ecuadorensis</u>	42
<u>Aldo-p</u>	<u>Aldolase - plastid isozyme</u>	<u>maxima x ecuadorensis</u>	41
<u>B</u>	<u>Bicolor fruit</u> . Precocious yellow fruit pigmentation; modified by <u>Ses-B</u>	<u>pepo</u>	34,35
<u>Bi</u>	<u>Bitter fruit</u> . High cucurbitacin content in fruit	<u>pepo</u>	2,15,43
<u>bl</u>	<u>blue fruit color</u> . Incompletely recessive to green	<u>maxima</u>	16
<u>Bu</u>	<u>Bush habit</u> . Short internodes; dominant to vine habit in young plant stage but recessive at maturity	<u>pepo maxima</u>	7,33,38

<u>Gene Symbol</u> <u>Preferred Synonym</u>	<u>Character</u>	<u>Species</u>	<u>Reference</u>
<u>cr</u>	<u>cream corolla</u> . Cream to nearly white petals for <u>cr/cr</u> , yellow for <u>cr/+</u> , and orange for <u>+/+</u> ; derived from <u>C. okeechobeensis</u>	<u>moschata</u>	27
<u>cu</u>	<u>cucurbitacin content</u> . Reduced cucurbitacin content	<u>pepo</u>	32
<u>D</u>	<u>Dark green stem</u> . Dominant to light green stem	<u>pepo</u>	14
<u>Di</u>	<u>Disc fruit shape</u> . Dominant to spherical	<u>pepo</u>	37
<u>Est</u>	<u>Esterase isozyme</u>	<u>maxima x ecuadorensis</u>	40
<u>Fr</u>	<u>Fruit fly (Dacus cucurbitae) resistance</u>	<u>maxima</u>	22
<u>G</u>	(a,m) <u>Gynoecious sex expression</u>	<u>foetidissima</u>	8,13
<u>Gal</u>	(Gal-1) <u>β-Galactosidase isozyme-1</u>	<u>maxima x ecuadorensis</u>	42
<u>Gal-2</u>	<u>β-Galactosidase isozyme-2</u>	<u>maxima x ecuadorensis</u>	42
<u>Gb</u>	<u>Green band on inner side of base of petal</u> ; dominant to no band	<u>pepo</u>	9
<u>gc</u>	<u>green corolla</u> . Green, leaf-like petals	<u>pepo</u>	39
<u>Got</u>	(Got-1) <u>Glutamine-oxaloacetate isozyme-1</u>	<u>maxima x ecuadorensis</u>	42
<u>Got-2</u>	<u>Glutamine-oxaloacetate isozyme-2</u>	<u>maxima x ecuadorensis</u>	42
<u>Gpi-c</u>	(Gpi-c1) <u>Glucosephosphate isomerase cytosolic isozyme-1</u>	<u>maxima x ecuadorensis</u>	42
<u>Gpi-c2</u>	<u>Glucosephosphate isomerase cytosolic isozyme-2</u>	<u>maxima x ecuadorensis</u>	42
<u>Gr</u>	(G) <u>Green rind</u> . Dominant to buff skin of mature fruit	<u>moschata</u>	26
<u>I-T</u>	<u>Inhibitor of the T gene for trifluralin resistance</u>	<u>moschata</u>	1

<u>Gene Symbol</u> <u>Preferred</u> <u>Synonym</u>	<u>Character</u>	<u>Species</u>	<u>Reference</u>
<u>Idh</u> (<u>Idh-1</u>)	<u>Isocitrate dehydrogenase</u> <u>isozyme-1</u>	<u>pepo</u>	6,18
<u>Idh-2</u>	<u>Isocitrate dehydrogenase</u> <u>isozyme-2</u>	<u>pepo</u>	6,18
<u>Idh-3</u>	<u>Isocitrate dehydrogenase</u> <u>isozyme-3</u>	<u>pepo</u>	6,18
<u>l</u> (<u>c</u>)	<u>light fruit color. Uniform light</u> <u>intensity of fruit pigmentation;</u> <u>modified by <u>St</u></u>	<u>pepo</u>	14,23,34
<u>l-2</u>	<u>light pigmentation of fruit - 2</u>	<u>pepo</u>	23
<u>Lap</u>	<u>Leucine aminopeptidase isozyme</u>	<u>maxima x</u> <u>ecuadorensis</u>	40
<u>lo</u> (<u>l</u>)	<u>lobed leaves</u>	<u>maxima</u>	10
<u>lt</u>	<u>leafy tendril. Tendrils with</u> <u>laminae</u>	<u>pepo</u>	29
<u>ly</u>	<u>light yellow corolla. Recessive</u> <u>orange yellow</u>	<u>pepo</u>	29
<u>M</u>	<u>Mottled leaves. Silver gray</u> <u>areas in axils of leaf veins</u>	<u>pepo</u> <u>maxima</u> <u>moschata</u>	3,28,31
<u>Mdh-m</u> (<u>Mdh-m1</u>)	<u>Malate dehydrogenase</u> <u>mitochondria isozyme-1</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>Mdh-m2</u>	<u>Malate dehydrogenase</u> <u>mitochondria isozyme-2</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>Mdh-c2</u>	<u>Malate dehydrogenase</u> <u>cytosolic isozyme-2</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>ms-1</u> (<u>ms₁</u>)	<u>male sterile-1. Male flowers</u> <u>abort before anthesis</u>	<u>pepo</u>	12
<u>ms-2</u> (<u>ms₂</u>)	<u>male sterile-2. Male flowers</u> <u>abort</u>	<u>pepo</u>	12
<u>n</u>	<u>naked seeds. Lacking a lignified</u> <u>seed coat</u>	<u>pepo</u>	15,30
<u>Per</u> (<u>Per-1</u>)	<u>Peroxidase isozyme-1</u>	<u>maxima x</u> <u>ecuadorensis</u>	42

