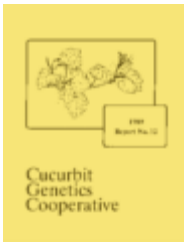
Cucurbit Genetics Cooperative



Report No. 12

July 1989

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Cucurbit Genetics Cooperative Report 12: vi-x (Introduction) 1989

Introduction

Comments from the CGC Coordinating Committee

The Call for Papers for the 1990 Report (CGC Report No. 13) will be mailed in August 1989. Papers should be submitted to the respective Coordinating Committee members by 31 December 1989. The report will be published by June 1990.

As always, we are eager to hear from CGC members regarding our current activities and the future direction of CGC.

It is a pleasure to acknowledge CGC members Marisa Maiero and Wayne A. Mackay for their assistance in assembling CGC Report No. 12 (1989).

- Gary W. Elmstrom: muskmelon
- Warren R. Henderson: watermelon
- J. Brent Loy: *Cucurbita* spp.
- Richard W. Robinson: other genera
- Todd C. Wehner: cucumber
- Timothy J. Ng, Chairman

Comments from CGC Gene List Committee

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

It is hoped that scientists will consult these lists as well as the rules of gene nomenclature for the Cucurbitaceae (HortScience 11:554-568, 1976; CGC Report 5:62-66, 1982) 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 of genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

- Todd C. Wehner: cucumber
- Michael Pitrat: muskmelon
- Warren R. Henderson: watermelon
- Richard W. Robinson: *Cucurbita* spp.
- Richard W. Robinson: other genera

Comments from the CGC 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 in 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.

- Todd C. Wehner: cucumber
- Edward L. Cox: muskmelon
- Billy B. Rhodes: watermelon
- Richard W. Robinson: *Cucurbita* spp.

Report of the Twelfth Annual CGC Business Meeting

10 August 1988

Michigan State University, East Lansing, MI

The 12th Annual Business Meeting of the Cucurbit Genetics Cooperative was held on 10 August 1987 in conjunction with the 85th Annual Meeting of the American Society for Horticultural Science at Michigan State University in East Lansing, MI. The meeting was called to order by J.D. McCreight, Chairman. Twenty- six members and guests were in attendance.

CGC Report No. 11 (1988) was mailed to members on 25 July 1988. The cost of printing and mailing CGC 11 was $1443.95. Twenty-two new members joined in 1987, making a total of 182 active members by the end of the year. Current CGC cash reserves totaled $3,089.64.

J.D. McCreight provided a summary of the Cucurbitaceae '88 EUCARPIA meeting. A meeting of European CGC members expressed an interest in having the CGC Report list international meetings as well as those in the U.S. They expressed interest in the cucurbit gene collections and encouraged geneticists to increase their activity in this area. They also expressed a desire to have an airmail option for the CGC Report in order to receive it in a more timely fashion.

Two CGC Coordinating Committee changes were announced. J. Brent Loy replaced Jack Juvick as Coordinating Committee member for *Cucurbita* spp. and Time Ng replaced J.D. McCreight as CGC Chairman.

Tim Ng assumed chairmanship of the meeting, introduced himself, and expressed his appreciation for the efforts of J.D. McCreight on behalf of CGC over the years. he next had those in attendance introduce themselves and mention their affiliations and research interests.

The subject of content of the CGC Report was brought up. Although the content of papers in the Report now extends beyond the original concept of having only genetic studies with cucurbit species published, the subject matter currently being accepted was agreeable to those present and the present policy will be continued. The policy of not allowing citation of CGC research reports without the author's permission for a period of five years was retained, as was the policy of publishing a *complete* gene list for each major cucurbit crop/species every four years. The 31 December deadline for submission of articles to CGC was also retained, and every effort will be made in 1989 to have CGC Report No. 12 mailed by April. A subsequent mailing will be made during the Summer of 1989 informing members of upcoming meetings of interest to cucurbit workers.

The cost of publishing the CGC Report increased in 1988. Also, U.S. postage rates increased substantially in April 1988 for books and printed materials, including the CGC Report. To offset the increased costs, it was moved and accepted that membership dues would rise by $1 per year effective in 1990. Hence, members renewing for 1989-90 would be billed $13 and those renewing for 1990-91 would be billed $14. An airmail option for non-U.S. subscribers would also be available beginning with renewals for 1989-90.

An announcement was made about the international meeting on "Evaluation and Enhancement of Germplasm of the Cucurbitaceae" which will be hosted by the U.S. Vegetable Laboratory, USDA-ARS, in Charleston, South Carolina, in November 1989. This meeting will be in conjunction with the joint meetings of: the Vine Crops Crop Advisory Committee, the National Muskmelon Research Group, the Watermelon Research Group, the Squash Breeders, and the Pickling Cucumber Improvement Committee. It will directly precede the Biennial Collaborators' Conference on Vegetable Breeding in the Southeastern United States. All Federal, state and private industry scientists involved or interested in research on cucurbit crops are invited. Tentative dates are 12-14 November 1989. Further announcements will published through CGC. ASHS, EUCARPIA, and individual working groups. CGC members can also be placed on a mailing list for further announcements by contacting: C.E. Thomas, USDA-ARS, U.S. Vegetable Laboratory, 2875 Savannah Hwy., Charleston, SC 29414.

Joe Norton displayed a honeydew melon developed in Iran, described its properties and availability, and sliced pieces for sampling as the CGC meeting was adjourned.

Cucurbitaceae '89

Evaluation and Enhancement of Cucurbit Germplasm

Location: Omni Hotel, Charleston, South Carolina USA

Dates: November 29 - December 2,1989

The purpose of Cucurbitaceae '89 is to provide a forum for the presentation and exchange of scientific information about germplasm evaluation and enhancement research activities on cucurbit crops (cucumber, muskmelon, pumpkin, squash, and watermelon). All persons engaged or interested in these research areas are invited to participate. Cucurbitaceae '89 will be hosted by the USDA - U.S. Vegetable Laboratory, and the official language will be English.

The scientific program will consists of invited papers by recognized authorities on topics related to evaluation and enhancement research in cucurbit corps, contributed presentation by meeting participants, and meeting of the follow groups:

- Cucurbit Crop Advisory Committee
- Cucurbit Genetics Cooperative
- National Muskmelon Research Group
- Watermelon Research Group
- Squash Breeders Group
- Pickling Cucumber Improvement Committee

For further details, including registration materials and information on travel and accommodations, guidelines for abstracts and posters, etc., contact: Dr. C.E. Thomas, USDA-ARS, U.S. Vegetable Lab, 2875 Savannah Highway, Charleston, SC 29414 USA.

US Watermelon Research Group

The 9th annual meeting of the Watermelon Workshop was held on 7 February 1989 in Nashville, Tennessee, with over forty participants in attendance. Doyle Smittle discussed the status of non-destructive measurement of maturity and quality of melons; it appears that availability of a commercially available unit is still sometime in the future. Don Hopkins discussed his work with growing watermelons in a monoculture, and Ray Martyn reviewed his work on induced resistance to Fusarium wilt. Lively discussions were also held on the topics of "Hollow Heart of Watermelon" and "Pollination of Triploids." The Watermelon Research Group will hold its next meeting in conjunction with Cucurbitaceae '89 in November-December of 1989, and will meet in Little Rock, Arkansas, on 4-6 February 1990.

US Cucurbit Crop Advisory Committee Update

The Cucurbit Crop Advisory Committee (formerly Vine Crops CAC) met in Madison, Wisconsin, in conjunction with the Pickling Cucumber Improvement Committee on 9 November 1988. In 1988, the Cucurbit CAC recommended that the National Plant Germplasm System (NPGS) fund four germplasm evaluation proposals and one germplasm enhancement proposal. These proposals included: verification of the species identity of *Cucurbita* accessions in the Regional Plant Introduction Stations; evaluation of cucumbers, muskmelons and *Cucurbita* for disease resistance; and transferring virus resistance from wild to cultivated muskmelon. In 1988. the committee completed and updated the five major sections (cucumber, muskmelon, watermelon, squash and pumpkin, and exotic species) and submitted its report to NPGS on the status and needs for cucurbit germplasm collection, storage, evaluation, and enhancement. NPGS requested a statement on the applicability of the Core Concept to cucurbit germplasm evaluation. The Core Concept addresses the problems of maintenance and efficient evaluation of large germplasm collection of routine evaluation; subsequent evaluation would focus on accessions in the larger collection indicated by the core evaluation as being likely sources for the desired traits. The Core Concepts is controversial and remains to be proven. The major concerns of the committee were the integrity of the accessions (relative to the original seeds) and the information in the Germplasm Resource Information Network (GRIN), and the acquisition of additional germplasm before the Center of Origin and Diversification are lost to development.

James D. McCreight, Chair

Cucurbit Genetics Cooperative Meeting in 1989

The Thirteenth Annual Business Meeting of the Cucurbit Genetics Cooperative will be held in conjunction with the 86th Annual Meeting of the American Society for Horticultural Science (ASHS) in Tulsa, Oklahoma, 29 July - 3 August 1989. Further information will be available in the Program & Abstracts issue for the ASHS Annual Meeting (HortScience vol. 24 (4)) when it is published. The Cucurbit Genetics Cooperative will also hold a meeting in conjunction with Cucurbitaceae '89 in Charleston, South Carolina, in November-December of 1989.

Other meetings of interest to CGC members

Group(s)	Date and Location	Contact Person
Cucurbit Crop Advisory Committee, National Muskmelon Research Group, Watermelon Research Group, Squash Breeders Group, Pickling Cucumber Improv. Committee	29 Nov - 2 Dec 1989; Charleston, South Carolina (Cucurbitaceae '89)	Dr. C.E. Thomas; USDA-ARS, U.S. Veg. Lab.; 2875 Savannah Highway; Charleston, SC 29414; USA; Tel: (803) 766-3761
Watermelon Research Group	4-6 February 1990; Little Rock, Arkansas	Dr. Gary W. Elmstrom; Univ. Florida Agr. Res. Ctr.; 5336 University Avenue; Leesburg, FL 32748; USA; Tel: (904) 787-3423

Corrigenda

In the article "Reactions of Muskmelon Genotypes to Races 1 and 2 of *Sphaerotheca fuliginea* in Israel," by Y. Cohen and H. Eyal [CGC 11:47-49, 1988], severity ratings in Table 1 for the genotype Charantais-T should be "+ + +" and "+ + +" for races 1 and 2, respectively, not "-" and "-" as they appeared.

In the abstract "Studies on Watermelon Germplasm Sources Resistant to Fusarium Wilt Disease at the Seedling Stage," by Wang Ming and Zhang Xian [CGC 11:68, 1988], in paragraph 1, sixth line, "5 x 10^3 spores" should read "5 x10^5 spores."

Cucurbit Genetics Cooperative Report 12:1-4 (article 1) 1989

Alternaria alternata f. sp. *cucurbitae* on Cucumber and Other Cucurbits

Demetrios John Vakalounakis

Plant Protection Institute, Heraklio, Crete, Greece

During the 1979 to 1980 crop season, a severe leaf spot disease of cucumber (*Cucumis sativus* L.) was noticed on greenhouse crops grown in some plastic houses in the Sitia Area, Lasithi, Crete, Greece, along the coastal strip between Koutsouras and Goudouras (10). Since then, it has spread to most of the cucumber growing areas in Crete, causing severe losses.

Symptoms appear in late autumn, mainly on the leaves of the middle and upper part of the plants. Necrotic flecks, surrounded by a chlorotic halo, appear on the leaf, and these enlarge to spots which may coalesce to form lesions up to 5 cm or more in diameter. The lesions appear circular in shape and bear black-brown fructifications of the pathogen. Severely-infected leaves become yellow, senescent, and die. No other part of the plant is affected. During the winter, when relative humidity in the plastic houses is high and plant vigor is reduced due to fruit bearing and unfavorable climatic conditions (reduced illumination and average air temperature lower than 15°C), infection progresses rapidly throughout the crop resulting in severe damage within a few days (11).

A long-chained *Alternaria spp*. with small spores was always observed on the old lesions of infected cucumber leaves. The same fungus was consistently obtained from samples taken from different plastic houses when pieces of infected tissue or spores from the spots were plated out on Petri dishes containing potato dextrose agar (PDA). The cultures of the fungus on PDA at 25°C under "daylight" fluorescent lamps have a dirty white color at the beginning, while later the center becomes gray. In a few days, the entire surface is covered with an abundance of spores. The spores on infected leaves or on cultures on PDA are produced in long chains on short conidiophores. They are brown but, when many of them have been produced on PDA, they look black with a velvety appearance. The dimensions of the spores either *in vivo* or *in vitro* (Table 1) agree fairly well with published descriptions of *Alternaria alternata* (Fr.) Keissler (8) and its synonym *Alternaria tenuis* Auct. (6, 7). The pathogen of the present disease is also similar to *A. alternata f. sp. lycopersici* which causes a stem canker of tomato (2). Alternaria infections similar to those described in this paper are very common on cucumber but are caused by *Alternaria cucumerina* (Ellis & Everh.) Elliott (synonym *Alternaria brassicae* f. *nigrescens* Pegl.) (1, 5) or *Alternaria pluriseptata* (Karst. & Har.) Jorstad [synonyms *Alternaria cucurbitae* Let. & Roum., *Stemphylium ilicis* Tengwall, *Ulocladium cucurbitae* (Let. & Roum.) Simmons, *Ulocladuim atrum* Press] (3, 9). However, both these fungi are morphologically distinct (1, 4, 5, 6) and are readily distinguishable from *Alternaria alternata* (11).

Of 62 cultivated and weedy species in 16 botanical families artificially inoculated and naturally infected in greenhouse experiments, 27 species belonging to the Cucurbitaceae were found to be susceptible to the pathogen (Table 2).

Table 1. Morphological characteristics of conidia of *Alternaria alternata* f. *sp. cucurbitae* from cucumber leaf spots in comparison with those of published descriptions of *A. alternata*, *A. cucumerina* and *A. pluriseptata*.

Spore measurements (µm)	Body length	Body width	Beak length	Spores (% with beaks)	Total length	No. of septa	Spores/chain
A. alternata							
Cucumber leaf spot	3804±12.0z (15-68) y	14.6±3.3 (9-24)	6.3±4.4 (1-21)	75	42.2±14.3 (15-73)	2-8	4-5
PDA	20.1±4.8 (12-29)	9.7±2.0 (6-14)	5.1±2.3 (3-12)	57	25.3±4.5 (17-34)	2-5	8
A. alternata **(Simmons, 1967)**							

Neotype specimen	30.9 (18-47)	12.6 (7-18)	up to 25	-	-	3-8	-
A. tenuis							
Medium	25.7 (7-70)	11.2 (6-20)	5 (1-58)	80	7-72	1-6	8
A. alternata f. sp. lycopersici							
Tomato	32.3±2.8 (18-50)	12.4±2.8 (7-18)	6.8±6.0 (2-20)	72	18-68	1-5	3-4
***A. cucumerina* (Jackson, 1958)**							
Host	57-87	18-21	106-135	-	-	5-9	-
A. pluriseptata (Hervert et al., 1980)							
Host	19-66	8-16	-	-	-	2-9	-
***Ulocladium atrum* (Simmons, 1976)**							
Medium	18.6 16.5-19.8	16.0 13.2-18.7	no beaks	-	-	1-3	1; sometimes 2

z Mean ± standard deviation.
y Numbers in parentheses indicate extreme values.

Table 2. Susceptibility of cucurbitaceous species and some belonging to other families to infection by *Alternaria alternata f. sp. cucurbitae.*

Species	**Disease severity**
CUCURBITACEAE	
Benincasa hispida (Thumb.) Cogn.	+++
Citrullus lanatus (Thumb.) Mansf.	++++
Cucumis africanus L. f.	+
Cucumis anguria L.	+++
Cucumis dipsaceus Ehrenb.	+
Cucumis ficifolius A. Rich	++
Cucumis hardwickii Royle	++++
Cucumis longipes Hook.	++
Cucumis melo L.	++++
Cucumis pustulatus	++++
Cucumis sativus L.	+++++
Cucurbita ficifolia B.	+
Cucurbita foetidissima Kunth.	+
Cucurbita lundelliana Bailey	++
Cucurbita maxima Duch.	+
Cucurbita mixta Pang.	+++
Cucurbita moschata (duch.) Duch. ex Poir.	+++
Cucurbita palmeta Wats.	+++
Cucurbita pepo L.	+++
Cucurbita sororia	+++
Cucurbita texana A. Gray	+
Ecballium elaterium (L.) A. Rich	+

Lagenarial leucantha Rusby var. *clavata Makino*	+++++
Lagenaria siceraria (Mol.) Standl. ssp. *asiatica* (Kob). Heiser	++++
Lagenaria vulgaris Ser.	+++++
Luffa cylindrica Roem.	++
Momordica charantia L.	+++
SOLANACEAE	
Capsicum annuum L.	-
Lycopersicon esculentum Mill.	-
Nicotiana tabacum L.	-
Nicotiana glutinosa L.	-
Solanum melongena L.	-
UMBELIFERAE	
Apium graveolens L.	-
Daucus carota L.	-
URTICACEAE	
Urtica urens L.	-
AMARANTHACEAE	
Amaranthus retroflexus L.	-
Gomphrena globosa L.	-
CHENOPODIACEAE	
Beta Vulgaris L.	-
Chenopodium album L.	-
Spinacia oleracea L.	-
COMPOSITAE	
Aster squamatus (Spr.) Hier.	-
Cichorium endivia L.	-
Chichorium intybus L.	-
Erigeron canadensis L.	-
Lactuca sativa L.	-
Lactuca serriola L.	-
Sonchus asper (L.) Hill.	-
CONVOLVULACEAE	
Convolvulus arvensis L.	-
CRUCIFERAE	
Brassica oleracea L. var. *capitata*	-
Raphanus sativus L.	-
CYPERACEAE	
Cyperus longus L.	-
var. badius Desf.	-
GERANIACEAE	
Erodium malacoides Willd.	-
GRAMINAE	

Setaria viridis P.B.	-
LEGUMINOSAE	
Glycyrriza glabra L.	-
Medicago polymorpha L.	-
Melilotus indica All.	-
Phaseolus vulgaris L.	-
Vicia faba L.	-
LILIACEAE	
Allium cepa L.	-
OXALIDACEAE	
Oxalis corniculata L.	-
ROSACEAE	
Fragaria vesca L.	-

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

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Cucurbit Genetics Cooperative Report 12:9-10 (article 2) 1989

Toxins: Potential Screening Aid for Selecting Anthracnose Resistance in Cucumbers

D.C. Linde, J.M. Shively and B.B. Rhodes

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Anthracnose (causal agent = *Colletotrichum lagenarium*) is one of the most important diseases of cucumbers, and cucurbits in general. An *in vitro* or greenhouse screening aid for selecting anthracnose resistance could be valuable if it saved time and money. Toxins are one class of screening aids investigated increasingly for selecting host resistance.

The chlorotic halo sometimes observed around the necrotic lesion caused by *C. lagenarium* suggests that one or more toxins may be involved in its pathogenesis. On the basis that lipid toxins have been isolated from liquid cultures of *C. nicotianae* (1, 2) and *C. capsici* (3), we attempted to isolate lipid toxins from shake cultures of race 2 *C. lagenarium.*

The fungus was grown in modified (40 g/l) sucrose) Czapek Solution liquid medium (4L) for 2 weeks on a shaker run at 150 rpm. Standard partition chromatography methods with ethyl acetate were used to obtain acidic and neutral lipid fractions from the hyphae, culture broth, and culture pellet. Only the acidic and neutral lipid fractions from the culture broth were found to inhibit cucumber and, to a greater extent, lettuce seed germination. When the 2 fractions were combined in ethanol and spotted on punctured tobacco and cucumber leaves, large necrotic lesions with chlorotic halos similar to anthracnose lesions were observed. The control (ethanol only) produced a small, almost transparent lesion.

N-hexane washes of the acidic and neutral lipid fractions contained no detectable toxic activity with the lettuce seed germination assay. Thin layer chromatography was used to purify the toxic fractions. A total of 3 acidic and 1 neutral lipid toxin fractions were identified. Their mobilities in several solvent systems are shown in Table 1.

The fungus was grown again in liquid culture (4L), and the acidic and neutral lipid fractions were obtained using partition chromatography as above. The two fractions were combined (total weight = 0.13 g) and suspended in 1 ml ethanol. One u1 was used in a leaf puncture assay in the greenhouse on 7 cucumber genotypes with varying levels of resistance to race 2 *C. lagenarium*. The leaf puncture assay was also used of 16 F2 cucumber plants segregating for anthracnose resistance. The 7 genotypes and 16 respective F3 families were inoculated in the field with the same isolate. No relationship was found between the lesion size caused by the leaf puncture assay and the field disease rating for either the 7 genotypes or the 16 F2 plants and their respective F3 families.

The high concentration of lipids used in the leaf puncture assay may have precluded a proportional response to the toxin. An alternative hypothesis is that the putative toxin is only one element of the pathogen's virulence.

Table 1. Rf values of the 4 toxic factions in 10 solvent systems on silica gel G thin layer chromatography.