<u>Gene Symbol</u> <u>Preferred Synonym</u>	<u>Character</u>	<u>Species</u>	<u>Reference</u>
<u>Per-3</u>	<u>Peroxidase isozyme-3</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>Pgi</u> (<u>Pgi-1</u>)	<u>Phosphoglucase isomerase isozyme-1</u>	<u>pepo</u>	6,18
<u>Pgi-2</u>	<u>Phosphoglucase isomerase isozyme-2</u>	<u>pepo</u>	6,18
<u>Pgi-3</u>	<u>Phosphoglucase isomerase isozyme-3</u>	<u>pepo</u>	6,18
<u>Pgm-c2</u>	<u>Phosphoglucomutase cytosolic</u> <u>isozyme-2</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>Pgm-p</u>	<u>Phosphoglucomutase plastid</u> <u>isozyme</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>Pm</u>	<u>Powdery mildew-resistance.</u> <u>Resistance to <u>Sphaerotheca</u></u> <u><u>fuliginea</u></u>	<u>lundelliana</u>	24
<u>r</u>	<u>recessive white. White fruit</u> <u>color</u>	<u>pepo</u>	14
<u>Rd</u>	<u>Red skin. Red external fruit</u> <u>color; dominant to green,</u> <u>white, yellow and gray</u>	<u>maxima</u>	20
<u>ro</u>	<u>rosette leaf. Lower lobes of</u> <u>leaves slightly spiraled</u>	<u>pepo</u>	21
<u>s</u>	<u>sterile. Male flowers small,</u> <u>without pollen; female flower</u> <u>sterile</u>	<u>maxima</u>	17
<u>Ses-B</u>	<u>Selective suppression of gene B</u>	<u>pepo</u>	36
<u>Skdh</u>	<u>Shikimate dehydrogenase isozyme</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>Sod</u> (<u>Sod-1</u>)	<u>Superoxide dismutase isozyme-1</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>St</u> (<u>1st</u>)	<u>Striped fruit. Longitudinal</u> <u>stripes on fruit, conspicuous</u> <u>if <u>1</u> but inconspicuous if <u>1⁺</u></u>	<u>pepo</u>	28
<u>T</u>	<u>Trifluralin resistance. Dominant</u> <u>to susceptibility to the</u> <u>herbicide; modified by <u>I-T</u></u>	<u>moschata</u>	1
<u>Tpi-c2</u>	<u>Triosephosphatase isomerase</u> <u>cytosolic isozyme-2</u>	<u>maxima x</u> <u>ecuadorensis</u>	42

<u>Gene Symbol</u> <u>Preferred Synonym</u>	<u>Character</u>	<u>Species</u>	<u>Reference</u>
<u>Tpi-p2</u>	<u>Triosephosphatase isomerase</u> <u>plastid isozyme-2</u>	<u>maxima x</u> <u>ecuadorensis</u>	42
<u>v</u>	<u>virescent.</u> Yellow-green young leaves	<u>maxima</u>	11
<u>W</u>	<u>White fruit.</u> Dominant to green mature fruit, partially epistatic to <u>Y</u>	<u>pepo</u>	38
<u>Wf</u>	<u>White flesh.</u> Dominant to cream flesh color	<u>pepo</u>	38
<u>Wt</u>	<u>Warty fruit.</u> Dominant to smooth	<u>pepo</u>	38
<u>Y</u>	<u>Yellow fruit color.</u> Dominant to green	<u>pepo</u>	38
<u>Ygp</u>	<u>Yellow-green placenta.</u> Dominant to yellow placental color	<u>pepo</u>	9
<u>ys</u>	<u>yellow seedling.</u> Lacking chlorophyll; lethal	<u>pepo</u>	21

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It is hoped that scientists will consult the above list as well as the rules of gene nomenclature for the Cucurbitaceae (5, 25) before choosing a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature were adopted in order to provide guidelines for the naming and symbolizing 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.