	Toxic Fraction y			
Solvent system[z]	**N1**	**Ala**	**Alb**	**A2**
Bz: EtAc: Ac 70 : 30: 1	0.250-0.0500	0.125-0.375	0.125-0.375	0.500-0.625
Bz: MeOH 90:10	0.47	-	-	-
Bz: EtAc 30:70	0.56	-	-	-
Et2	0.50-0.60	0.00-0.10	0.30-0.50	0.00-0.10

100				
EtAc 100	0.50	0.00	0.00	-
CHCl3: MeOH 90:10	0.58	-	0.00	-
Acetone 100	-	0.00-0.30	0.00	-
EtOH 100	-	-	0.10-0.30	-
EtOH: MeOH	-	-	0.70-0.80	-
MeOH 100	-	-	1.00	-

[z] BZ = benzene, EtAc = ethyl acetate, Ac = acetic acid, CHCl3 = chloroform, Et2 = diethyl ether, MeOH = methanol, EtOH = ethanol.

[y] 'N' = neutral lipid toxic fraction, 'A' = acidic lipid toxic fraction.

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Cucurbit Genetics Cooperative Report 12:7-8 (article 3) 1989

Tolerance of Cucumber to Chloramben Herbicide

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USDA, ARS Department of Horticulture, University of Wisconsin, Madison, WI 53706

Lack of an efficacious chemical weed control system is a major factor which limits yield in commercial cucumber (*Cucumis sativus* L.) production in the United States. This is particularly true of once-over, mechanically-harvested acreage where uniform emergence and flowering, and plant growth at close spacings can be dramatically affected by weed competition (4).

Bensulide, DCPA, CDEC, naptalam, paraquat, trifluralin and chloramben are currently registered for use in commercial cucumber production in the United States (9). Bensulide, CDEC, and naptalam often give poor weed control (5, 6) and DCPA causes severe damage when surface-applied prior to crop emergence (4). Paraquat, being a contact herbicide, is only suitable for removing weeds for seedbed preparation and does not provide control for an extended period of time (9). Moreover, since the suggested safe use of chloramben requires the addition of activated charcoal as a saftening agent (8), which adds costs of time and materials, it has received limited use among growers (Personal communication, H.J. Hopen, 1988).

Given these restrictions and/or the poor performance of these herbicides, it would be useful to identify germplasm possessing herbicide resistance or tolerance. Although chloramben (3-amino-2, 5-dichlorobezoic acid) provides excellent grass and broadleaf weed control (8,9), crop tolerance and genotypic variability is low (1, 3). We felt it prudent to survey the U.S. cucumber collection for chloramben tolerance. If tolerant accessions were identified, this would allow for the development of a resistant population for use in breeding programs.

The germplasm collection was surveyed by planting 25 seeds of each accession (753) in each of 20 replications arranged in a randomized complete block design at Hancock, WI (sandy loam soil) in 1987. After planting, chloramben 75DF was surface applied at 6.72 kg/ha to half of the plots. After 12 hours, 13 mm of water was applied through overhead sprinkler irrigation. Treated seedlings were compared to controls 1 and 3 weeks after emergence, and rated for chloramben injury on a 10 point scale (1=seedling death, 5=moderate to severe, and 10=no injury). All plants showed some injury. Plants with mean values of 7 to 9 (Table 1) were classified as tolerant. These plants were transplanted to the greenhouse and random-mated.

The mechanism of resistance and/or tolerance to chloramben in these plants is unclear. Several mechanism have been proposed to explain tolerance to chloramben. Stoller (7) suggests that plants tolerant to chloramben sustain higher internal chloramben concentrations and conjugate absorbed chloramben more rapidly than susceptible species. Colby (2) hypothesized that tolerance is a function of the binding of the chloramben in the roots of more tolerant species. In this scenario, chloramben bound in the roots reduces phytotoxicity in the leaves in tolerant plants; hence, there is less translocation of chloramben.

Our objective was to develop a population tolerant to chloramben form which inbred lines with acceptable horticultural characteristics could be developed. An elite population is being develop form chloramben tolerant lines (Table 1) through recurrent half-sib family selection. After initial selection, near-isogenic tolerant and susceptible lines will be developed. Not only will these lines be of value in hybrid production, but near-isogenic lines may allow for further elucidation of tolerance mechanisms.

Table 1. Plant introductions in the U.S. cucumber germplasm collection which were classified as tolerant to chloramben herbicide (6.72 kg/ha) at Hancock, WI in 1987.

PI no.	Origin	Varietal Name
173892	India	Khira
482464	Zambia	-

275411	Netherlands	Lange Groene Broei
179676	India	Kakri
279464	Japan	Kara-Aonaga-Fushinari
1649502	Turkey	-
436609	Peoples Rep. China	Tsin Sanz Yen 15919
279465	Japan	Natsufushinari
277741	Netherlands	Green Spot Super

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Cucumber Yield Improvement Through Breeding in the Southeast U.S.A.

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It is of interest to cucumber breeders in the U.S.A. to determine how much progress they have made in the improvement of yield. yield is a complex trait, and is affected by sex expression, disease resistance, and cultural practices, as well as other factors. All of those have been improved over the past decades by researchers in public and private institutes.

In a survey of cucumber breeders made in 1987, the primary objectives for trait improvement listed were yield, disease resistance, fruit quality, and earliness/sex expression (2). With emphasis on many traits, progress on any one will be slow because selection for many traits simultaneously reduces the selection intensity on each trait. In my recurrent selection program, 14 populations are subjected to a 5% selection intensity (20 families kept out of 400 tested) for yield, earliness, quality, and disease resistance (mostly anthracnose, downy mildew and gummy stem blight). Actual selection intensity is approximately 40% for yield and 50% each for earliness, quality and disease resistance (.05=.40 x .50 x .50 x .50). Thus, one would not expect much progress to be made where many traits are being selected simultaneously.

Nevertheless, progress has been made for yield, even after accounting for the contributions of improvement in cultural practices, sex expression and disease resistance. For example, the cultivars released form the public breeding programs run sequentially by Carroll Barnes, Richard Lower, and me in the Carolinas have lead to continuously improved yield even though many releases study was to estimate the improvement made for yield in pickling cucumbers grown in the southeast U.S.A.

Methods. Five cultivars which are most similar in type (gynoecious hybrid pickling cucumbers with resistance to the southern foliar disease) developed in the Carolinas over the last 2 decades were grown in trials in Clinton, NC under standard cultural practices (1). The trials were run in the spring when there was no foliar disease load, and in the summer when anthracnose, downy mildew and gummy stem blight were moderate to severe. The trials were run in 1981 through 1985 using 3 replications and 6 harvests. No summer trials were run in 1982 and 1983.

Irrigation was used to supplement rainfall. Weeds, diseases and insects were controlled as needed using labeled pesticides. Weight of all fruits produced, regardless of size (most being grade 2 and 3 with a diameter of 27 to 50 mm), were summed over harvests to get total yield. Yield was regressed on release date to determine the progress made per year. Release date is not completely accurate in determining when the material was developed. 'Raleigh', for example could have been released in 1985 if it had been given top priority in the program.

Results. It is interesting to note that the cultivars do not keep the same rank for any one trial as the overall mean (Table 1). In general, yield increased with each subsequent release, with an average of 0.4 t/ha each year of breeding. That yield progress was made even though other traits (such as fruit color, fruit shape, and length: diameter ratio) were being improved.

Since the 5 cultivars were tested under the same cultural practices, and had similar sex expression and disease resistance, progress in yield must have been due to direct improvement of the trait. Thus, I conclude that we have not hit a yield plateau in cucumber breeding, but have been working on so many traits that progress on each one of them is slow.

Table 1. Yield (t/ha) of disease resistant, gynoecious pickling cucumber hybrids grown in field trials (spring and summer) in Clinton, NC.[z]

		1981		1982	1983	1984		1985		
Release date	**Cultivar name**	**Spr**	**Sum**	**Spr**	**Spr**	**Spr**	**Sum**	**Spr**	**Sum**	**Mean**

1969	Explorer	19	20	29	34	31	22	38	37	28.8
1973	Carolina	20	10	28	33	41	21	45	38	29.6
1975	Calypso	21	21	32	34	42	30	41	41	32.7
1979	Regal	24	18	40	38	41	27	48	41	34.6
1987	Raleigh	26	26	37	34	33	27	52	48	35.5

[z] Data are means of 3 replications and 6 harvests.

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Source-Sink Relationships in Cucumber

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Average yield of cucumber (*Cucumis sativus* var. *sativus* L) in the United States has increased from approximately 65 (1920) to 200 (1980) bushels per acre (1). Much of that yield improvement was the result of improved cultural practices, gynoecious sex expression, and disease resistance (5,6). Knowledge of plant physiology will help in the direct improvement of yield. A fruit developing from the first pollinated flower on the cucumber plant inhibits the development of subsequent fruits. It is not known whether this inhibition is due to a substance which is translocated from the fruit, or to a substrate-limited source-sink relationship (2,4,7).

Unlike var. *sativus, Cucumis sativus* var. *hardwickii* (R.) Alef. possesses a sequential fruiting habit (3), and therefore has potential for increasing fruit yield in cucumber (9). Inbred lines derived from var. *sativus* x var. *hardwickii* matings have been developed in my program (10). Although the fruit quality of these lines is commercially unacceptable (11), their fruit yielding abilities are significantly higher than standard cultivars (10).

In order to gain more information concerning the fruit setting nature of var. *hardwickii*, an experiment was designed to compare the morphological and photosynthetic characteristics of a standard var. *sativus* inbred (WI 1606), a var. *hardwickii* accession (PI 215589), and a var. *sativus* x var. *hardwickii* derived inbred (WI 5551). It was thought that these comparisons would provide information concerning the role of source-sink relationships in cucumber.

Seeds of WI 1606, WI 5551, and PI 215589 were planted in 10 replications (single plant), each equidistantly spaced 2.7 m apart (center to center) in a randomized complete block design. Fruit, seed, and plant (stem + leaf) dry weight, as well as fruit and seed number per plant were recorded at maturity (100 days after sowing). Harvested tissues were dried at 60°C for 7 days. The net CO2 assimilation rate of the 4th (leaf #1) from the terminal whorl was recorded 3 weeks after sowing on cloudless days using an LI 6000 portable gas analysis system (Li-Cor, Inc., Lincoln, Nebraska). Photosynthetic rates of the 4th and 6th (leaf #2) leaves were measured at 5 and 6 weeks, while rates of the 4th, 6th, 8th (leaf #3) and 10th leaf #4) leaves were measured 7, 8, 9 and 10 weeks after sowing. The LI 6000 consists of a battery powered nondispersive infra-red gas analyzer, a porometer, a communications divide, and a dedicated datalogger. When a leaf is placed into the monitoring chamber, CO2 concentration decreases as CO2 assimilation occurs. Net carbon assimilation rate is calculated based on leaf area, changes in CO2 concentration and air flow rate.

Stem weight per plant as well as fruit number per plant was significantly higher in PI 215589 when compared to the other inbred lines (Fig. 1). However, the seed number and weight per fruit of PI 215589 was significantly lower than for WI 1606. The means of WI 5551 for most characters were intermediate (seed weight per 500 seeds) to the parents, or closer to WI 1606 (stem and fruit weight, fruit number) than to PI 215589. There were no significant differences observed in the mean net CO2 assimilation rate (AR) among leaves or between inbred lines during the growing season. Mean AR fell dramatically in all lines when flowering (weeks 7 to 8) and fruit development began, but the magnitude of this decrease was similar in all three lines. Although this decrease may be associated with lower irradiance in weeks 9 to 10 (1017±431 mmols/m^2/s) when compared to weeks 3 to 8 (1569±281 mmol/m^2/s), irradiance was greater than light saturation (300-500 mmols/m^2/s) for cucumber.

A significantly higher proportion of photosynthate was translocated to the fruit in WI 1606 when compared to the other lines (Table 1). In contrast, the percent of dry weight of leaf and stem tissue was higher, in PI 215589 (9 and 38% respectively) when compared to WI 1606. While the portion of assimilates in the leaf and stem in WI 5551 was similar to that of WI 1606, contribution to fruit development was 10% lower. PI 215589 typically flowers 2 weeks later than the other lines in days to anthesis (approx. 51 days in Wisconsin). The effect of this difference in maturity date on assimilate partitioning was minimized by delaying the harvest 100 days after sowing.

Consistent differences in the direction (+ or -) of phenotypic corrections in traits between lines may indicate dissimilarities in

their physiologic nature. Different significant correlations in directions between lines were observed for fruit number and weight/500 seeds, weight/500 seeds and stem weight, and see weight/500 seeds and seed number (Table 2). Negative correlations in fruit number and weight/500 seeds and seed number were negatively correlated in PI 215589 and positively so in WI 1606.

These calculated associations along with observed differences in carbohydrate partitioning between lines suggest that they are physiologically different. It appears that PI 215589 has the ability to set large number of fruits containing small but numerous seeds. On the other hand, WI 1606 does not. Although AR among inbred lines is similar, PI 215589 partitions more of its photosynthate to leaves and stems when compared to the other inbred lines examined, suggesting that sinks and/or their strengths are dissimilar. A similar finding was reported by Ramirez and Wehner (8). The fact that WI 5551 is higher yielding than WI 1606, but partitions significantly more assimilates to seeds than to fruit suggest that: i) Seeds may be a significant sink; and ii) Seed maturation may be related to the observed reductions in fruit size. One could hypothesize that selection for fewer seeds per fruit in populations having high fruit number per plant may result in derived inbreds partitioning more assimilates to the mesocarp of the fruit, thereby resulting in larger length/diameter ratios.

Table 1. Dry weight percentage of plant tissue of a C. *sativus* var. *sativus* (WI 1606), a C. *sativus* var *hardwickii* (PI 215589) and a derived var. *sativus* x var. *hardwickii* (WI 5551) inbred line grown at Hancock, WI.[z]

	Proportion of plant by weight (%)[y]		
Plant part	**WI 1606**	**WI 5551**	**PI 215589**
Fruit	50a	40b	14c
Leaf	22b	22b	31a
Stem	16c	18b	54a
Seed	12d	20a	1c

[z] Different letters within a row indicate that mean percent values are significantly different (5%) using LSD test.
[y] WI 1606 = C. *sativus* var. *sativus* inbred; PI 215589 = C. *sativus* var *hardwickii*; WI 5551 = var. *sativus* x var. *hardwickii* derived inbred.

Table 2. Phenotypic correlations between dry weights of tissue of a C. *sativus* var. *sativus* (WI 1606), a C. *sativus* var. *hardwickii* (PI 215589) and a derived var. *sativus* x var. *hardwickii* (WI 5551) inbred line grown at Hancock.

	Inbred line or accession [z]		
Parameters correlated	**WI 1606**	**WI 5551**	**PI 215589**
Fruit no. vs. seed wt./500 seeds	-0.56*	0.33	0.67*
Seed wt./500 seeds vs. stem. wt.	0.62*	-0.01	0.83**
Seed wt./500 seeds vs. seed no.	0.60*	0.38	-0.63*

[z] WI 1606=C. *sativus* var. *sativus* inbred; PI 215589=C. *sativus* var. *hardwickii*; WI 5551=var. *sativus* x var. *hardwickii* derived inbred.
*, ** Indicates that correlation coefficients are significant at 5 and 0.1%, respectively.

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Delayed Pollination Successful for Cucumbers in North Carolina Greenhouse

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Munger reported that pistillate flowers of cucumber could be pollinated successfully up to 24 hours after they opened in the Philippines and in his New York greenhouses, but not in his New York fields (2). Lower and Edwards (1) recommended that pistillate flowers be pollinated on the morning they open, up until noon. Generally, bee activity falls off after 12 noon due to the heat in the summer of the southern U.S. cucumber production areas.

Usually, we make all of our pollinations on the morning the pistillate flowers open because it is more comfortable to work then. Also , field pollinations made in some years (where the maximum temperature was above 35°C) failed if they were made after 10 am. In July, our greenhouses reach 40°C in the afternoon, even with shading and a water-cooled ventilation system. Therefore, we doubt that delayed pollinations would be successful in the summer greenhouse. However, it is occasionally useful to pollinate pistillate flowers 12 to 24 hours after they open. We have found this to be possible, and have taken data to show the effect of the delay on seed set.

Methods. Plants of Gy 14A and 'Marketmore 80F' were grown in 150 mm diameter pots in the North Carolina State Univ. greenhouses at Raleigh, NC. Plants were planted in January and pollinated in February to March. Temperatures were maintained at 23 to 30°C during the day and 19 to 21°C at night. Newly-opened pistillate flowers were pollinated at 8 am, 12 noon, 4 pm and 8 am on the following day for each cultigen, setting one fruit per plant. The experiment was replicated 4 times.

Results. Generally, all of the pollination treatments were successful, and resulted in 46 to 242 seeds per fruit. There appeared to be a slight reduction in the number of seeds per fruit, and in the number of successful pollinations as pollination was delayed (Table 1). However, there were larger differences among replications than among pollination treatments, and the treatments were not significantly different.

For convenience and comfort, we intend to continue our practice of pollinating pistillate flowers on the morning they open (7 am to 12 noon). However, we will make delayed pollinations when necessary, since one would expect nearly as much success under our spring and fall greenhouse conditions.

Table 1. Effect of pollination time on seed number and fruit set of cucumbers grown in the greenhouse in Raleigh, NC.[z]

Time of day	Hours after flower opening	Cultigen	Seeds/fruit	No. fruits set/4
8:00	0	Gy 14A	204	4
8:00	0	MM 80F	126	4
12:00	4	Gy 14A	179	3
12:00	4	MM 80F	202	3
16:00	8	Gy 14A	148	4
16:00	8	MM 80F	68	3
8:00	24	Gy 14A	81	4
8:00	24	MM 80F	183	3
LSD (5%)	-	-	*NS*	-
Mean	-	-	*148*	-

CV (%)	-	-	*43*	-

[z] Data are means over 4 replications of 1 fruit each of 2 cultigens, Gy 14A (gynoecious pickle inbred) and Marketmore 80F (gynoecious slicer inbred).

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Seed Weight of Cucumber Cultivars

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It is useful to know the weight of cucumber seeds for cultivars being grown for research and production, since many operations are done by weight even though it is number that is of interest. For example, to achieve the proper stand, Knott's Handbook for Vegetable Growers recommends planting 2 to 3 lb/A of seed (1). The handbook bases that recommendation on its published value of 1100 seeds/ounce for the typical cucumber cultivar.

We have observed differences in seed weight among cultivars, and were interested to know how our measurements compared with the published estimates. The objective of this study was to compare seed weights for cucumber cultivars commonly used for field production in the U.S.A.

Methods. Seeds of 9 different breeding lines and cultivars (collectively referred to as cultigens hereafter) were obtained from seeds companies and the N.C. State Univ. breeding program. The cultigens were chosen to represent pickling and slicing fruit types, northern and southern adaptation, and compact, determinate and little leaf plant types. Seeds were divided into 4 lots of 500 seeds to provide replication for the measurement of seed weight. Seed weight was then converted into number of seeds per gram, ounce and pound for use by those who use those measures.

Results. There was a large range in number of seeds per ounce among the 9 cultigens and 4 samples counted (Table 1). Cultivars had between 904 and 1291 seeds per ounce in the sample counted, ranging 18% below to 17% above the figure of 1100 seeds per ounce published by Lorenz and Maynard (1).

The only cultigen that did not fit the general trend for seed size was the compact (*cp cp*) type. In addition to small vine size, that gene results in plants with small, deformed seeds, and a low percentage of germination. There are more than twice as many seeds per ounce (approx. 2600) of the compact type compared to the other cultigens (approx. 1100).

In summary, the published number of seeds is very close to the value we measured for the cultigens here (excluding the small-seeded compact type). However, the specific cultigen being used can diverge significantly from the general value of 1100 seeds per ounce.

Table 1. Seed number per gram, ounce, and pound for 9 cucumber cultigens of 5 different types.[z]

		Seeds/g		Seeds/oz		Seeds/lb	
Cultigen	**Type**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Sprint 440	Slicer	32	32-33	921	904-929	14731	14464-14872
Pioneer	Pickle	34	34-34	955	953-958	15283	15252-15324
Calypso	Pickle	35	33-36	991	947-1027	15851	15150-16434
Sumter	Pickle	36	35-36	1018	1002-1029	16291	16028-16458
Dasher II	Slicer	37	36-37	1037	1035-1042	16600	16554-16676
M 21	de de	39	37-39	1098	1050-1118	17562	16799-17886
Poinsett 76	Slicer	45	45-46	1278	1268-1291	20541	20286-20655
Little John	ll ll	46	46-47	1313	1303-1323	21005	20845-21176
Compact	cp cp	92	86-98	2611	2444-2790	41784	39102-44644
	LSD (5%)	2	-	72	-	1146	-
	CV (%)	4	-	4	-	4	-

	Mean (all cultigens)	44	-	1247	-	19951	-
	Mean (compact excluded)	38	-	1076	-	17222	-

[z] Data are from 4 replications of 500 seeds each. Cultigens are pickling, slicing, compact (cp), determine (de) or little leaf (ll) types.

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Electrophoretic Examination of *Cucumis sativus* L. and *Cucumis melo* L.

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The genus *Cucumis* contains 30 species of which only two species, *C. sativus* (cucumber) and *C. melo* (muskmelon), are extensively cultivated. While *C. melo* has chromosome number of x=12, and *C. sativus* has x=7, the attempts to produce interspecific hybrids between the two have not succeeded (2,3). An isozymic analysis was designed to compare the two taxa and determine whether justification exists to classify them as a single genus. Taxa were compared using peroxidase (PRX), glutamate oxaloacetate transaminase (GOT) and esterase (EST).

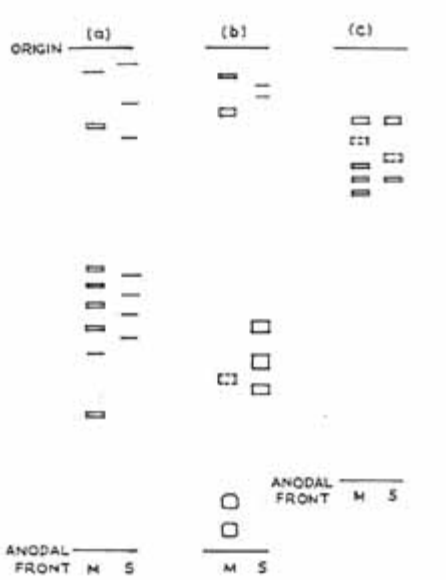
Methods. Polyacrylamide gel electrophoresis was performed using varietal slab gels (ADCO, India) at 5°C under 40 MV in the following manner. Peroxidase (PRX) was sampled from the root and hypocotyl region of 4 to 5 week-old seedlings. Gels consisted of 7% acrylamide, and electrophoresis was carried out using a Tris-chloride gel buffer (pH 8.3). Gels were stained according to Conklin and Smith (1). Glutamate oxaloacetate transaminase was sampled from 3 to 4 day-old seedling, and electrophoresis was performed using 9.5% gels. Gel and electrode buffers were the same as those used for peroxidase, and staining procedures were those of Shaw and Koen (6). Esterase was sampled from 3 to 4 day old seedling and extracts electrophoresed on a 7.0% gel. Gel and electrode buffers were the same as above, and staining was performed according to Shaw and Koen (6).

Results. In the peroxidase system, *C. sativus* was lacking in the fastest moving PRX which was present in all the *Cucumis* species (x=12; data not shown). The allozymes of *C. sativus* were observed at PRX2, PRX3 and PRX4, corresponding to the three loci of *C. melo*. However, allozymes of *C. sativus* were not similar in mobility to those of *C. melon* (Fig.1).

The two taxa shared a common band at GOT4. This allozyme was common to the 13 *Cucumis* species studied and absent in the other general in the *Cucurbitaceae* like *Citrullus, Luffa, Momordica, Praecitrullus, Lagenaria* (data not presented). The allozyme at GOT1 present in *C. melo* was absent in *C. sativus*. The allozyme at GOT2 of *C. sativus* had identical mobility with the hybrid isozyme at GOT2 in *C. melo*. However, since the banding pattern at GOT2 in *C. melo* was identified to be a hybrid type, the allozyme at GOT2 of *C. sativus* was treated as having a different subunit constitution than that of the hybrid allozyme of *C. melo*. The allozymes at GOT3 also differed in mobility. There were no similarities between the two taxa in esterase zymograms.

Data suggest that there is little similarity between *C. melo* and *C. sativus* for the 3 enzymes studied. However, isozyme constitution at GOT4 in both species was characteristic of the genus *Cucumis,* and justifies their classification under the genus *Cucumis*. This conclusion contrasts to that of Pangalo (4), who suggested that the two *Cucumis* species should be elevated to generic status because of their wide variability, non-crossability, and chromosome number differences. Also, Ramachandran and Seshadri (5) consider *C. sativus* cytogenetically very different from *C. melo.* Our data (common band at GOT4) of *C. melo* and *C. sativus* lends support to the proposition that these widely divergent taxa remain under one genus.

Fig. 1. Comparison of zymograms of *C. sativus* (S) and *C. melo* (M) for peroxidase (a), esterase (b), and glutamate oxaloacetate transaminase (c).



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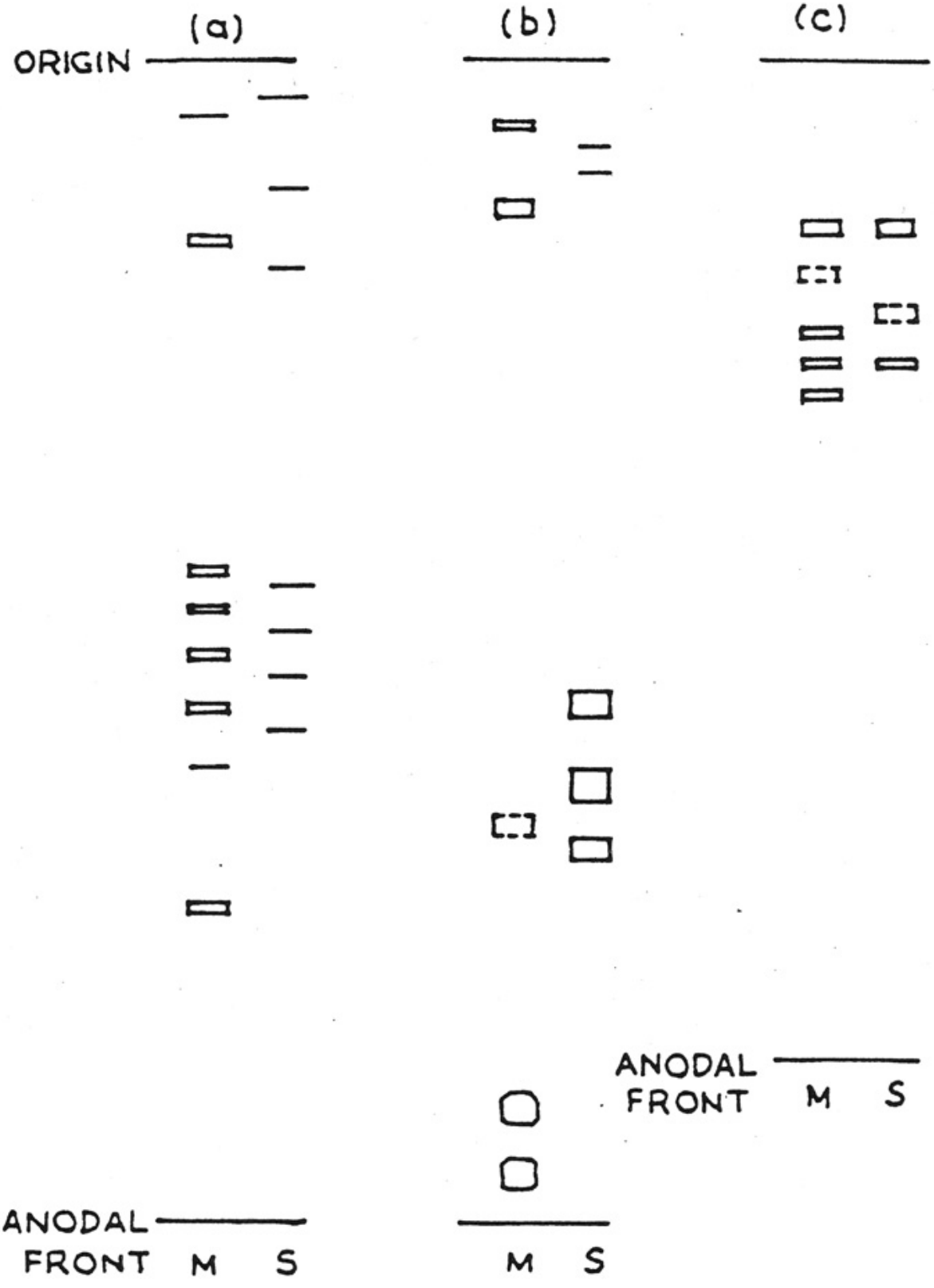
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Cucurbit Genetics Cooperative Report 12:20-22 (article 9) 1989

Improvements of *in vitro* Growth of Cucumber

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In general, *in vitro* culture of cucumber (*Cucumis sativus* L.) meets with several problems. In regeneration studies, adventitious buds as well as somatic embryos develop poorly into plants, which show structural abnormalities and absence of apex formation (1,3). The *in vitro* culture of complete plants is hampered by vitrification, precocious flower formation, and cessation of growth (2). In order to improve this, in vitro growth of small shoot tips and axillary buds was studied. Attention was paid to the effect of better aeration during the continuous culture of cucumber plants.

Seeds of *C. sativus* cultivar Hokus (Rijk Zwaan, De Lier) and *C. sativus* var. *hardwickii* (IVT Gene bank number 0777) were aseptically germinated in honey jars on 40 ml Murashige-Skoog medium with 3% (w/v) sucrose and 0.6% (w/v) agar (Oxoid Bacteriological). The jars were closed with white, partially transparent plastic lids. Three seeds were incubated per jar. The cultures were grown at a 16 hour photoperiod (Philips TL 84, 1 Klux in the jar) at 25°C day / 23°C night. Shoot cuttings, including the cotyledons, were excised from the seedlings 8 days after germination and subcultured on fresh media. After 2 weeks, shoot tips and nodal cuttings were collected from the plants obtained.

Three experiments were designed. In experiment 1, shoot tips of different sizes (2, 3 and 5 mm in length) were compared for their growth capacity. In experiment 2, nodal cuttings with internodes of different length (2, 10 and 20 mm) were examined for the ability of the axillary buds to develop into complete plants. In both experiments, the period of culture was 4 weeks, under the same conditions as described for the seedlings.

Experiment 3 was designed to study continuous *in vitro* culture of cucumber plants by successive subculturing. Each subculture was started from nodal cuttings with a 15 mm internode. Effects of growth conditions were studied. Aeration was changed using 3 methods of closing the jars: a plastic lid, one layer of vitafilm (Good Year) and 3 layers of vitafilm. Light was reduced by covering the jars with layers of cheese cloth.

Shoot tips. As a consequence of choosing main axes of different length from 'Hokus' plants, the size of the basal leaf of the cuttings differed considerably (Table 1). All cuttings survived incubation, but the amounts of growth and plant formation were different. The large cuttings generally developed into normal plants with 4 full-grown leaves having blade lengths up to 40 mm. In contrast, most cuttings of 2 and 3 mm in length initially showed arrest of growth. After growth had started, very compact plantlets developed with 2 to 4 small leaves with blades about 10 to 20 mm in length. Upon subculture, these plants did not regain normal growth, but instead formed numerous flower buds in the axils.

Nodal cuttings. As consequence of the varying length of the internodes attached, the distance rom axil to medium was different (Table 2). Normal plants developed from the long cuttings of 10 and 20 mm length. The short cuttings, however, formed compact plants with small leaves. In general, the features of these plants were similar to those grown from shoot tips.

Table 1. Capacity of plant formation *in vitro* of *Cucumis sativus* cv. Hokus shoot tip cuttings of different sizes. The plants obtained after 4 weeks are classified by length.[z]

Cutting size (lengths in mm)		**Classes of plant length (%)**		
Main Axis [v]	**Blade of basal leaf**	**50-10 mm**	**10-50 mm**	**50-75 mm**
2	0	90	5	5
3	3-7	80	10	10
5	10-16	0	14	86

[z] Each treatment comprised 21 shoot tips.

[y] Length measured from apex to base.

Table 2. Capacity of plant formation *in vitro of Cucumis sativus* cv. Hokus nodal cuttings with internodes of different lengths. The plants obtained after 4 weeks are classified by length.[z]

		Classes of plant length (%)		
Internode length (mm)	**Distance from axil to medium (mm)**	**5-10 mm**	**10-50 mm**	**50-75 mm**
2	0	90	10	0
10	5-7	0	10	90
20	15-17	0	5	95

[z] Each treatment comprised 21 shoot tips.

Continuous in vitro culture. In the initial culture, the nodal cuttings yielded plants that grew well, but after 2 and 3 subcultures, plants developed which showed several irregularities such as abundant flower formation, vitrification and stunted growth. The leaf color became light green. These problems were more obvious in 'Hokus' than in the *C. sativus* var. *hardwickii* accession. Sealing the culture jars with vitafilm instead of using the plastic lids considerably improved the condition of the plants. One layer of the film proved to be better than 3 layers. In that treatment, plants were produced having vital, dark green leaves and without flower bud formation, but plant extension growth as well as leaf size were reduced. Moreover, the culture medium desiccated rapidly. These problems could be overcome by covering the jars sealed with one payer vitafilm with one layer of cheese cloth, which reduced the light to approximately 1 klux, and by application of 5 ml sterilized water on top of the solid medium. Under these conditions continuous culture of cucumber plants was successful.

From the results in this study, we concluded that cucumber cultures that grow well can be obtained by starting from relatively large cuttings (shoot tip or nodal.) Cultures started from small cuttings proved to be less successful. As can be deduced from the experiment with the nodal cuttings, the distance from the culture medium rather than the size of the cuttings appeared to be important. Apparently, close contact of the apex or axillary bud with the culture medium prevents their normal development into plants, possibly because of a disturbance in functioning of the endogenous hormones. This might also be an explanation for the poor development into plants of adventitious buds and somatic embryos of cucumber under normal tissue culture conditions. The continuous culture of cucumber plants is improved considerably under special conditions, viz. culturing in jars sealed with a thin vitafilm instead of closing them with an air-tight lid. This suggests that the culture needs aeration. Cucumber plants in culture apparently produce certain harmful gases, such as ethylene, which can diffuse through the thin vitafilm.

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Haploid Gynogenesis in *Cucumis sativus* Induced by Irradiated Pollen

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A very efficient method of doubled haploid production is now commonly used in muskmelon (*Cucumis melo* L.) breeding programs (2). This method consists of the induction of gynogenesis *in situ* with gamma-ray irradiated pollen, then followed by rescue of haploid embryos by *in vitro* culture.

My first attempts to apply the same method in cucumber (minicucumber type) were promising and produced viable gynogenetic haploid plants. (1). this study was undertaken in order to develop the method for cucumber.

The gynogenetic induction and development process is similar in muskmelon and cucumber. In the two cases, when an irradiation from 300 to 1000 Gy was applied, the pollination with such irradiated pollen induced normal development of fruit and seed coats. In one fruit, only a small number of seeds were not empty. Three weeks after pollination with irradiated pollen, these seeds contained either a single embryo, which was haploid, or an undifferentiated structure that was probably a pseudo-endosperm or an aborted embryo. Embryo and endosperm were never observed together in the same seed. Some haploid embryos had reached the cotyledon stage, while other embryos were less differentiated (globular, heart shaped, or torpedo stage).

In cucumber, a great variation in the rate of gynogenetic induction was recorded among fruits. This heterogeneity was observed regardless of genotype studied (minicucumber type with different levels of parthenocarpy). The mean rate of viable plants was about 3 per 1000 seeds if all the developed seeds produced in fruits after pollination with irradiated pollen were taken into account. However, after pollination with normal pollen in the minicucumber type under our culture conditions, only 30 to 60% of seeds were full. Therefore, for each genotype, the real rate of viable gynogenetic plants might be calculated according to the mean number of ovules susceptible to be fertilized. This rate in the minicucumber type was near 1%.

Cucumber haploid plants were propagated *in vitro* by successive microrootings. Spontaneous diploidization was frequent in root meristems especially when plants had undergone several cycles of microrooting. These plants grew rapidly and normally in soil and produced staminate and pistillate flowers which were generally smaller than diploid ones. Furthermore, their petals were not joined together at the corolla base. The plants remained haploid and produced pollen grains typical of haploid plants. Chromosome doubling was obtained by colchicine treatment of haploid cuttings in *vitro*. Doubled haploid plants produced normal and fertile pollen and normal seeds.

Further studies are in progress to i) increase the production of viable haploid plants, ii) apply the soft X-ray radiography technique to detect haploid embryos in immature seeds as it has been shown in melon (3), and iii) perform the technique of chromosome doubling.

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Preliminary Data on Haploid Cucumber (*Cucumis sativus* L.) Induction

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Pollination of muskmelon (*Cucumis melo* L.) with irradiated pollen resulted in the production of haploid embryos which developed into haploid plants *in vitro* (2). The same method was used to induce haploid cucumber embryos (3). In the latter case pollination with irradiated pollen (400 to 600 Grays, 60 Co) followed by ovule culture gave 0.3 percent viable plants. These plants were haploid (x=7) when first meristems were evaluated and exhibited mixoploid chromosome numbers (with some 3x and 4x cells) later. No details of varieties, media or plant numbers were published. The objective of this study was to determine whether a haploid cucumber F1 cultivar (2n=2x=14) could be obtained using this method.

Methods. Pistillate flowers of the cultivar Polan F_1 were pollinated with pollen which had been subjected to one of two levels of irradiation (900 or 300 Grays, 60 Co.). All flowers pollinated with normal pollen produced fruits with on average 400 seeds each. After irradiation of pollen, fewer fruits developed with 250 seeds per fruit. All control seeds contained normal embryos, while only 13 embryos were produced after irradiation of pollen with 300 Grays (Table 1). Eighteen to 20 days after pollination, these embryos (heart to cotyledonary stage) were excised from seeds and cultured on E20 medium (2). Embryos were smaller than those of the control, with abnormalities on cotyledons (with respect to size, position and color) and in proper embryo development.

From these 13 embryos, 8 plants were obtained. These plants were transplanted onto a P medium (1) which promoted further development. The chromosome number of four of these plants was estimated in root and stem meristems using the Feulgen method. After four weeks in culture these four plants were micropropagated and their chromosome number was estimated a second time (Table 2).

Results. It was found that, after micropropagation of plant n°3 and plant n°4, a number of cells in new root meristems had undergone spontaneous chromosome doubling. The four remaining plants exhibited tetrological changes and were difficult to micropropagate.

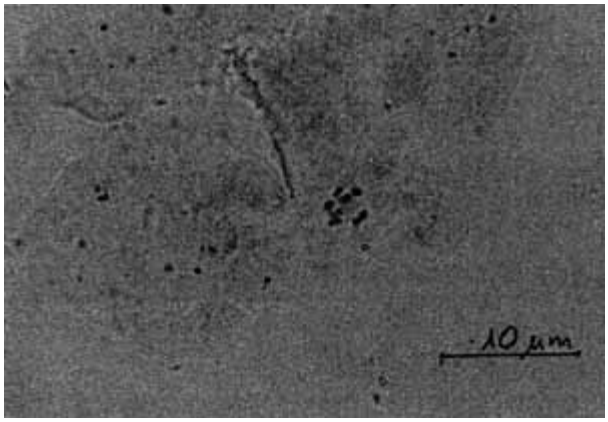
Table 1. Number of fruits and embryos obtained after pollination of 'Polan' F_1 with irradiated pollen.

Grays	Pollinated flowers	Number of fruits	Embryos
900	10	5	0
300	10	6	13
Control	5	5	All seeds with embryos

Table 2. Number of chromosomes before and after micropropagation in mitotic division and numbers of plants in clones.

Plant number	Chromosome number		Number of plants in clones
	Before prop.	After prop.	
1	7	7	13
2	7	7	11
3	7	7 and 14	7
4	7 and 8	7 and 14	11

Fig. 1 Chromosomes in root meristem of haploid n=x=7 cucumber plant.



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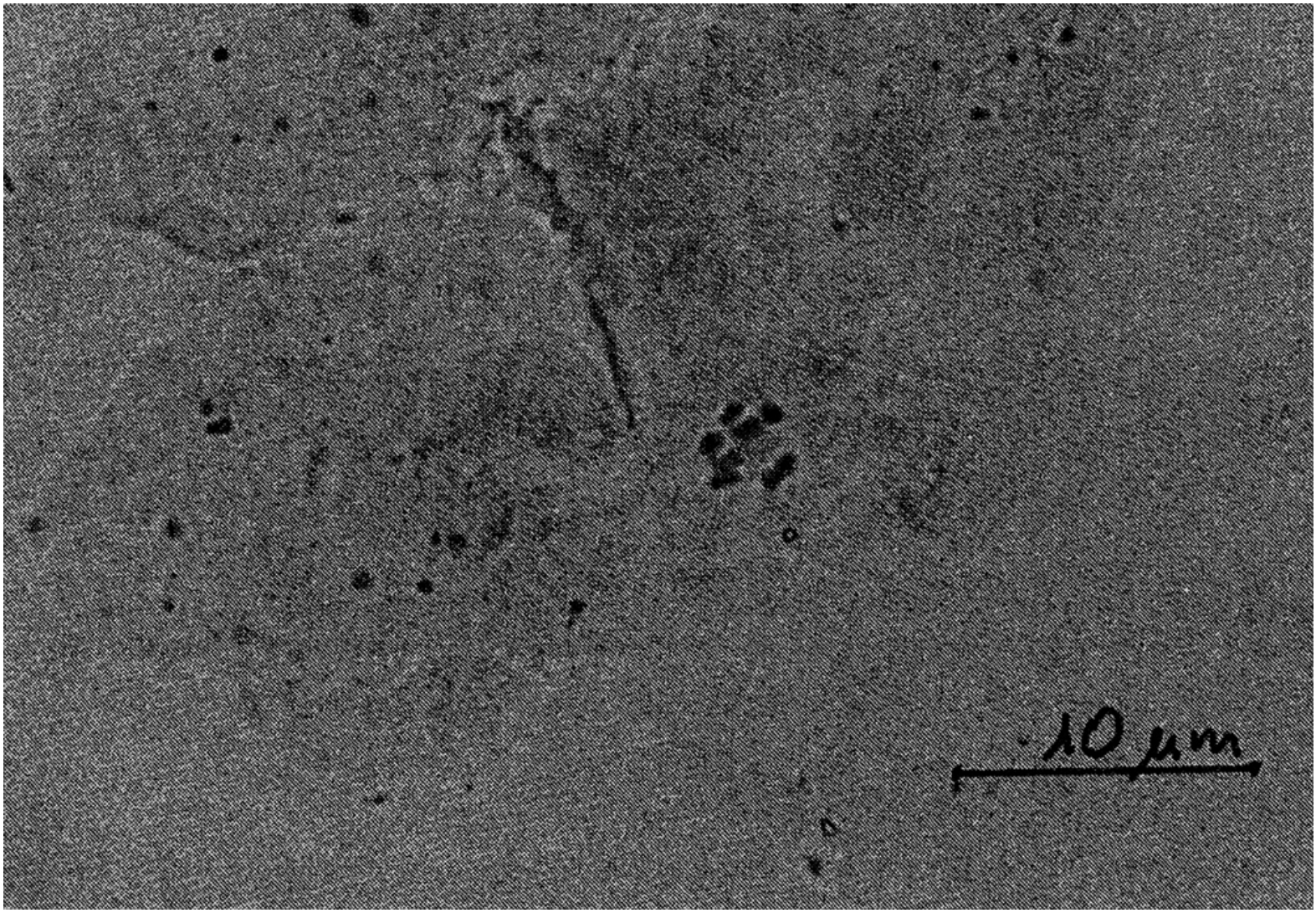
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Cucurbit Genetics Cooperative Report 12:26-29 (article 12) 1989

Isolation and Culture of *Cucumis metuliferus* Protoplasts

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In the southeastern United States, approximately 12% of the potential cucumber yield is lost to root knot nematodes (*Meloidogyne* spp.). Screening of the *Cucumis sativus* germplasm revealed no resistant accessions (4). Within *Cucumis*, the species *C. metuliferus* has shown medium- to high-level resistance to root knot nematode (3). Traditional sexual hybridization techniques have been unsuccessful in producing hybrids between *C. sativus* and *C. metuliferus* (1). Protoplast fusion is one possible method of overcoming the barriers which exist between these two species. Before fusion work can take place, techniques for protoplast isolation and culture of *C. metuliferus* need to be established. The objective of this study was to develop a procedures for protoplast isolation and culture of *C. metuliferus* protoplasts.