Gene List Committee:

<u>Cucumber:</u>	T.C. Wehner
<u>Muskmelon:</u>	M. Pitrat
<u>Watermelon:</u>	W.R. Henderson
<u>Cucurbita spp.:</u>	R.W. Robinson
<u>Other Genera:</u>	R.W. Robinson

Resolution and notes of the organization meeting, 28 October 1976, at the Denver Hilton, Denver, Colorado, U.S.A.

The following resolution was adopted by research workers interested in organizing a Cucurbit Genetics Cooperative:

The Cucurbit Genetics Cooperative is organized to develop and advance the genetics of economically important cucurbits.

Membership to this Cooperative is voluntary and open to workers who have an interest in Cucurbit Genetics (an invitation to participate is extended to all Horticulturists, Entomologists, Plant Pathologists, Geneticists, and others with an interest in Cucurbits).

Reports of the Cooperative will be issued on an annual basis. The reports will include articles submitted by members for the use of the members of the Cucurbit Genetics Cooperative. None of the information in the annual report may be used in publications without the consent of the respective authors for a period of five years. After five years, the information may be used in publications without the consent of the authors.

Further, dues for the Cucurbit Genetics Cooperative (CGC) will be \$2.50* per year and will be used to defray cost of preparation and mailing of the annual report. Members from outside the U.S.A. are encouraged to pay dues in at least two-year increments because of bank charges incurred for clearing checks. Only postal money orders or checks drawn on U.S. banks are acceptable. The annual report will include four sections: Research Notes, Stocks and Germplasm Desired or for Exchange, Membership Directory, and Financial Statement. Other sections will be added in future reports as desired, i.e. gene lists, linkage groups, etc.

In accordance with the above resolution, we requested than an invitation to join the CGC be published in the following:

Agronomy News	Journal of Economic Entomology
Euphytica	Journal of Heredity
HortScience	Phytopath News

We are most pleased to acknowledge the assistance of the editor of these publications.

* Dues Structure and Biennial Membership, effective 1986:

<u>Subscriber</u>	<u>Dues Biennial Membership</u>	<u>Back Issue Fee</u>
Individual	\$12.00	\$ 6.00
Libraries	\$24.00	\$12.00

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 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 sp., 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.

Approvals:

W.P. Bemis
W. Bemis

Joseph D. Norton
J. D. Norton

R. W. Robinson
R. W. Robinson

W. E. Henderson
W. E. Henderson

Ken Robbins
M. L. Robbins

R. L. Lower
R. L. Lower

Approvals:

W.P. Bemis
W. Bemis

Joseph D. Norton
J. D. Norton

R. W. Robinson
R. W. Robinson

W. E. Henderson
W. E. Henderson

Ken Robbins
M. L. Robbins

R. L. Lower
R. L. Lower

CGC 11:105 (1988)

CGC 11:106 (1988)

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 indefinitely, 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 provision of the By-Laws or any other 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.

Approvals:

W.P. Bemis
W. Bemis

Joseph D. Norton
J. D. Norton

R. W. Robinson
R. W. Robinson

W. R. Henderson
W. R. Henderson

Ken Robbins
M. L. Robbins

R. L. Lower
R. L. Lower

W. P. Bemis
W. Bemis
(Cucurbita sp.)

W. R. Henderson
W. R. Henderson
(Watermelon)

Joseph D. Norton
J. D. Norton
(Muskmelon)

Ken Robbins
M. L. Robbins
(Cucumber)

R. W. Robinson
R. W. Robinson
(Other genes and species)

R. L. Lower
R. L. Lower, Chairman

Cucurbit Genetics Cooperative Membership Directory

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FINANCIAL STATEMENT

31 December 1987

<u>Balance</u> on 31 December 1986		\$2,412.59
<u>Receipts</u>		
Dues and Back Issues	1,398.50	
Interest	122.20	1,520.70
<u>Expenditures</u>		
Report #10 ^z	1,111.31	
Membership Invoices ^z	43.34	
Report #11 (Call for Papers) ^z	152.39	
Reprinting Reports #5 and #6	<u>435.47</u>	<u>1,742.51</u>
<u>BALANCE</u> on 31 December 1987		\$2,190.78

^zPublishing and mailing