Methods. Cucumis metuliferus PI 482454 seeds were sterilized using the industrial disinfectant LD (Alcide Corporation, Norwalk, Conn. USA 06851) for 30 minutes at the suggested rate of 1:1:10 for base, activator, and double glass-distilled water, respectively. Seeds were rinsed 5 times with sterilized water, and placed onto C1 medium (Table 1) and incubated in the dark at 30°C. After 84 hours, seedlings were placed in a growth room held at 22°C and 16 hours of light (8,000 lux). Twenty four hours before protoplast isolation, seedlings were transferred back to 30°C in darkness.

An enzyme solution was prepared consisting of 0.7 mM KH_2PO_4, 7 mM $CaCl_2$ * $2H_20$, 0.5 M mannitol, 3 mM MES [2-(N-morpholino)ethanesulfonic acid], 2% cellulysin(Cal. BioChem.), and 0.5% macerase (Cal. BioChem.). This solution was then mixed at a 1:1 ratio with C2 medium (Table 1) as described by Durand et al. (2), modified by adding an additional 230 mg/l $CaCl_2$ * 2H20 (5). Ten ml of enzyme-C2 solution were added to 0.5 grams of cotyledons (5 to 7 days old), which were then vacuum infiltrated at 9.33 kPa for 20 seconds. The infiltrated tissue was put into sterile 50 ml flasks on a gyrator run at 60 rpm at 25°C in the dark. After 6 hours of digestion, the protoplasts were separated by gently swirling the 50 ml flasks. Protoplasts were isolated from cell walls and other debris by filtering through sterilized miracloth (Cal. BioChem.).

Protoplasts were washed 3 times with C2 medium by centrifuging at 100 g for 3 minutes. Viability was determined using a fluroscein diacetate stain (7). Protoplasts were cultured in 5 ml of C2 medium at a density of $1x10^5$ protoplast/ml, in 10 x 60 mm petri plates, and incubated in the dark at 25°. Five days after protoplast isolation, half of the plates were moved to a 30°C chamber in the dark. Seven days after protoplast release, 1 ml of C3 (Table 1) medium was added to each plate. Fourteen and 21 days after isolation, 1 ml of C4 (Table 1) medium was added to each plate. Protoplast culture plates were briefly swirled daily to increase aeration.

Estimates of plating efficiency (percentage of protoplast which had undergone cell division) were made 8 to 10 days after isolation. Plating efficiency was estimated by visual observation of 5 samples per plate, at 320X magnification. Plating efficiency was calculated by counting the number of cells with clearly defined (1 or more) cell divisions. Using the sample results, total number of divided cells per plate was calculated. This number was then compared to the total number of protoplasts in the plate ($5x10^5$) to produce an estimate of plating efficiency. Approximately 3 weeks after isolation, the number of microcalli per plate (clumps of 8 to 64 cells which appeared to have originated from 1 cell) were estimated. The number of microcalli per plate was estimated by counting the number of microcalli in 5 samples per plate (100X magnification), and calculating an approximate number per plate from the random visual counting. Experiment 1 was a randomized complete block with 4 replications.

In experiment 2, protoplasts were isolated and cultured using the methods described above. After 3 weeks, microcalli

suspensions were pipetted onto C5 medium (Table 1) containing different amounts of 2,4 dichlorophenoxyacetic acid (2,4-D), indoleacetic acid (IAA), kinetin (kin), and benzylaminopurine (BA) (Table 2). The callus cultures were maintained at 22°C in the dark for 3 weeks before being rated for percentage of the petri plate covered with callus. Callus color was rated 1 to9 (1-3 = white, 4-6 = yellow, 7-9 = brown). For both experiments, protoplast viability and number of protoplasts isolated per gram of tissue were determined. Experiment 2 was a randomized complete block with 4 replications.

Results. Protoplast viability (as determined by fluroscein diacetate staining) was consistently between 80 and 100%, and the number of viable protoplasts isolated per gram of tissue was 8.2±2.5 X 10^6. In both experiments, protoplasts rapidly regenerated cell walls and underwent cell division. Cell wall regeneration was determined by observed changes in protoplast shape, and actual cell division. In experiment 1, protoplasts cultured at 25°C had a plating efficiency PE) of 4%. Protoplasts cultured at 30°C had a PE of 7%. Analysis indicated there was a significant difference between the 2 temperatures for plating efficiency. After 3 weeks of culture at 25°C, each plate had an average of 3970 microcalli, while culture of protoplasts at 30°C produced an average of 5025 microcalli per plate.

In experiment 2, medium A3 (Table 2) was best for producing a large amount of yellow, friable calls. the color ratings showed no significant differences among media, but protoplasts cultured at 30°C were significantly whiter. Callus color appeared to indicate potential for continued proliferation because callus with ratings above 5 usually had little or no continued growth, even when transferred to fresh media. Although no plant regeneration recurred from any of the 4 media, medium A3 provided the means for producing large amounts of callus which could subsequently be transferred to a embryo inducing medium.

From these two experiments, successful isolation of a large number of viable protoplasts, and regeneration of cell walls of *C. metuliferus* protoplasts was achieved. A rapid method of producing protoplast-derived callus, suitable for possible plant regeneration was also found. In future experiments, we will attempt to increase the plating efficiency of isolated *C. metuliferus* protoplasts, and regenerate plants from culture.

Table 1. Components used for culture media for *C. metuliferus* protoplasts.[z]

	Medium				
Component	**C1**	**C2**	**C3**	**C4**	**C5**
Mannitol	-	0.3 M	0.15 M	-	-
2,4-D	-	0.5 mg/l	0.5 mg/l	0.5 mg/l	-
Kinetin	-	1.0 mg/l	1.0 mg/l	1.0 mg/l	-
Agar (W/v)	0.8%	-	-	-	0.8
Salts and vitamins	1/2 MS[y]	Mod. DP[x]	Mod. DPD	Mod. DPD	Mod. DPD
Sucrose (g/l)	15.0	17.1	17.1	17.1	17.1

[z]All media were adjusted to a pH of 5.8.

[y] Murashige and Skoog salts (6).

[x] Durand, Potrykus and Donn medium (2) modified by Jia et al. (5).

Table 2. Results of callus production from *C. metuliferus* protoplasts.[z]

			Protoplast culture temperature			
			25°C		30°C	
Code no.	**Media Components[y]**		**% plate covered**	**Color rating**	**% plate covered**	**Color rating**
A1	0.01 mg 2,4-D	1.0 mg BA	3.0	6.1	5.0	5.0
A2	0.20 mg IAA	0.5 mg BA	0.0	-	3.0	5.5
A3	0.25 mg 2,4-D	0.5 mg KIN	10.0*	5.3	8.0	4.4
A4	0.50 mg 2,4-D	1.0 mg KIN	2.0	5.3	5.0	3.7

[z] Data are means of 4 replications.

[y] Base medium was C5 (Table 1).

*Significant at 5% level.

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Cucurbit Genetics Cooperative Report 12:29-34 (article 13) 1989

Isolation and Culture of Protoplasts of *Cucumis sativus* and *Cucumis metuliferus* and Methods for Their Fusion

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The introduction of disease resistance into pickling cucumber (*Cucumis sativus* L.) is an essential component of all cultivar development programs. Efforts continue to identify new sources of resistance to major disease problems, such as root-knot nematodes (*Meloidogyne incognita*) and to viruses, such as zucchini yellow mosaic (ZYMV) and watermelon mosaic (WMV). The wild African horned cucumber (*Cucumis metuliferus*) PI 292190 has been shown to carry resistance to *M. incognita* and to ZYMV and WMV-1 (7, 15, 16) but not to WMV-2 (15). Efforts to introgress this germplasm by conventional sexual crosses have not yielded any success due to severe incompatibility barriers (3, 10). Somatic hybridization by protoplast fusion has been one approach that investigators have shown to be successful in transferring traits of interest from distantly related species to cultivated species in *Brassica* (14), *Daucus* (5), *Lycopersicon* (13), *Nicotiana* (4) and *Solanum* (1, 2, 6, 17). In this report, we describe results from studies aimed at establishing a procedure for the isolation and fusion of mesophyll protoplasts of two species, *C. sativus* and *C. metuliferus*. The ultimate goal is to identify somatic hybrids which may bring in traits of interest from*C. metuliferus* into a *C. sativus* background.

Plant materials. Seeds of *C. sativus* Gy 14 and *C. metuliferus* PI 292190 were dipped into 70% ethanol for 20 sec. followed by a 20 min soak in a 20% solution of commercial bleach (Clorox, 5.25% sodium hypochlorite) to sterilize them, followed by 3 rinses in sterile, distilled water. The seed coats were excised under sterile conditions and the embryos transferred to Magenta boxes containing 50 ml of hormone-free Murashige and Skoog (12) basal medium (MS) with full strength macroelements and microelements, myo-inositol (100 mg/l), thiamine HCL (0.8 mg/l), 3% sucrose, and 0.65% Phytoagar. The pH of the medium was adjusted to 5.8 with 1 N KOH prior to autoclaving at 15 psi for 16 min. Boxes were incubated in a walk-in growth chamber set at 18/24°C night/day temperature regime with 16 hr/day photoperiod provided by cool-white fluorescent lamps (intensity of 160 mEM^{-2} sec^{-1}). These *in vitro* cultures were used to provide plant materials for protoplast isolation. The first to third leaf from 15 to 21 day-old seedlings were used as the source of mesophyll protoplasts.

Isolation and purification of protoplasts. True leaves (1 g) were cut into 1-2 mm wide X 10 mm long strips with a scalpel under sterile conditions and placed in 20 ml of the enzyme solution in a 100 X 25 mm petri dish. The optimal concentration of pectinase (Sigma) and cellulysin (Calbiochem) required for both Gy 14 and for *C. metuliferus* was 0.5% and 1.0%, respectively. These resulted in yields of 5 to $6x10^6$/g tissue. Enzyme solutions were prepared in modified MS medium containing half-strength major salts, full complement of minor salts and vitamins, 2% sucrose and 0.25M mannitol. The enzyme solution was sterilized by filtration using a syringe (B-D disposable) and Nalgene disposable filter unit (0.22 mM pore size). Tissues were incubated overnight (15 to 16 h) in the dark at 24±2°C on a reciprocating shaker set at 60 rpm. The resulting suspensions was passed through multilayers of sieve cloth (pore sizes from 50 to 300 mM to separate protoplasts from undigested plant debris. Two rinses in basal medium containing mannitol and centrifugation at 1200 rpm were conducted to remove the enzyme solution and purify the protoplasts. Protoplasts were concentrated as a dark green band at the meniscus of the Babcock bottles following purification. The pellet was removed with a Pasteur pipette and resuspended in basal medium and protoplasts were diluted to the desired density (2.5 to 3.0 x 10^4/ml) for fusion.

Protoplast fusion. One-half ml of the protoplast suspension of each species was mixed in a 60 X 15 mm petri dish and 1 ml of the following fusion treatments were tested: PEG M.W. 8,000 (8) at a concentration of 15% (in final volume), with or without 1% DMSO, for 20 min; high pH/Ca for 15 to 20 min (solution comprised of mannitol, 80 g/l; $CaCl_2 \cdot 2H_20$, 7.35 g/l; glycine, 3.75 g/l; pH 10.0) (9). Following the treatments, the protoplast suspension was washed 2 to 3 times with basal medium to remove the fusigenic agents, and protoplasts were concentrated to a density of 2.5 to $3.0x10^4$/ml.

Protoplast culture. Protoplasts of *C. sativus* at a density of 2/5 to 3.0x10^4/ml or at 0.5 to 0.6x10^4/ml, *C. metuliferus* protoplasts alone, and a mixture of the two species, were plated without any fusion treatment, and following the treatments described above, in MS medium with half strength major salts, full complement of minor salts and vitamins, 2% sucrose, and 0.25 M mannitol. Hormonal requirements were provided by 2,4-D/BA at 5.0/5.0 mM or NAA/BA at 5.0/2.5 mM. The suspension (2 ml) was added to soft agarose (0.4%) in 35 X 10 mm petri dishes. All dishes were incubated in the dark at 24 to 26°C in a growth chamber for the first 7 days, and then transferred to a 16 h photoperiod provided by cool-white fluorescent lamps, intensity of 100 mEM^{-2} sec^{-1}. The days to first division, second-third division, and formation of minicalli were assessed for each species and fusion treatment. An additional treatment was imposed on these platings, namely that of a nurse culture (11). This was achieved by placing droplets of the protoplast suspension (with or without fusion treatments) in the center of the petri dish and placing along the periphery of the dish a 1.0 to 1.5 cm zone of mesophyll protoplasts of *C. sativus* at a density of 2.5 to 3.0 x 10^4ml, avoiding contact with the protoplasts under experimentation by providing a cell free circular zone about 1.5 cm wide.

Results. The protoplast isolation procedure described gave high yields of good quality protoplasts of both species. Without imposing any fusion treatment, protoplasts of *C. sativus* formed minicalli within 13 days when plated at a density of 2.5 to 3.0 X 10^4/ml (Table 1). At a lower density of 0.5 to 0.6 X 10^4ml, a nurse culture system was essential to promote sustained divisions. With *C. metuliferus*, only first cell divisions were observed and there was no development of minicalli. When a mixture of these two species was plated out, division and regeneration of *C. sativus* was inhibited by the presence of *C. metuliferus* protoplasts (possibly due to a dilution of the plating density), but this was overcome by the presence of the *C. Sativus* nurse culture system (Table 1). When fusion treatments were imposed, their effects were determined on control protoplasts of *C. sativus* as well as in mixtures. Both PEG and high pH/Ca delayed the onset of divisions and development of minicalli in *C. sativus*, and this was partially overcome by the presence of the nurse culture (Table 1). Fusion frequencies were estimated to be around 5 to 6% for PEG and 2 to 5% for high pH/Ca. the presence of 1% DMSO in PEG was detrimental, since it caused cell enlargement and rupturing of the protoplast membrane. In mixtures of the two species with PEG or high pH/Ca as the fusigenic agent, (Fig. 1) cell divisions of fused and unfused cells were observed (Gif. 2) and minicalli developed (Fig. 3). These have been subcultured into callus proliferation medium containing full strength MS salts containing the same hormonal combinations, with 3% sucrose and 0.65% Phytoagar.

Discussion. Sustained division of mesophyll protoplasts to produce callus, which eventually gave rise to plantlets (unpublished) was accomplished for *C. sativus* but not in *C. metuliferus*. The lack of regeneration of *C. metuliferus* is advantageous in fusion studies, since only *C. sativus-C sativus* or *C. sativus-C metuliferus* fusions would be selected. Since low plating density affected the extent of protoplast divisions, which occurs because fusion followed by washings dilutes the initial plating density, a nurse culture system was employed in this study. Nutrients or compounds released by the adjacent growing cells enhanced division of mixed cells. Although protoplasts were used to provide the nursing effect, suspension culture cells can also be substituted (unpublished). The nurse culture system also minimized the extent of delay of cell divisions due to the fusion treatments.

PEG 8000 yielded higher fusion frequencies than high pH/Ca in this study. The callus developing from fusion mixtures would be of the *C. sativus-C metuliferus* hybrids. Because high plating densities are required for growth, individual isolation of potential hybrid cells cannot be accomplished. However, hybrid callus or plants regenerated from them should be distinguishable from *C. sativus* by morphological differences, chromosome numbers and isozyme banding patterns. The results described here are the first step toward accessing the desired traits of disease resistance from *C. metuliferus.*

Table 1. Response of protoplasts of *Cucumis sativus* and *C. metuliferus*, alone or in mixture, to a nurse culture and fusion treatments.

		Days required to obtain				
Species		**Nurse culture**	**Cell wall formation**	**First division**	**Second-third division**	**Mini callus**
No Fusion Treatment						
C. sativus [z]		-	1-2	4-5	6-7	13
		+	1-2	4-5	6-7	13
C. sativus[y]		-	3-4	10-20	>20	-
		+	2-3	5-6	9-10	20

C. metuliferus[z]		-	5-6	8-10	-	-
		+	5-6	8-10	-	-
Mixture		-	3-4	10-20	>20	-
		+	2-3	7-10	14	28
With Fusion Treatment						
C. sativus	PEG	-	2-3	5-7	10-14	22-28
		+	2-3	5-6	9-12	20
	High pH/Ca	-	2-3	5-7	10-14	22-28
		+	2-3	5-6	9-12	20
Mixture	PEG	+	3-4	7-8	12-16	28
	High pH/Ca	+	3-4	7-8	12-16	28

[z] Plating density of 2.5 to 3.0 X 10^4/ml.

[y] Plating density of 0.5 to 0.6 X 10^4/ml.

Fig. 1. Mixture of protoplasts of *Cucumis sativus* (S) and *C. metuliferus* (M).

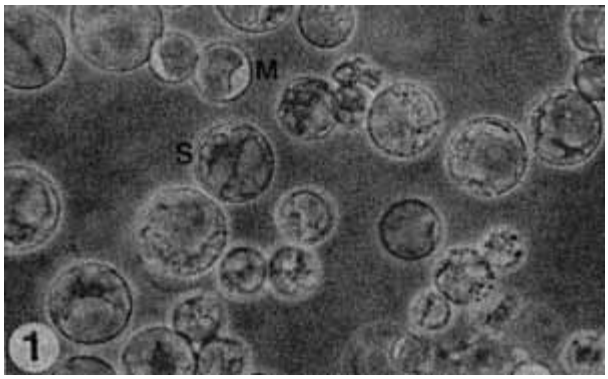


Fig. 2. Close-up of fusion of protoplasts of the two species.

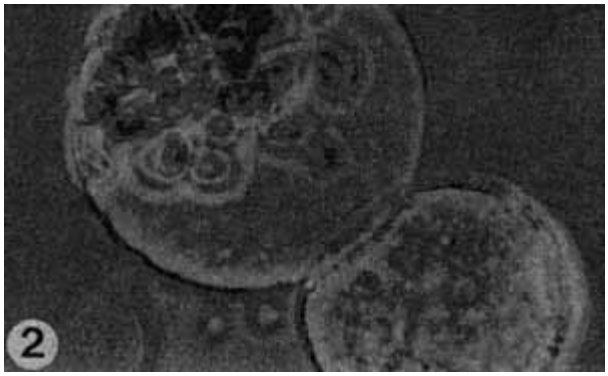
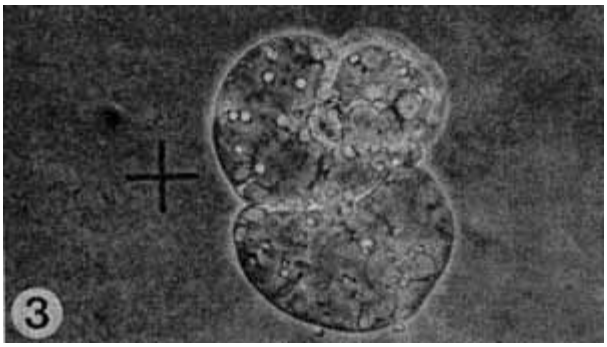


Fig. 3. Cell division of fused cells.



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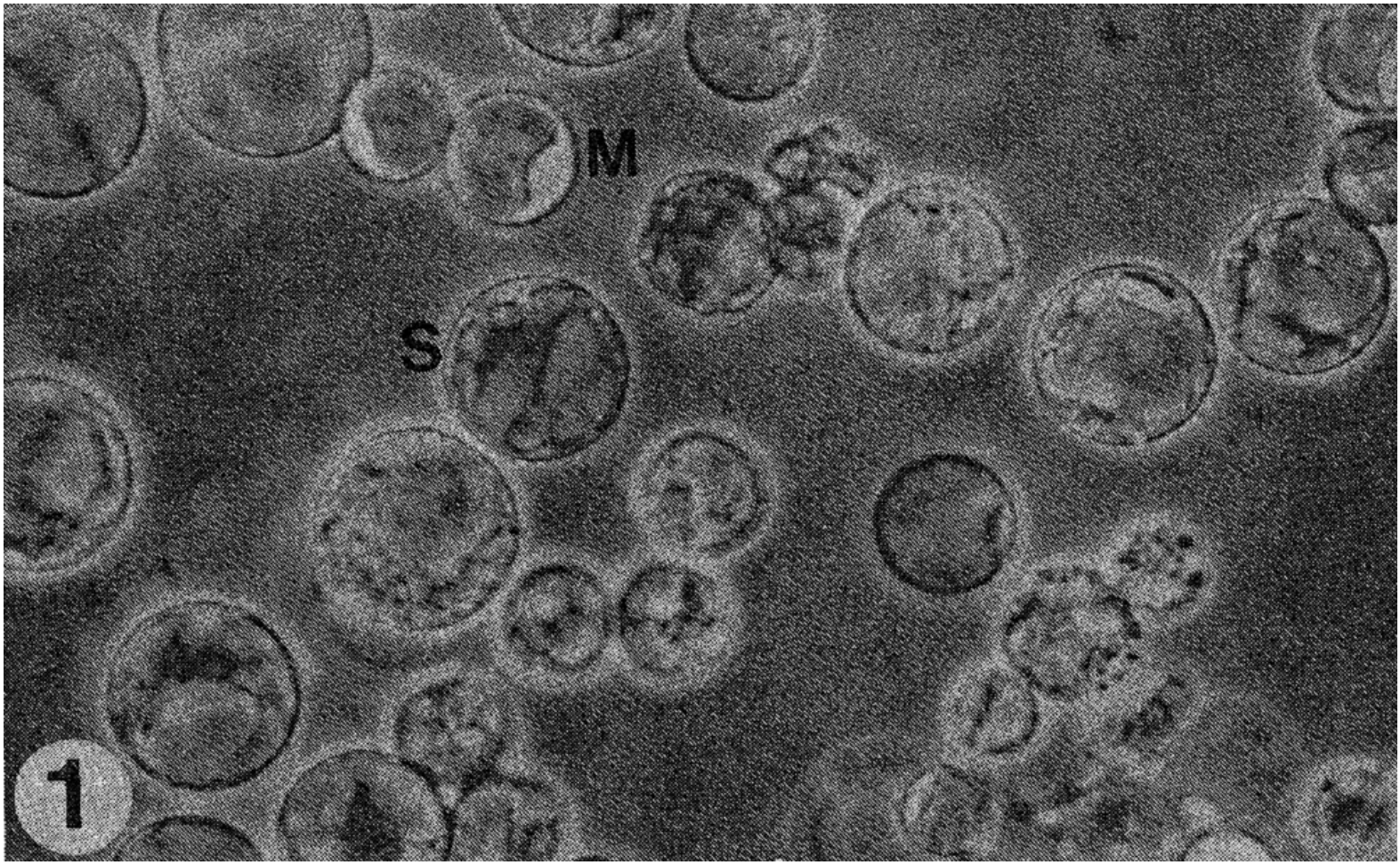
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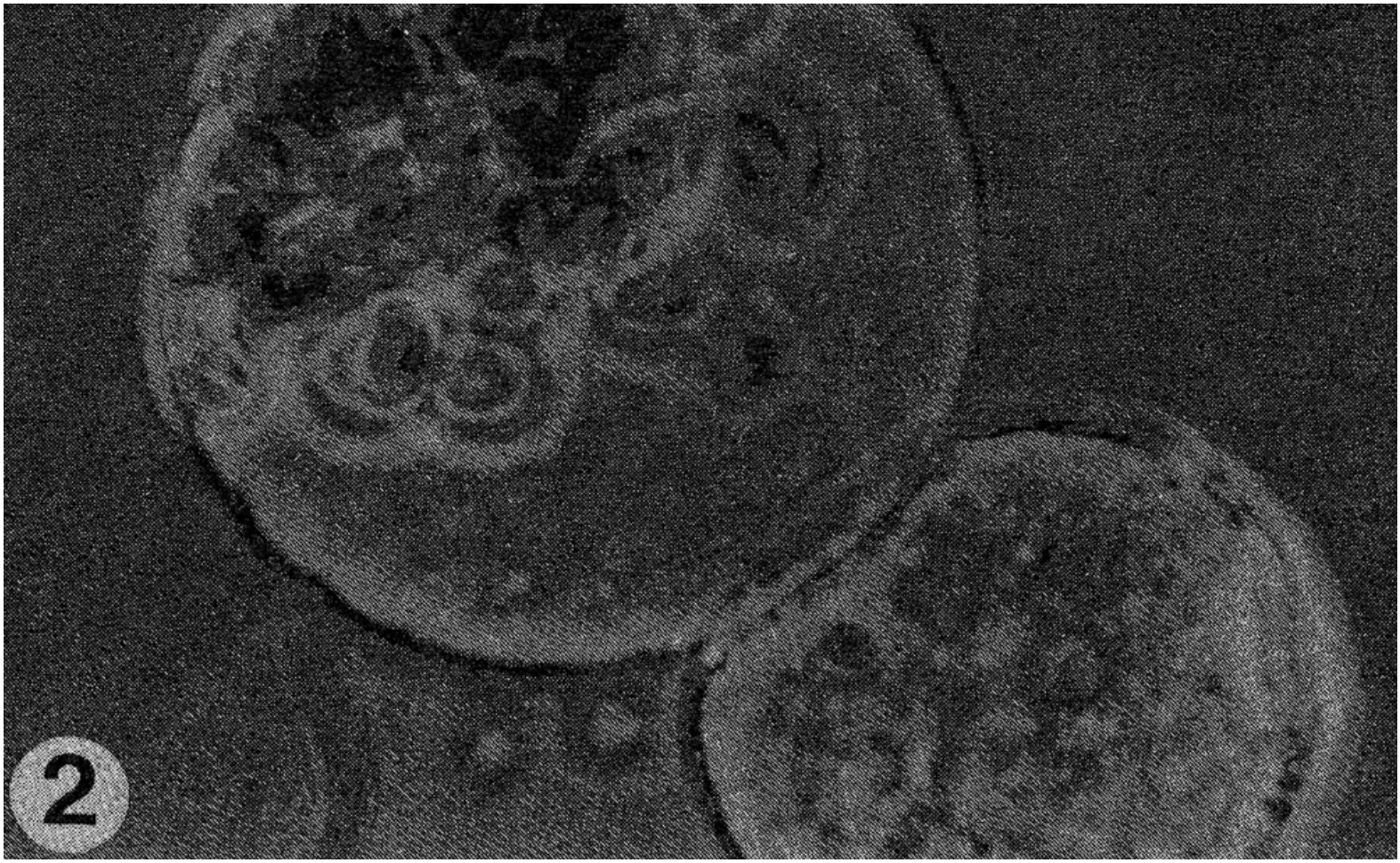
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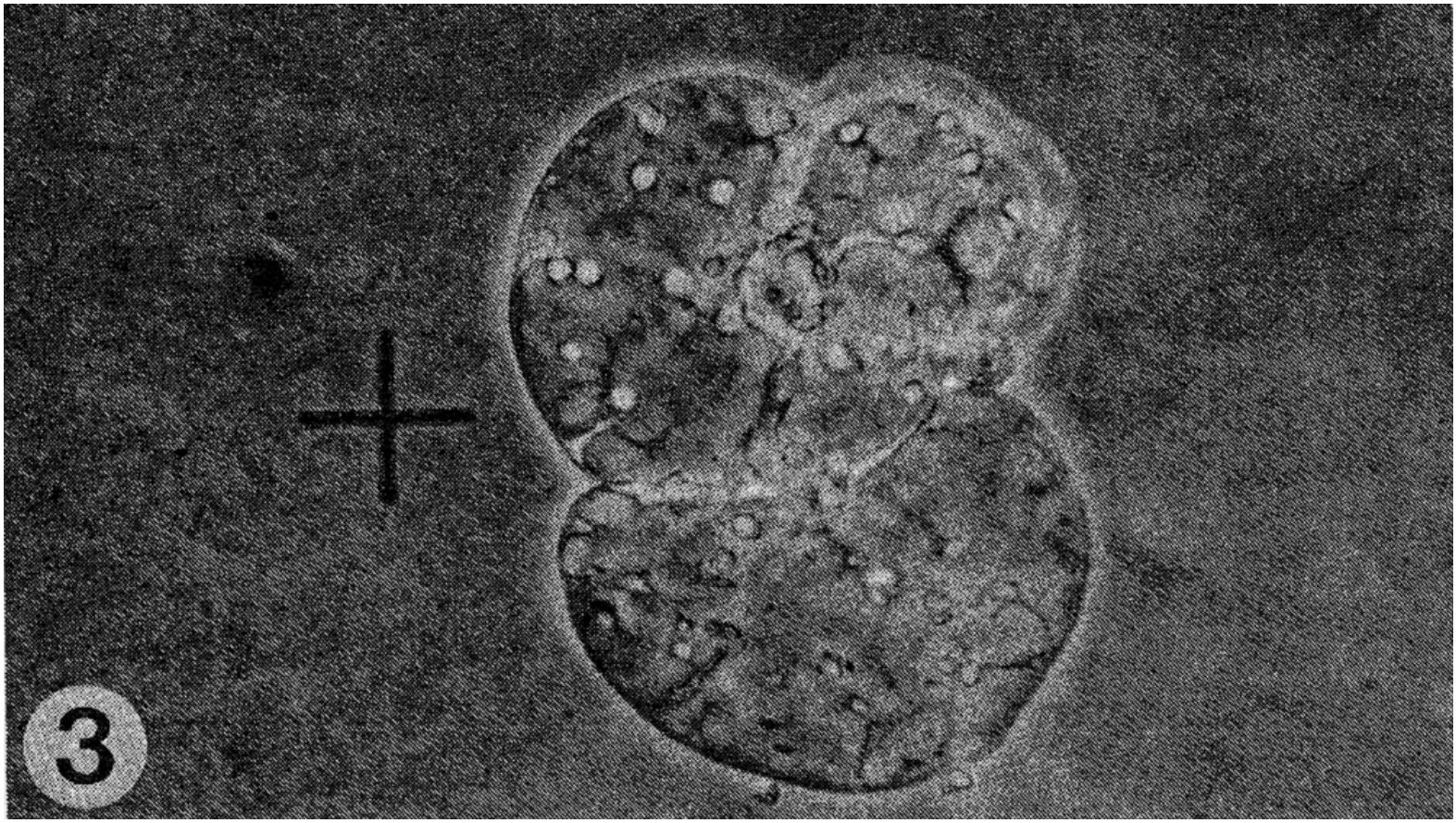
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Cucurbit Genetics Cooperative Report 12:35-36 (article 14) 1989

Transformation of Cucumber with *Agrobacterium rhizogenes*

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Agrobacterium rhizogenes includes hairy root disease in many dicotyledonous plants. The root including ability is conferred to plant cells by bacterial genes (T-DNA) which are transferred from the root including (Ri) plasmid of the bacterium to the plant genome. The Ri plasmid of the often used agropine type strains of *A. rhizogenes* consists of two distinct transformation elements, designated TL- and TR-DNA. TL-DNA contains genes relevant for hairy root induction, whereas TR-DNA contains genes involved in the production of auxin. However, recent reports indicate that TR-DNA alone can cause production of hairy roots (1). Transformation of cucumber (*Cucumis sativus* L.) with *A. rhizogenes* has been reported only once (4). An agropine type strain was used. The results showed that the frequency of Ri T-DNA transfer into cucumber was rather low and that mostly only a small part of TL- or TR-DNA was integrated in the plant genome. This contrasts with *A. rhizogenes* transformation of other plant species, as most Ri-plants analyzed so far contain both TL- and TR-DNA, whereas sometimes the integration of TL-DNA alone is found (1). In this paper, we present preliminary results of *A. rhizogenes* transformation of cucumber inbreds Gy 3 and 'Straight Eight'.

Methods Hypocotyl explants were inoculated on the basal wound with agropine type *A. rhizogenes* strain LBA-9402 and inserted upside down in a Murashige-Skoog solid medium supplemented with 3% (w/v) sucrose. Developing roots were excised from the hypocotyl explants and root cultures were made on the same medium for examining autonomous growth and expression of hairy root phenotype, i.e. excessive formation of lateral roots and partial nongeotropism. After 3 weeks of culture, growing roots were divided in 1 cm explants which were rechecked on a medium without hormones for another 3 weeks. Subsequently, root clones with the hairy root phenotype were tested for agropine and mannopine production. Opine positive clones were subcultured on media with hormones for induction of plant regeneration. Two embryo-inducing media were used; (M1) MS with 5 µM 2,4-D + 5 µM BA (4), and (M2) MS with 4 µM 2, 4-D + 4 µM BA.

Results. The 2 inbred lines reacted rather similarly. After 6 weeks of culture, 75% of the inoculated hypocotyl explants showed root formation from the treated wound surface. No rooting was ever observed from the control explants. A total of 174 roots was excised from the hypocotyl explants, and 58% showed rapid growth on a medium without hormones. Large clones could be obtained from these roots, which clearly expressed the hairy root symptoms. The test on opine synthesis was carried out for only 25 fast growing root clones. In 20% of these clones, opines could be detected. Upon subculture on hormone containing media, these roots formed gray, slowly-growing callus on M1, whereas on M2 a more vital callus was formed from which regular protuberances of an embryonic, yellow callus developed. We transferred the yellow callus into a liquid medium of the same hormone composition as M2. Several somatic embryos appeared in this medium, but until now it was impossible to regenerate plants.

In the root cultures, evident differences were found between the roots derived from the *A. rhizogenes* infected cucumber hypocotyl sections. Approximately 40% of the roots did not show the hairy root phenotype. These roots probably originated as an indirect result of the process of transformation. Approximately 60% of the roots exhibited autonomous growth, accompanied by expression of the hairy root phenotype. This suggests that only these roots contained genes of the Ri T-DNA responsible for hairy root formation, i.e. TL-DNA, TR-DNA, TR-DNA or both. Since only a low percentage of the fast growing roots actually produced opines, the integration of TR-DNA seems to be less common than that of TL-DNA. In agreement with the results obtained by Trulson et al. (4) it is concluded that the integration of T-DNA via *A. rhizogenes* transformation in cucumber is a rather complicated event resulting in roots with different parts of the T-DNA. A careful analysis of the integrated DNA by Southern hybridization is needed to evaluate this phenomenon.

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Cucurbit Genetics Cooperative Report 12:37-38 (article 15) 1989

Tolerance Reaction of Muskmelon to Inoculation with *Fusarium oxysporum f. sp. melonis* Races 0 and 1

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Intermediate resistances have already been described with *Fusarium oxysporum f. sp. melonis* race 2. This study deals with a case of tolerance to races 0 and 1 found in a line 'Vilmorin 109'.

The line 'Vilmorin 109' was crossed with a very susceptible (to race 0 and 1) netted line 'Vilmorin 110'. None of both lines had gene (*Fom-1*) nor (*Fom-2*). Both can be considered homozygous (inbreeding for 9 generations).

Two types of inoculation were tested: A) plantlets were removed from seedling pots at the cotyledon stage; roots were pruned to about 20 mm and dipped for 1 mn in *Fusarium* suspension (10^5 conidia/ml). Then they were transplanted into growing trays and placed in a growth chamber (day/night, 24/18°C, 14/10 hours). B) 5 ml of the same suspension were poured at the basis of each plantlet at the cotyledon stage and placed in the same growth chamber.

Susceptible and resistant controls were respectively cv. Charentais T. and cv. Vedrantais for race 0 and cv. Vedrantais (*Fom-1*) and 'Vilmorin 108': (*Fom-2*) for race 1.

Virulence of both races was studied on the two parental lines an on the hybrid for one month. Two *Fusarium oxysporum* isolates of each race were also studied but they were not statistically different for their pathogenicity. The symptom scale is: (1) no symptom, (3) symptom of physiological disorders, (5) beginning of yellowing, (7) entire plantlet yellowing, (9) plantlet death (fig. 1a, b. c, d).

In every four experiments, 'Vilmorin 109' appeared statistically different from the susceptible and the resistant controls (see table 1). On the other hand the symptoms became visible faster on 'Vilmorin 110' than on the two controls. The level of the F1 ('Vilmorin 109' x 'Vilmorin 110') was between the level of the two parents indicating a partially dominant gene action. This intermediate level, obvious with race 0, was impossible to detect with the race 1 and method A (fig. 1c) but could be seen with method B (fig. 1d). This agreed with the work of Latin and Snell (1) and emphasized that different results could be obtained with different inoculation methods.

In the case of emergence of a new race of *Fusarium* or the spreading out of the race 1, 2, these "minor" genes, beside the 2 genes (*Fom-1*) and (*Fom-2*), will perhaps play a prominent part in muskmelon breeding programs.

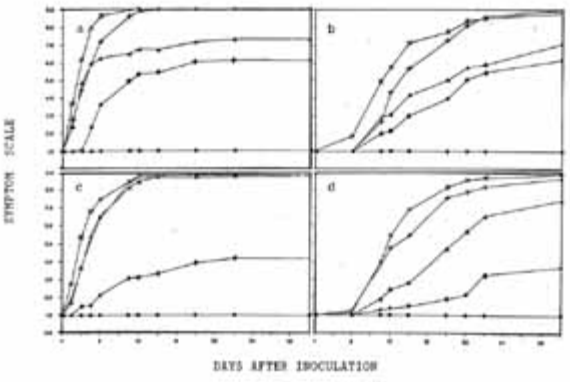

fig.1 Virulence of Fusarium oxysporum f.sp. melonis on five cultivars ; (a) race 0 inoculation method A. (b) race 0 inoculation method B. (c) race 1 inoculation method A. (d) race 1 inoculation method B. ■ : resistant control, + : susceptible control . ♦ : "Vilmorin 109", x : "Vilmorin 110", ▲ : F1 ("Vilmorin 109" x "Vilmorin 110").

Table 1. Newmann-Keuls test on symptom scale (P = 0.05).

	Days after inoculation			
	6 8 14	**11 14 22**	**6 8 14**	**11 14 22**
Cultivars	**fig. 1A**	**fig. 1b**	**fig. 1c**	**fig. 1d**
'Vilmorin 110'	A A A	A A A	A A A	A A A
Susc. Control	B B A	B AB A	AB A A	A B A
Fl (109 x 110)	B B B	B BC B	AB A A	B C B
'Vilmorin 109'	C C C	B C B	BC B B	B D C
Resis. control	C D D	B D B	D B C	B D D

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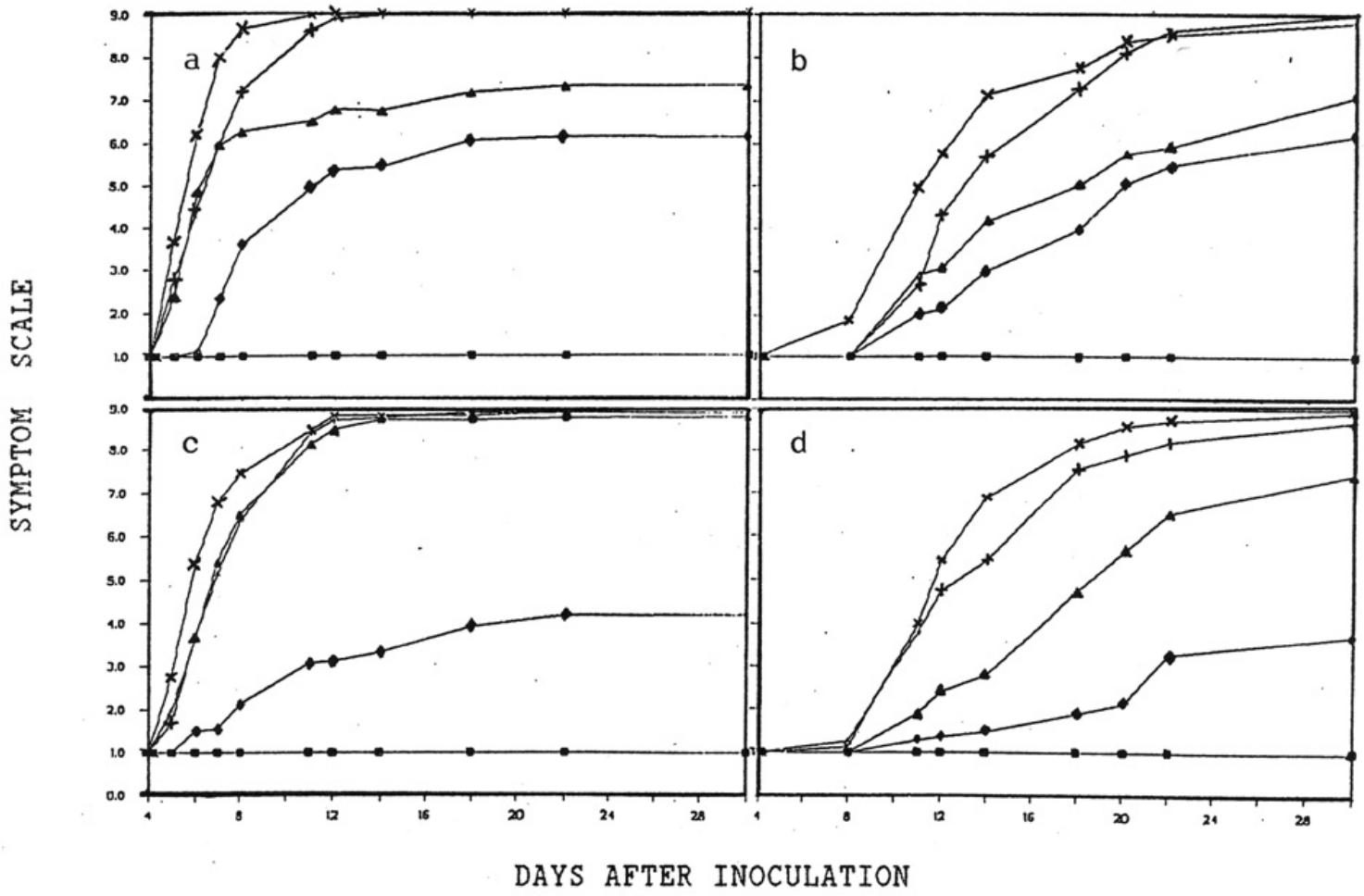

fig.1 Virulence of Fusarium oxysporum f.sp. melonis on five cultivars ; (a) race 0 inoculation method A, (b) race 0 inoculation method B, (c) race 1 inoculation method A, (d) race 1 inoculation method B. ■ : resistant control, + : susceptible control , ♦ : "Vilmorin 109", x : "Vilmorin 110", ▲ : F1 ("Vilmorin 109" x "Vilmorin 110").

Cucurbit Genetics Cooperative Report 12:39-40 (article 16) 1989

Resistance to *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. in Spanish Muskmelon Cultivars

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In 1987 and 1988 several cultivars of Spanish type melons were inoculated with *Sphaerotheca fuliginea* race 1 to determine possible sources of resistance to this pathogen. The cultivars chosen were those who in previous years were free of disease symptoms under natural conditions of infection.

Two methods of artificial inoculation were employed; both used a dry inoculum. In one, a small mass of spores was placed on the leaf surface with a scalpel (M. Pitrat, personal communication). This method allowed a visual check of the efficiency of inoculation success. In the other, the spores were applied by dusting the leaves (1). The inoculum was a strain of *S. fuliginea* race 1 isolated in the Estacion Experimental "La Mayora" (Malaga, Spain) (3).

Table 1. Response of different Spanish melon cultivars against *S. fuliginea* race 1.

Cultivars	Artificial inoculation	Natural inoculation	Observations
AN-C-36	R	+	Piel do Sapo type
C-C-3	S	+	Type not ascribable
AN-C-57	R	0	Yellow type
AN-C-39	S	+++	-
MU-C-44	S	+++	-
AN-C-7	S	+++	-
PI 1224112 B	R	0	Resistant races 1, 2, and 3
PMR 6	R	0	Resistant races 1 and 2
AN-C-68	R	0	Yellow type
PMR45	R	0	Resistant race 1
E-C-14	S	+++	-
AN-C-08	S	+++	-
AN-C-42	R	0	Type not ascribable
J-22112-C	S	+++	-

R resistant; S sensitive.
+++ the symptoms appeared from the start of cultivation period.
+ mild symptoms appear at the end of the cultivation period.
0 no symptoms were observed.

The same cultivars were grown in a polyethylene greenhouse on a sandy soil with drip irrigation in a field with a previous history of powdery mildew. No fungicide applications against the fungus was carried out and the plants were left to be infected naturally. The sensitive genotypes acted as a source of inoculum throughout the cultivation period, and melon genotypes with known resistance to the three races of *S. fuliginea* were used as testers (2).

Crosses have been initiated between the resistant cultivars AN-C-42, AN-C-68 and AN-C-57, and the muskmelon varieties of commercial importance of the Yellow and Piel do Sapo types to study the genetic of this resistance and ways of introducing

it.

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Transmission of the Causal Agent of Muskmelon Yellowing Disease

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Since 1982, a yellowing disease has seriously affected muskmelon (*Cucumis melo* L.) crops cultivated under polythene greenhouses on the southeast coast of Spain. It now seriously affects the profitability of muskmelon growing in this area because it considerably decreases the numbers of fruits per plant and the average fruit weights.

The symptomology of the affected plants is of two types: one starts with small yellow spots on the leaves; the other shows up as an intense yellow stain at the base of the leaf stalk. In each case, the disease spreads until the whole of the leaf, except the veins, is yellowed (1). In both cases, the symptoms start on the old leaves and progress to the younger ones.

The observation that there is a close relationship between the presence of greenhouse white-fly *Trialeurodes vaporariorum* and the appearance of the disease and the symptoms described suggests that muskmelon yellowing disease may be the same as that previously described in Japan (5), Holland (4), France (3) and Bulgaria (2). In each of these works, the cucumber yellows virus (CuYV) is ascribed as the causal agent of the yellowing in spite of the fact that no virus particle was isolated.

To determine the optimum conditions for carrying out controlled infections in experiments in order to select genotypes which might be used to introduce resistance to this yellowing disease into commercial varieties usually cultivated in this area, the three following possible types of disease transmission were studied: a) transmission by *Trialeurodes vaporariorum*, b) mechanical inoculation and c) seed transmission.

The experiments were carried out at temperatures between 25°C (max) and 11°C (min), with a relative humidity of 70% and a 16:8 hr light:dark cycle. The vegetable material employed was *Cucumis melo* var. Piel do Sapo.

The conclusions arrived at from the results were that the greenhouse white-fly *Trialeurodes vaporariorum* acts as the vector causal agent of this yellowing disease. Under the conditions in which the experiments were carried out, at least 40 days were required to confirm transmission. The symptoms observed in the infected plants were identical to those described above. The disease was not transmitted by mechanical inoculation of the infected extract.

No case of seed transmitted disease was observed in the 100 plantings obtained from seeds of diseased muskmelon plants which were previously inoculated using *T. vaporariorum* as vector.

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Search for Sources of Resistance to Yellowing Disease in *Cucumis* spp.

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The unpromising results obtained from earlier experiments seeking sources of resistance to muskmelon yellowing disease in a large collection of different muskmelon (*Cucumis melo* L.) cultivars under conditions of natural infection led to this present search for wild species with resistance to this disease.

In addition to three wild species -- *Cucumis zeyheri*, *C. anguria* var. *longipes*, and *C. myriocarpus* (A) and (B) -- shown the year before to have satisfactory resistance to yellowing disease (2), this present work studied six new wild species of the genus *Cucumis*. The two sensitive cultivars Piel de Sapo and Bola de Oro were used as controls.

Previous work in this laboratory (3) demonstrated that the greenhouse whitefly *Trialeurodes vaporariorum* is the vector of transmission of the causal agent of yellowing disease; consequently, in this work, the populations of whitefly on each species were estimated. The 12 species (Table 1) were cultivated in the same polyethylene greenhouse in sandy soil with drip irrigation.

Table 1. Incidence of yellowing disease and presence of whitefly *Trialeurodes vaporariorum.*

Species	Yellowing symptoms	Whitefly population
Cucumis myriocarpus (A)	1/10	+
Cucumis myriocarpus (B)	10/10	+++
Cucumis zeyheri	10/10	+++
Cucumis anguria var. *longipes*	1/10	++
Cucumis anguria var. *anguria*	0/10	+
Cucumis africanus	0/10	+++
Cucumis meeusii	0/4	++
Cucumis dipsaceus	0/10	+++
Cucumis figarei	0/10	+
Cucumis melo var. *agrestis*	3/10	++
Piel de Sapo (*)	10/10	+++
Bola de Oro (*)	10/10	+++

(A) Resistant line. (B) Sensitive line. (*) Controls (*C. melo* cultivars). n/n = Plants with symptoms / Plants observed.

Cucumis zeyheri exhibited resistance the year before (2), but was found to be sensitive in this present work. The appearance of symptoms of yellowing in some generally resistant accessions suggests the need for controlled artificial inoculations using *T. vaporariorum* as the vector.

Cucumis melo var. *agrestis* showed good resistance. Because this accession belongs to *C. melo*, it is the most interesting one to introduce the yellowing resistance into cultivated muskmelons.

In the experiments with *Cucumis africanus* and *C. dipsaceus* no symptoms were observed although the populations of whitefly were similar to those of the controls. It can be supposed that these accessions are resistant to the disease

transmission by *T. vaporariorum*, but it is necessary to prove this behavior using controlled infections before making such an assertion.

A study has been initiated of the genetics of the resistance to yellowing found in *C. myriocarpus*. Likewise, there is on-going a program seeking to transfer the genes for disease resistance discovered in some wild species to commercial cultivars. This bridge was designed to exploit the known interspecies compatibilities of the *Cucumis* genus described in (1).

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Resistance to Yellowing Disease in Muskmelon

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Cultivation of greenhouse muskmelon on the south east coast of Spain is being seriously affected by a yellowing disease (3). The disease is transmitted by greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) (5). The causal agent has not been detected yet, but other whitefly transmitted diseases of cucurbits that cause similar symptoms are virus diseases (1, 2, 4, 6). All the muskmelon hybrids and cultivars grown in the area have shown high levels of susceptibility. Therefore in 1985 we initiated a program to search sources of resistance.

We have evaluated 189 accessions of Spanish landraces from 1985 to 1988. The accessions were distributed through 5 tests. These were carried out during the 1985, 1986 and 1987 seasons in Algarrobo-Costa (Malaga) and during the 1987 and 1988 seasons in El Egido (Almeria). The incidence of the yellowing disease in both localities is very high. The tests were made under natural infection conditions.

Only one accession, which belongs to 'Tendral' type and which was evaluated during 1988 in El Egido, behaved as resistant/ The observed resistance have to be confirmed under controlled inoculation conditions. The remaining accessions were notably affected. The 'Piel de Sapo' and 'Tendral' types landraces have a tendency to show susceptibility levels which are slightly lower than 'Amarillo' and 'Rochet' types.

In the season of 1986 we evaluated other muskmelon genotypes of non-Spanish origin. These were 'Nagata Kim Makuwa', 'Muchianskaja', 'Miel Blanc', 'Freeman's cucumber', 'Kafer Hakin', PI 161375, PI 157084 and PI 157080. All of them were susceptible to yellowing disease but 'Nagata Kim Makuwa'. PI 161375 and PI 157084 showed levels of symptomology lower than the Spanish landraces. The behavior of these three genotypes during the seasons of 1987 and 1988 were heterogeneous since some plants which belong to them displayed a high susceptibility whereas others were slightly affected (Table).

Also from among the plants which belong to progenies derived from the crosses between highly susceptible parents ('Galia' and 'Piel de Sapo') and 'Nagata Kim Makuwa' or PI 161375 there were always some of them slightly affected whereas the remaining ones were seriously affected.

It is important to state that all the plants of the highly susceptibility accessions (188 Spanish landraces and 6 non-Spanish genotypes) showed severe symptoms.

In the season of 1987 we started to test wild cucurbits species since we thought that the only possible thing to do was to resort to these species as sources of resistance to yellowing disease. But during the 1988 season, in Algarrobo, the majority of plants of an accession of *Cucumis melo* var. *agrestis* showed resistance under natural severe infection conditions. Only 3 of the 13 plants displayed slight symptoms of yellowing disease. If the behavior of this accession and the previously mentioned landrace were confirmed, the present prospect of muskmelon breeding for resistance to yellowing disease could be substantially changed.

Table 1. Incidence of yellowing disease in the genotypes which were slightly affected during the 1986 season.

Genotype	Locality and season of test	Incidence of yellowing disease[z]
Nagata Kim Mikuwa	Algarrobo 1987	14/9
	El Egido 1987	3/18
	Algarrobo 1988	5/10
PI 161375	Algarrobo 1987	18/7

	El Egido 1987	16/4
	Algarrobo 1988	2/11
PI 157084	Algarrobo 1987	15/0
	El Egido 1987	15/0
	Algarrobo 1988	6/4

[z] a/b: slightly affected plants/seriously affected plants.

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A Screening Procedure for ZYMV Resistance in Muskmelons

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Breeding for disease resistance is often restricted due to the lack of reliable and efficient screening procedures. Resistant individuals may be identified by symptomatology, bioassay, and/or serology; however, in some situations, these methods are of limited applicability. Zucchini yellow mosaic virus (ZYMV), a recently reported virus in the potyvirus group ($), has caused appreciable economic losses in many cucurbit species. The economic impact of this virus has been especially significant on various types of melons growing in the irrigated semi-arid Imperial Valley of California. Melon line PI 414723 has been identified as a source of dominant, single gene resistance to this virus (7). Our group has been utilizing this material in a breeding program designed to transfer ZYMV resistance into the western shipping-type muskmelon. We have previously reported a procedure to vegetatively propagate the breeding progenies, as well as a two-step evaluation of this material (3). In this report, we describe a procedure based on the use of cDNA probes to screen for ZYMV resistance in our laboratory. Similar procedures have been developed for the identification of other viruses and viroids and have been found to be highly sensitive and efficient (1, 6, 8.).

cDNA copies of several regions of the ZYMV genome were developed by AJR according to standard procedures of cDNA cloning (5). The cDNA clones were tested for their specific homology to the ZYMV genomic RNA and not to several closely related potyviruses by hybridization (DNA/RNA) before using them as diagnostic probes. Out of approximately two hundred clones tested, several dozen had a high degree of homology and selectivity for our ZYMV isolate. The sensitivity was found to be in the picogram range of viral RNA.

For screening purposes, the breeding progenies were vegetatively propagated (3). The plants were mechanically inoculated with ZYMV freshly extracted by grinding leaves of a source squash plant ('Early Prolific' zucchini) in a 10 mM Potassium Phosphate buffer, pH 7.0 with 1% (w/v) celite as an abrasive. It has been observed that the virus infects most efficaciously under greenhouse conditions if the plants are infected at the 2-3 new leaf stage after being repropagated. The systemic mosaic symptoms appear within 2-3 weeks post-inoculation.

Leaves of these plants are harvested (1-5 gm of tissue) and ground in liquid nitrogen. The frozen powdered tissue is soaked in 12 ml of a 1 X SET solution [1% SDS (sodium dodecyl sulfate), 1 mM EDTA, 25 mM TRS-HCL pH 7.5], and 0.5 ml of 10 mg/ml Protease K for approximately 2 hr at 37°C. The extract is centrifuged at 10,000 *g* for 15 min and the pellet discarded. The supernatant is treated with 0.5 ml of 10 M ammonium acetate and 25 ml of 95% ethanol and the nucleic acids allowed to precipitate at -20°C. The pellet is collected by centrifugation, as described above, air dried, and redissolved in 1X SET and reprecipitated. This is repeated 2-3 times until its spectroscopic analysis showed it to be fairly pure nucleic acid (260/280 ratio greater than 1.7). Finally, the pale green pellet is resuspended in 0.5 ml of 1X SET and the nucleic acid content measured by spectroscopy. All samples are first adjusted for the same amount of nucleic acid, then denatured with 7.5% formaldehyde at 65°C for 10-15 min, brought to 10X SSC (1.5 M Sodium Chloride and 150 mM Sodium Citrate pH 7.0) and finally spotted under vacuum onto nitrocellulose paper in a 96-well manifold (Bethesda Research Laboratories). The nitrocellulose paper is baked at 80°C for 90 min to fix the nucleic acids onto this paper. The unbound portion of the nitrocellulose paper is blocked by prehybridization at 65°C overnight with 100 µg/ml denatured salmon sperm DNA and 5X Denhardt's solution (1% polyvinylpyrolidone, 1% ficoll, and 1% Bovine Serum Albumin) in 0.5% SDS and 6 X SSC. Hybridization to the nicktranslated (P^{32}labelled according to 5) cDNA probe of ZYMV is carried out in the same solution, this time with probe at 65°C, now for approximately 2 days. Unhybridized probe is stringently washed off the paper prior to autoradiography; first 2 times in 2X SCC and 0.1% SDS at 65°C for at least 30 min each 2 then additional times in 0.1X SSC and 0.1% SDS at 65 ° C, for similar times.

The data presented in Fig. 1 is a representative example. Lanes 1-12 are as follows: 1-3 are uninfected healthy controls; 4, 5, and 6 are inoculated F_2 plants without symptoms; 7 is a 'Top Mark' plant with symptoms; 8-11 are field samples showing some type of mosaic symptoms and lane 12 is a squash plant infected with ZYMV as a positive control. The presence of spots at lanes 4, 6, 7, and 12 and absence at 1-3 show that this probe is capable of hybridizing to ZYMV. The absence at lane 5 reflects resistance in this F_2 segregate, while ZYMV detection in lanes 4 and 6 may suggest tolerance in these F_2 segregates. The lack of detection at lanes 8-11 indicates that this probe does not hybridize to false positives. These field samples had mosaic symptoms apparently from an infection by another virus and not from ZYMV. Samples from other field plants, not connected with this study, but infected with ZYMV have tested positive with this probe (AJR, personal communication).

The relative intensities of the dots shown in Figure 1 were quantified using a LKB Ultroscan XL laser densitometer. These values are presented in Table 1 as the area of the dot's peak adjusted for 1 µg of total nucleic acid applied. These figures reflect the conclusions discussed above while allowing for a comparison of the relative titer of virus in each sample. The virus levels in the symptomless F_2 plants seen in lanes 4 and 6 are approximately 30% of the levels found in the susceptible plant presented in lane 7.

the resistance to ZYMV has been considered as a Mendelian character. The data presented by Pitrat (7), based on symptomatology, can be explained by a single dominant gene (*Zym*) and is similar to Tobacco Mosaic Virus resistance found in *Nicotiana glutinosa* (conferred by the *N* gene) (2). While preliminary, our data suggest a more complex genetics. The finding of tolerance, as well as the ability to easily sort infected F_2 plants in the greenhouse, on the basis of severity of symptoms, into several classes is similar to Ryegrass Mosaic Virus resistance in rye grass (9). This resistance is considered polygenic.

The authors again wish to stress the preliminary nature of our data, however, we also wish to stress the power of the molecular probe. Not only can the cDNA probe be used to detect and quantitate a specific viral presence, and thus be useful in a breeding program such as ours, but the molecular probe can be used to help elucidate the genetics of resistance as well as to identify a possible source of viral potential in an otherwise healthy population.

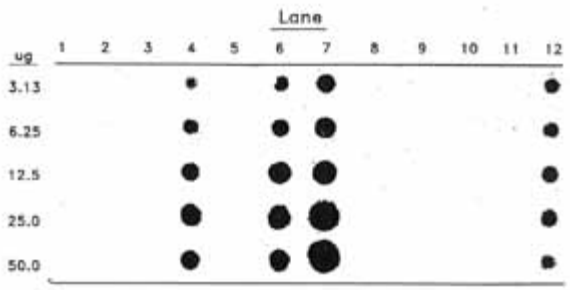

Figure 1. Dot blot analysis of plant extracts hybridized to a radiolabelled cDNA probe to ZYMV isolated from the Imperial Valley of California. Pleas see text for lane designations. µg refers to the amount of total nucleic acids applied to each dot in that row.

Table 1. Relative quantitation of ZYMV levels from dot blot analysis presented in Figure 1.

Lane [z]	**Rel. amount** [y]	**Lane** [z]	**Rel. amount** [y]
1	0.000	7	0.774 ± 0.084
2	0.000	8	0.000
3	0.000	9	0.000
4	0.249 ± 0.019	10	0.000
5	0.000	11	0.000
6	0.215 ± 0.006	12	0.110 ± 0.015

[z] Please see text for lane designations.

[y] Relative amounts are in absorbance X peak width (mm) adjusted for one microgram of total nucleic acid.

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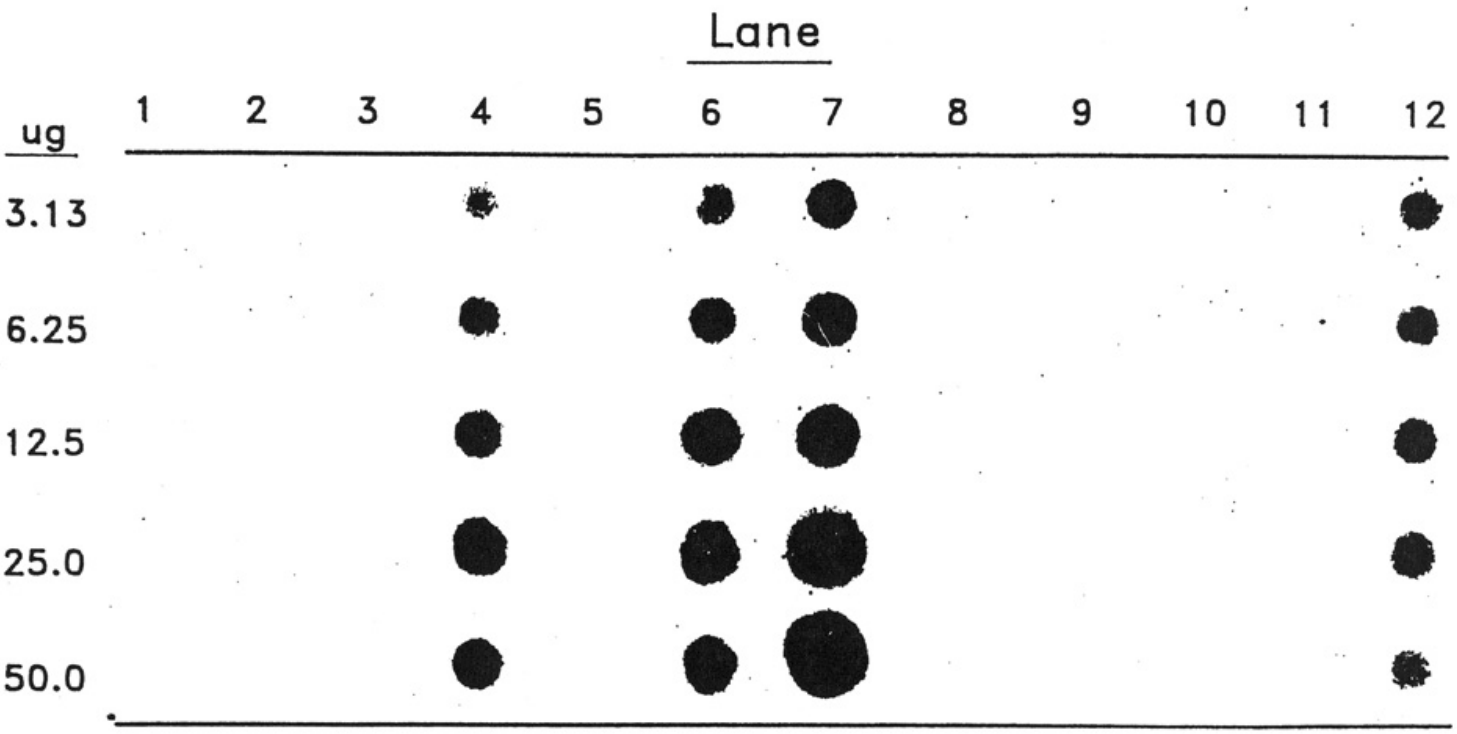
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Cucurbit Genetics Cooperative Report 12:50-52 (article 21) 1989

Low Temperature Germination in Muskmelon is Dominant

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Muskmelon seed (*Cucumis melo* L.), like most domestic cucurbits, requires 17 to 18°C for germination (threshold temperature). At lower temperatures the membrane transfer from the 'dry' to the 'wet' phase is very slow, resulting in destructive leakage of solutes (H. Nerson, unpublished data). The existence of germplasm with low-temperature germination potential in muskmelon has previously been reported (1, 2). This report provides preliminary information regarding the inheritance of low temperature germination in this species.

The birdsnest inbred line P202, which possesses an ability to germinate at 15°C (2), was crossed with Noy-Yizreel (NY), an indeterminate cultivar which does not germinate at this temperature. The F_1's were produced in a greenhouse (Winter 1985) and the F_2's in a field nursery (Spring 1985) at Newe Ya'ar Experiment Station (northern Israel). Seeds were kept at 10°±2°C and 45-55% RH for three years before evaluation of germinability. Four replicates of 25 seeds were germinated in 9 cm petri dishes on Whatman No. 2 blotting paper moistened with 4 ml deionized water under optimal (28°C) and low (15°C) temperatures in the dark. In a greenhouse 10, 15 and 50 replicates (10 seeds each) of parents, and reciprocal F_1 and F_2 progeny, respectively, were sown in 3 liter pots containing a soil : sand : peat (2:1:1) medium for an emergence test. The maximum day and minimum night temperatures ranged between 25 to 32°C and 18 to 21°C, respectively. Germination (radicle length > 3mm) and emergence (cotyledon above soil) were recorded during a 3 week period to determine final percent and rate (mean days germination - MDG, and mean days emergence - MDE).

The preliminary results (Table 1) demonstrate the low-temperature germination in P202 is dominant. It could be hypothesized that more than one dominant gene (perhaps 2) are involved in the expression of this character. This hypothesis is currently being tested using BC_1 families. Germination rates in reciprocal F_1 progeny indicates that there is a significant maternal effect. Progeny of P_2 X P_1 matings having NY maternal tissues are slower to germinate than their reciprocals. This effect was essentially eliminated in F_2 progeny germination. The maternal effect in the F_1 was probably limited to radicle initiation, since there was no significant difference in emergence rate (MDE) at the suboptimal temperature tested.

Table 1. Germination, and emergence percentage and rate of P202 (P_1) and NY (P_2) muskmelon and their F_1 and F_2 progeny.

		Percent Germination		Mean days to Germinate		Emergence	
		28°C	15°C	28°C	15°C	Percentage	MDE[1]
P_1	(P202)	100±0	98±2	1.18±0.08	7.06±0.10	91±5	7.32±0.72
P_2	(NY)	91±2	2±2	2.86±0.34	-	85±8	9.96±1.14
F_1	(P_1X P_2	94±2	87±8	1.21±0.08	8.34±0.26	85±10	6.71±0.91
F_2	(P_1X P_2)	75±15	82±9	2.67±0.70	13.48±0.58	85±8	7.90±1.19
F_2	(P_1X P_2)	97±2	87±5	1.09±0.06	9.65±0.59	94±3	6.90±0.75

F_2	(P_2X P_1)	98±2	89±3	1.02±0.03	8.48±0.50	96±1	7.01±0.86

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Ethylene Production by Germinating Seeds of Different Sexual Genotypes of Muskmelon (*Cucumis melo* L.)

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The use of gynoecious genotypes has been proposed as a method for muskmelon hybrid seed production. The identification of gynoecious plants is necessary for the introduction of this character into agronomically interesting lines; in order to save time and space this identification should be done as early as possible.

It is known that cucumbers and muskmelon gynoecious lines produce more ethylene than monoecious genotypes (1, 3). Ethylene production from cotyledonary disks of cucumber changes with different sexual expressions (5). Germinating seeds of gynoecious cucumbers produce more ethylene than monoecious, andromonoecious or hermaphroditic lines (4). In this study, we tried to assess whether it was possible to identify different muskmelon sexual genotypes by measuring the ethylene produced by germinating seeds.

The plant material used in these experiments were made up of the following cultivars or lines: 'Piel de Sapo' and 'Invernizo', both andromonoecious local cultivars, line 8502, a monoecious local line, and the gynoecious line WI 998.

Ten seeds of each of the above muskmelon lines or cultivars were placed on moistened filter paper and introduced into 12.5 ml glass flasks sealed with a rubber serum cap and maintained at 30±0.5°C. Five replications were performed on each line or cultivar and the number of germinated seeds were counted 3 days later. One ml of the internal gas was taken from each flask with a chromatographic syringe and the ethylene contents of that gas determined by gas chromatography.

During the germination, seeds of the gynoecious 'WI 998' produced more ethylene than the other sexual genotypes, among which no significant differences were found (Table 1).

Table 1. Mean ethylene production (nl) by germinated seeds of four sexual muskmelon genotypes,

Genotype	**Total C_2H_4 production (nl)**	**C_2H_4 production/germinated seed**
Piel de Sapo	1.2	0.14 a[z]
Invernizo	0.8	0.10 a
8502	0.9	0.11 a
WI998	1.9	0.21 b

[z] Means followed by different letters are significantly different (Newman-Keuls' test, p=0.05).

Thus, it seems possible to identify the gynoecious line WI 998 by measuring the ethylene produced by germinating seeds. This agrees with Rudich et al. (4), who found that germinating seeds of gynoecious monoecious and hermaphroditic plants. It will be necessary in the future to assess whether this higher ethylene production in germinating seeds of WI 998 will be kept when the gynoecious trait is introduced into other genetic backgrounds.

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Cucurbit Genetics Cooperative Report 12:53-54 (article 23) 1989

Flesh Calcium Content of Group Inodorus and Group Reticulatus Muskmelon (*Cucumis melo* L.) Fruits

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Honeydew and casaba (group Inodorus) muskmelons tend to have extended storage lives when compared to netted (group Reticulatus) muskmelons (5). Decline in storage is usually manifested by flesh softening and breakdown, and by shriveling and discoloration of the rind. Genetic differences in storage life may be attributable in part to differences in the timing and magnitude of the ethylene climacteric in the different types of muskmelons (2, 4).

Calcium may also be involved in regulating ripening in muskmelons. Higher calcium concentrations can retard ripening and senescence activities in many climacteric fruit tissues (1). The slower decline in flesh firmness of ripening fruits with higher calcium concentrations has been attributed to the ability of calcium to combine with pectin to form a calcium pectate in cell walls (3). The current study was initiated to determine whether differences in flesh calcium concentrations existed among different types of muskmelons, and whether these differences might be related to fruit longevity in storage.

Two casaba, two honeydew and two netted cultigens of muskmelon were grown under identical conditions in Salisbury, Md. Six ripe fruits of each cultigen were harvested on the same day. ripeness was determined on netted types by abscission of the fruit from the vine, while ripeness of honeydew and casaba melons was determined by fruit softening at the blossom end. Fruits were transported back to College Park, Md., and three fruits of each cultigen were sampled immediately for percent dry weight and flesh calcium content. The remaining three fruits were stored for 7 days at 10°C and 95% RH, then sampled. For calcium determinations, ashed tissue samples were dissolved in boiling 5N HCl, filtered, and subjected to atomic absorption and emission spectrophotometry.

Flesh dry weight and calcium concentration for the six cultigens are presented in Table 1. The analyses of variance for the effects of muskmelon type and storage on dry weight and calcium content are presented in Table 2. Percent dry weight was similar among the different fruit types but increased during storage, probably as a result of fruit dehydration. Calcium, on both a fresh and dry weight basis, was significantly affected by muskmelon type. However, the casaba cultigens, which have the longest storage life, had the lowest calcium concentrations. In particular, the casaba 'MaryGold', which can be stored for over two months in a marketable state (personal observation), had the lowest calcium concentration among all lines. Honeydew, which are intermediate in storage ability between casaba and netted types, had the highest calcium concentration.

Although this study was preliminary in nature, it seems unlikely that major differences in the rate of fruit ripening and senescence among group Inodorus and group Reticulatus muskmelons can be simply explained on the basis of flesh calcium content.

Table 1. Flesh dry weight and calcium content in honeydew, casaba and netted muskmelons at harvest or stored for 7 days at 10°C.

		Dry Weight (% fw)		Flesh Calcium Content			
				µg Ca/g fw		µg Ca/g dw	
Muskmelon type	**Line**	**Fresh**	**Stored**	**Fresh**	**Stored**	**Fresh**	**Stored**
Casaba	MD8562	0.14	0.14	1.07	0.91	7.82	6.78
	MaryGold	0.13	0.14	0.85	0.91	6.55	6.44
Honeydew	MD85100	0.13	0.15	1.46	1.26	11.54	8.50
	MD8599	0.13	0.14	1.40	0.88	10.90	6.70

Netted	MD8540	0.12	0.13	1.05	1.02	8.55	8.17
	MD266	0.12	0.15	1.15	1.02	8.52	6.73

Table 2. ANOVA for effect of muskmelon type on dry weight and calcium.

Dependent variable	**Muskmelon type**	**Storage**
Flesh dry weight	NS	*
Calcium (fw basis)	*	NS
Calcium (dw basis)	*	*

NS, * indicate not significant, and significant at the 5% level.

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Direct and Indirect Regeneration of *Cucumis melo* L. from Cotyledon Culture

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Successful *in vitro* selection requires the regeneration of plants from unorganized tissue. We have previously reported the regeneration of three cultivars of muskmelon ('Hales Best', 'Iroquois' and 'Perlita') using a modification of the protocol described by Moreno et al. (3). However, regeneration with other growth was low for 'Perlita' and 'Iroquois'. Regeneration with other growth regulator combinations has been reported (1, 4). To improve low regeneration efficiency alternate growth regulator combinations were tested.

Surface disinfestation and removal of cotyledons was accomplished as previously described (2). Cotyledons were plated on 25 ml of medium contained in 15x90 mm petri dishes. The basal medium consisted of Murashige and Skoog salts and vitamins, 3% sucrose, 0.8% Phytoagar supplemented with 0.0, 0.5, 1.0, 2.5, or 5.0 $mg \cdot l^{-1}$ benzyladenine (BA) and 0.0, 0.1, 0.25, 0.5, or 1.0 mg 1^{-1} naphthalene acid (NAA) in factorial combination. Rooting medium consisted of basal medium supplemented with 0.001 mg 1^{-1} NAA dispensed into 55X70 mm jars (42.5 ml). The pH was adjusted to 5.7 to 5.8 with NaOH and HCL prior to autoclaving for 20 minutes at 121°C, 124 kPa.

Primary callus initiated on basal medium supplemented with either 0.5, 1.0, or 2.5 $mg \cdot l^{-1}$ BA was subcultured on basal medium supplemented with either the same level of BA or the next two higher levels of BA. Cultures from treatments producing less friable or morphogenic callus were then subcultured in the same manner for each new level of BA.

Cotyledon cultures were grown for 28 days either in the dark or under 16 hr photoperiods from cool white fluorescent lamps (~50 $\mu Em^{-2}s^{-1}$) at 25°C. Subcultured callus was transferred every 28 days. Cultures with shoots were transferred to basal medium with 0.1 $mg \cdot l^{-1}$ BA for shoot elongation. Shoots were excised from cotyledons or callus clumps and rooted under 16 hr photoperiods from cool white fluorescent lamps (50 $\mu Em^{-2}s^{-1}$). Rooted shoots were transferred to sterile 1:1 Jiffy mix:soil contained in Plant Cons (Flow Laboratories, McLean, VA 22102) When shoots and roots began active growth, plants were transferred to 6" plastic azalea pots and acclimated in a greenhouse mist chamber for seven days before placement under in vivo conditions.

The effect of the absence or presence of light was similar to that previously reported on medium supplemented with kinetin and indoleacetic acid (IAA). Cotyledons cultured in the light formed green or white callus while those cultured in the dark formed friable white callus. However, unlike the kinetin-IAA medium there was direct regeneration of shoots from cotyledons on basal medium supplemented with 0.0, 0.1, or 0.25 $mg \cdot l^{-1}$ BA combined with 0.0, 0.1, or 0.25 $mg \cdot l^{-1}$ NAA, and 5.0 $mg \cdot l^{-1}$ BA combined with 0.0 $mg \cdot l^{-1}$ NAA (Fig. 1). In general shoot number decreased as NAA concentration increased. Media lacking BA formed progressively more roots with numerous root hairs as the NAA concentration increased. This pattern of root growth was the same for cotyledons grown both in the dark or the light.

All three cultivars developed shoots from subcultured callus when transferred as follows: 0.5--0.5--0.5 or 0.5--0.5--1.0 $mg \cdot l^{-1}$ BA. Other successful treatment combinations were as follows: 'Hales Best' and 'Perlita' 1.0--1.0--1.0 $mg \cdot l^{-1}$ BA. In general when callus was transferred to higher levels of BA friable nonmorphogenic callus overgrew the shiny green morphogenic callus previously formed.

We previously reported that 'Hales Best' had the highest morphogenic potential on basal medium supplemented with kinetin and IAA (2). On basal medium supplemented with BA and NAA, 'Perlita' had the highest morphogenic potential, followed by 'Hales Best' and 'Iroquois'. Optimum BA and NAA levels varied with cultivar. For indirect regeneration, 'Perlita' also had the

highest morphogenic response followed by 'Hales Best' and 'Iroquois'.

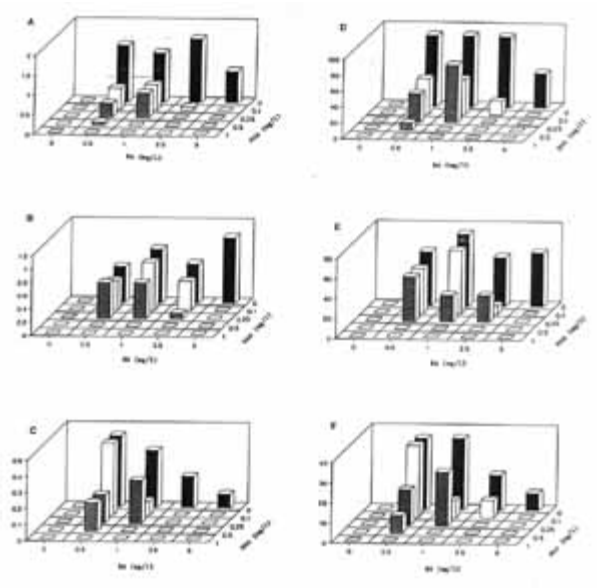

Figure 1. A-C) Average rating of eleven light-grown cotyledons. Rating scale 0=no shoots, 0-1=1-10 shoots, 1-2=11-20 shoots. A) 'Perlita' B) 'Hales Best' C) 'Iroquois'. D-F) Percentage of eleven light-grown cotyledons forming shoots. D) 'Perlita' E) 'Hales Best' F) 'Iroquois'.

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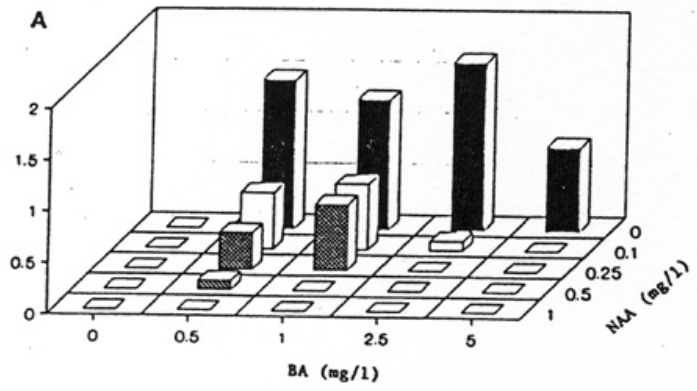
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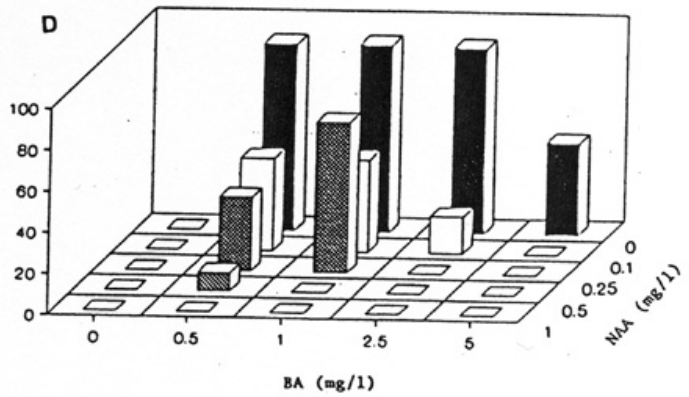
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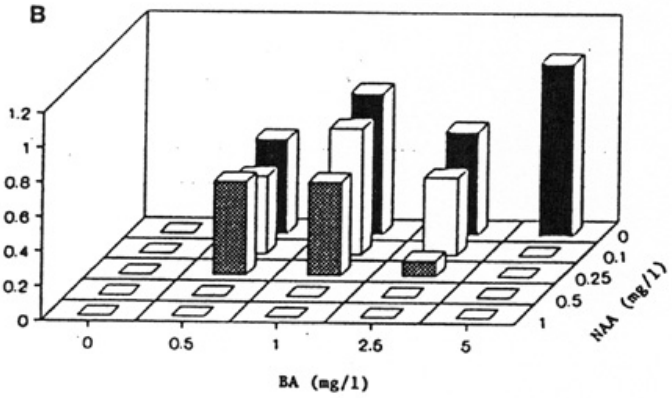
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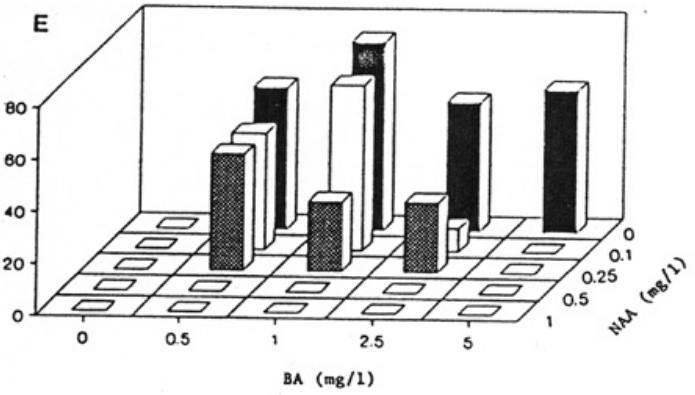
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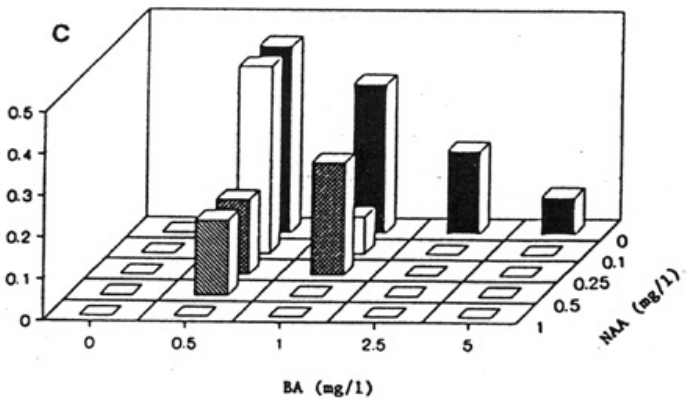
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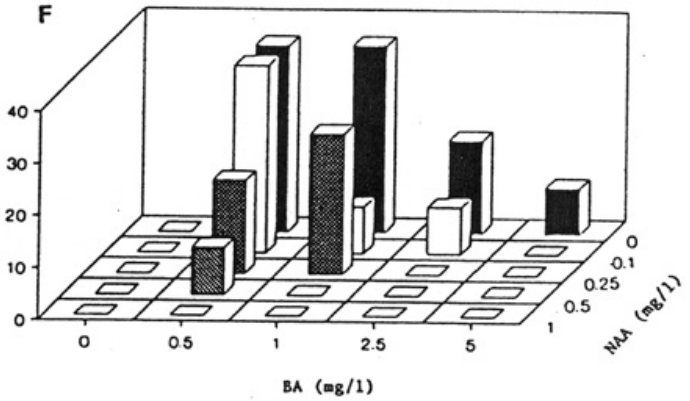
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A Second Look at the *Glabrous Male-Sterile* (*gms*) Character in Watermelon

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Watts (5) recovered the *gms* character from irradiated seed in 1957 and reported on the variant in 1962. Although the variant behaved as a single recessive gene, there were two notable exceptions. One glabrous plant grown in the greenhouse produced enough pollen to set 35 seed in a fruit on a homozygous normal plant. Watts was not able to recover subsequent progeny from this cross. He also noted that a single selfed heterozygote produced a 1:1 ratio of hairy:glabrous instead of a 3:1 ratio as expected. the class with fewer individuals than expected was the homozygous normal class.

Production of *gms gms* gametes in a tetraploid line carrying the *gms* character is reduced (1, 3). However, the variant segregated faithfully in the tetrasomic condition (4). Ray and Sherman (2) suggested that chromosome desynapsis was the cause of male sterility in the *gms* prototype.

We now have four lines derived from a single *glabrous, male-fertile* variant of the *gms* material. Three of the lines exhibit some male fertility. Female fertility was related to male-fertility in these lines. Male flowers with viable pollen occur two or more weeks after the appearance of the first female flower.

The *gms* variant is more than a well-behaved Mendelian recessive. Glabrousness is recessive to hairiness, but sterility and glabrousness are not pleiotropic effects of the same gene. Male-sterility and female-sterility are related, suggesting that the meiotic process is flawed (2). The extremely late development of male fertility in new recombinants may provide a far superior system for hybrid seed production than previously envisioned.

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Inheritance of Orange Flesh Color in Watermelon

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A variety of flesh colors are present in watermelon: red, orange, yellow, and white. Porter (3) reported yellow flesh from 'Golden Honey' was recessive, *y* to red flesh, *Y* . Poole (2) showed that yellow flesh, which he termed golden yellow, was also from 'Golden Honey' as well as from 'Yellow Flesh Ice Cream', was controlled by a single recessive gene, *y*. Poole demonstrated that Canary yellow from 'Honey Cream' was controlled by a single dominant gene, *C*, to pink flesh, *c* from 'Dove'. Shimotsuma (5) found that two pairs of genes with epistasis controlled white, yellow and red flesh color derived from 1) a cultivated form of *Citrullus lanatus*; 2) a bitter, wild type of *C. lanatus*; and 3) a non-bitter wild *C. lanatus*. White flesh was controlled by a single dominant gene, *Wf*, to yellow and red flesh; thus *Wf* = *B* and *Wf* = *bb* both gave white flesh; yellow flesh was dominant to red flesh and was expressed only when *wfwf* was present, thus yellow = *wfwf B_*, and red flesh was the double homozygous recessive,*wfwf bb*. An F_2 phenotype segregation ratio would thus be 12 white: 3 yellow: 1 red.

In the present investigation the inheritance of orange flesh derived form 'Tendersweet Orange Flesh' ('Tendersweet OF') was studied in crosses with red flesh from 'Dixielee' and 'Sweet Princess', and yellow flesh from 'Golden Honey'. Following the flesh color symbols referred to earlier and those given by Robinson et. al. (4) and by Henderson et. al. (1) *y* will be used in this study to designate yellow flesh from 'Golden Honey' and *Y* red flesh color.

The F_1 of 'Dixielee' x 'Tendersweet OF' was red, indicating dominance of red flesh to orange flesh. The F_2 and BC_1 data support a single gene hypothesis whereby orange flesh was recessive to red flesh (Table 1). Chi-square values are all non-significant indicating a good fit to the single recessive gene hypothesis for orange flesh to red flesh. Also an orange fleshed F_2 selection segregated 3:1 for red to orange flesh as would be expected in 2/3 of the red selections.

A test for heterogeneity for Chi-square goodness of fit (Table 2) showed that each family segregated in a similar direction and was similar to the pooled value in both the F_2 and backcross generations. Thus, reliability can be placed in the pooled data e.g. a deficiency in a character of one family was not cancelled by a surplus in another family.

In the test for allelism (Table 3) orange flesh was dominant to yellow flesh in the cross 'Tendersweet OF' x 'Golden Honey'. Further, red flesh was dominant to yellow flesh in the cross 'Golden Midget' (red flesh) x 'Golden Honey' (yellow flesh). It is tempting to hypothesize a multiple allelic system as is shown in Table 1 and 2 whereby *yy* = yellow flesh, y^oy^o or y^oy = orange flesh and *Y_* = red flesh. However, a dihybrid system with epistasis has not been ruled out and awaits the F_2 and backcross data. Tentatively the symbol y^o is given to orange flesh which is recessive to red flesh (*Y*) but dominant to yellow flesh (*y*).

Table 1. Segregation and Chi-square goodness of fit test for watermelon flesh color in the cross 'Tendersweet Orange Flesh' (orange) x 'Dixielee' (red).

			Flesh color Offspring (no. plants)		Expected ratio		
Generation	**Parents**	**Hypothesized parental genotype(s)[z]**	**Red**	**Orange**	**Red:Orange**	**Chi-Square**	**Probability**
P_1	Dixielee	*YY*	8	0	1:0	-	-
P_2	Tendersweet OF[y]	y^oy^o	3	0	1:0	-	-
F_1	Dixielee x Tendersweet OF	Yy^o	12	0	1:0	-	-

F_2	Dixielee x Tendersweet OF F_1(self)	Yy^o (self)	21	11	3:1	1.50	.50-.75
BC_1	F_1 x Dixielee	Yy^o x YY	46	0	1:0	0	1.00
BC_2	F_1 x Tendersweet OF	$Yy^o + y^oy^o$	47	65	1:1	2.89	.05-.10
F_3	F_2 - red selection (self)	$Y\_$ (self)	11	3	1:0 or 3:1	0.10	.75-.90
F_3	F_2 - orange selection (self)	y^oy^o (self)	0	10	0:1	0	1.00

[z] Tentative flesh color genotypes: $Y\_$=red - dominant to orange and yellow; y^oy^o or y^oy=orange - recessive to red, dominant to yellow; yy=yellow - recessive to both red and orange

[y] Tendersweet OF='Tendersweet Orange Flesh'

Table 2. Heterogeneity test for Chi-square goodness of fit test for F_2 and backcross generations for watermelon flesh color in the cross, 'Dixielee' x 'Tendersweet Orange Flesh'.

Generation	**df**	**Chi-square**	**Probability**
F_2			
Sum of two chi-squares	2	1.66	.25 - .50
Pooled	1	1.50	.10 - .25
Heterogeneity	1	0.16	.50 - .75
Backcross (F_1 x Tendersweet OF)			
Sum of four chi-squares	4	6.73	.10 - .25
Pooled	1	2.89	.05 - .10
Heterogeneity	3	3.84	.25 - .50

Table 3. Allelism tests for watermelon flesh color.[z]

		Flesh Color (number of plants)		
Cross	**Family**	**Red ($Y\_$)**	**Orange (y^oy^o or y^oy)**	**Yellow (yy)**
Tendersweet OF[y] (y^oy^o) x Golden Honey (yy)	1	0	17	0
	2	0	4	0
	Total	0	21	0
Tendersweet OF (y^oy^o) x Golden Midget (YY)	1	16	0	0
	2	13	0	0
	Total	29	0	0
Golden Honey (yy) x Golden Midget (YY)	1	12	0	0
Tendersweet OF (y^oy^o) x Sweet Princess (YY)	1	19	0	0

Golden Honey (*yy*) x Sweet Princess (*YY*)	1	9	0	0
Golden Midget (*yy*) x Sweet Princess (*YY*)	1	14	0	0

[z] Yellow derived from 'Golden Honey', orange from 'Tendersweet Orange Flesh' and red flesh color from 'Golden Midget' and 'Sweet Princess'.

[y] Tendersweet OF = 'Tendersweet Orange Flesh'

[x] Tentative gene symbols:

- *Y* = red
- y^o = orange (recessive to *Y* dominant to *y*)
- *y* = yellow (recessive to *Y* and y^o)

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Influence of Handling and Nitrogen Nutrition on Flowering and Growth of Watermelon Transplants in the Greenhouse

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Efficient, early production of female flowers in watermelon *Citrullus lanatus* (Thunb.) Matsum. and Nakai is of keen interest to those making controlled crosses or otherwise attempting to produce fruit in a greenhouse setting. I noted on numerous occasions that seedlings started in peat pellets and transplanted at a very late stage were early and precocious flowers. Manipulation of this stress response might prove useful to those requiring greenhouse-grown watermelon fruit, since earlier female formation could led to more rapid fruit set and moreover, better control of the vining habit.

Seeds of 'Charleston Gray #5' (CG) and 'Bush Charleston Gray' (BGG) were sown in Jiiffy-7 peat pellets held in plastic flats. The flats were watered to runoff daily until the pellets were removed for transplanting to 1-gallon pots (2:1 vermiculite:peat) at either the two-true-leaf stage (early) or 3 weeks afterwards (late); these constituted the 2 levels of the handling treatment. the third treatment was fertilization level, altered by adding 100 ml of 0, 200 or 400 ppm N three times a week as reagent-grade ammonium nitrate in double-distilled water. A completely randomized design of a complete 2x2x3 factorial, replicated 3 times, was used. Flower counts were made at 3 weeks following the delayed transplanting date (approximately 6 weeks from seeding) and again at 6 weeks, at which time the plants were also harvested for dry weight determination. Flower counts at 6 weeks excluded the first 8 nodes on each plant.

Results are presented in Table 1. The two cultivars clearly differed for flowering and growth. CG plants were, as expected, larger and had more flowers, but BCG seemed to produce earlier female flowers (significantly larger node). Average dry plant weight of the two cultivars was similar (although significantly different), but differences in the number of male flowers produced per plant indicate a different flowering response for the two types. Nonetheless, femaleness was not significantly different for these two cultivars. These results seem to suggest that the bush type produces fewer flowers than the vining type, but in the same male-female proportion, and on a shorter, stockier plant.

Time of transplanting had the most dramatic effect of any factor. Late planting significantly reduced growth and flower development of the plants. However, these same seedlings produced the earliest female flowers by a wide margin (fifth node as opposed to ninth). Transplant timing also seemed to reduce the numbers of flowers produced and femaleness (% female flowers) at the earlier measurement date. It seems likely that differences in the number of flowers produced was a direct result of plant development differences, as plant dry weight was severely lowered by delaying transplanting. Femaleness was not significantly different for the two timings at the later measurement date.

Increasing N resulted in small but statistically significant increases in male flowers at six weeks and female flowers at three weeks. Here again the flowering response may be attributable to plant growth, since plant dry weight was highly influenced by N level. Earliness of female development was increased significantly by increased N level, although not substantially. N level promoted femaleness at the three-week measurement, but not at the later date. Although significant nitrogen x time interactions were found for two variables, the data (not shown) merely tended to show a much stronger influence of nitrogen in the late planted seedlings, no doubt an outcome of their poor initial nutritive condition.

Response of earliness to N level was opposite to that indicated by the timing data, and suggests that delayed transplanting causes more than simple nitrogen stress. Certain environmental influences, such as daylength, temperature and application of growth regulators have well-documented influences on watermelon sex expression (2, 3, 4). A field study (1) on watermelon showed little difference in date of first female anthesis under N rates of 0-150 1b/A. Higher N rate did increase the number of females/plant, but percent females was not recorded. Sex expression in *Cucumis* is known to be influenced by

environmental factors, but a recent report (5) showed no effect of increased fertilization on sex expression and earliness of gynoecious cucumber lines. It seems possible that differences between the handling regimes is attributable to more than nutrient stress. Moisture, which was undoubtedly less stable in the delayed transplants, may be involved.

The results suggest that female flowering can be accelerated by late transplanting, but probably at the expense of general plant vigor. Application of N appeared to alter flowering mainly by altering growth response, but the promotion of female earliness by increasing N contradicts earliness induced by late transplanting, a condition one might expect to be related to nutrition. Nutritional differences due to other (unmonitored) consequences of ammonium nitrate application (pH, etc.) are also plausible.

The previously mentioned field study (1) showed fruit set in watermelon to be reduced by low N application rates. A priority of further work will be to test whether stressed watermelon plants can set and produce fruit with appropriate late nutrition.

Table 1. Main treatment and interaction effects for cultivar, transplant timing and nitrogen regime on flowering and growth of watermelon transplants.

Main effect	# Males 3 wk	# Males 6 wk	# Females 3 wk	# Females 6 wk	Node bearing first female	% Female 3 wk	% Female 6 wk	Plant dry weight (g)
Cultivar (Cv)								
Charleston Gray	12.4	16.9	1.3	1.6	9.5	7.4	12.1	8.8
Bush Ch Gray	9.3	7.2	1.7	1.4	6.4	13.2	16.4	8.2
Significant [z]	ns	**	ns	ns	**	ns	ns	*
Transplanting								
Early	17.2	14.4	2,7	2.3	9.3	14.5	11.6	12.6
Late	4.6	9.8	0.3	0.8	5.0	6.1	17.2	4.5
Significant	**	**	**	**	**	*	ns	**
N level (ppm)								
0	8.0	9.6	1.1	1.3	8.6	9.1	14.8	7.0
200	12.3	13.1	1.4	1.5	8.2	7.7	12.9	7.6
400	12.4	13.6	1.9	1.9	6.6	14.3	15.3	10.9
Significant linear	ns	*	**	ns	*	ns	ns	**
Significant quadratic	ns	ns	ns	ns	ns	*	ns	**
Interactions								
Cv x Time	ns	ns	ns	**	ns	ns	ns	*
N x Time	*	ns	ns	ns	ns	**	ns	ns
N x Cv	ns	ns	ns	ns	ns	ns	ns	ns
N x Cv x Time	ns	ns	ns	ns	ns	ns	ns	ns

[z] Separation by F-test, ns=not significant at 5% level, *=significant at the 5% level, **=significant at the 1% level. analysis performed on transformed data as needed to account for lack of homogeneity of treatment variance.

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Studies of Watermelon Germplasm Resources and Breeding. III. Correlation between Parents and their F_1 Hybrids, Phenotypic Correlation Among Characters and Path Analysis

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Abstract. Twenty-one (21) watermelon lines and their 19 F_1 hybrids were tested for correlation analysis between parents and their F_1 hybrids, phenotypic correlation and path coefficient analysis. The results indicated that significantly positive correlation existed between midparents and F_1 hybrids for fruit yield per plant, fruit numbers per plant, fruit weight, soluble solids content, resistance, and extremely significant correlation was found between high parents and F_1 hybrids for resistance. There has been a significant correlation between fruit weight and fruit yield per plant, shoot thickness and fruit weight, resistance and soluble solids content. The results obtained from path coefficient analysis suggested that fruit weight has an obviously direct effect on fruit yield per plant and indirect effects on shoot thickness and fruit yield per plant mainly via fruit weight, apart from the direct effect by itself.

Key Words: watermelon; germplasm resources; heterosis; correlation; path analysis

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Cucurbita moschata Half-sib Families Collected in Puerto Rico and the Dominican Republic

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Half sib families from seed of 38 fruits originating from the Dominican Republic and 12 fruits from Puerto Rico were evaluated in replicated field trials in Puerto Rico from 1986 to 1988. In the Dominican Republic fruits were collected from farmer's field with the cooperation of the Centro Sur de Desarrollo Agropecuario (CESDA), San Cristobal, Dominican Republic. An attempt was made to collect a variety of fruit types with good pulp color (yellow-orange) and thickness. In Puerto Rico fruits were collected both from types distinct in shape or skin color from the traditional Puerto Rican cultivar 'Borinquen'. 'Borinquen' from six different seed sources as well as some Puerto Rico Agricultural Experiment Station breeding lines (selected from 'Borinquen') were also included in the trials. Certain families in the first and second trials were eliminated due to poor germination or seedling vigor. Twenty-one to 23 entries were evaluated in each of three trials in a randomized complete block design with three or four replicates. Three (trials 2 and 3) or four (trial 1) plants per plot were spaced 20 ft apart within rows and 25 ft apart between rows. Tropical genotypes of *C. moschata* are extremely large and sprawling plants with vines that easily reach 50 ft in length. To contain their growth, plants were wound around their identifying stake until female flowers appeared. Nevertheless, it was often difficult to distinguish between plants within a plot or even between plots at harvest.

From this very limited sample of genotypes we found nearly every shape and color fruit imaginable. Pear, oblong, ovate, oblanceolate, obovate, oval, cuneate, elliptic, gourd-shaped, globe, round, and flat fruits were observed among these families. Within families four or five different shapes were not uncommon. Puerto Rican consumers give little importance to fruit shape when purchasing pumpkin although round, globe or flat shapes are preferred (1).

Skin colors ranged from nearly black to dark green to mottled green and white ("pinta" in Spanish) to mottled pale orange and white. However, not a single fruit of thousands evaluated had the buff color of 'Butternut'. In Puerto Rico skin color is somewhat important to consumers (the "pinta" color is preferred) but not nearly as important as pulp thickness and pulp color (1).

These families were vary variable in terms of fruit size, yield per plant, number of fruit per plant, pulp color and pulp thickness. Mean family fruit size ranged from 2.4 kg to 6.4 kg. Some individual fruit weighed more than 10 kg. Average family yield per plant ranged from 3.8 kg to 47.1 kg. Mean number of fruit per plant varied from 1 to 9. Average family pulp thickness varied from 2.2 cm to 4.8 cm. Flesh color ranged from light yellow to dark orange.

Chi-square tests of independence in a sample of 842 fruits from all families indicated that there was an association between fruit shape and pulp color (X^2 = 77.4, degrees of freedom - 35, P=0.005). Flat fruits were more often associated with dark green skin color than were other fruit shapes. Dark skinned fruits generally had good pulp color compare to other skin types.

Over-all trials, number of fruits per plant was highly correlated with yield (r=0.81). Fruit size and pulp thickness were only intermediately correlated with yield (r=0.42 and 0.43, respectively). Increased fruit size was not associated with number of fruit per plant (r = -0.06). No correlation was found between pulp color and pulp thickness (r-0.03).

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Inheritance of Mottled Leaf in *Cucurbita moschata*

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The mottled leaf trait in the genus *Cucurbita* has been described as silver gray areas in axils of leaf veins controlled by a single dominant gene, *M*, in *C. maxima, C. moschata* and *C. pepo* (1, 2, 3). Modifier genes have been reported as extending and/or intensifying the character expression, and at least five phenotypes were described (2, 5). According to Shifriss (6) cell position and environment also contribute to variation in mottling.

Shifriss (4, 5, 6, 7) reported an association between mottled leaf trait and an escape mechanism against aphid-transmitted virus diseases. Mottled leaf plants either repelled aphids similarly to aluminum mulch or slowed speed of virus multiplication.

In the cv. Pira-Moita (*C. moschata*), we observed a great range of expression for the trait, from non-mottled to highly mottled plants. Contrasting lines were isolated after three selfing cycles, and six generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) were compared from the cross, highly mottled leaf (P_1) x non-mottled leaf (P_2). Plants were evaluated for degree of mottling at first leaf stage, and data analyzed by chi-square (Table 1).

Table 1. Inheritance of mottled-leaf character in different generations from the cross between two lines, highly mottled leaf (P_1) and non-mottled leaf (P_2), derived from cv. Pira-Moita (*Cucurbita moschata*).

	Number of plants				
Generation	**Mottled leaf**	**Non-mottled leaf**	**Tested ratio**	**X^2**	**P**
P_1	all	-	1:0	-	-
P_2	-	all	0:1	-	-
F_1	all	-	1:0	-	-
F_2	627	214	3:1	0.09	0.75-0.90
BC_1	819	2	1:0	-	-
BC_2	549	581	1:1	0.91	0.25-0.50

All of the F_1 plants showed intermediate phenotypes, moderately mottled leaves, indicating partial dominance. The chi-square test indicated good fit to a 3:1 ratio for F_2 generation and to a 1:1 ratio for BC_2 generation, corroborating earlier published studies. A single, partially dominant gene confers mottled leaf, but there are modifier genes affecting the character as indicated by the continuous variation in mottled expression in F_2.

Besides, the possible partial protection of *Cucurbita* plants against aphid-transmitted virus diseases, the trait may be useful as a seedling genetic marker.

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List, Description and Interactions of the Genes Affecting Fruit Color in *Cucurbita pepo*

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Cucurbita pepo L. contains a fascinating array of fruit colors. A few genes affecting fruit exterior color have been identified, and their preferred symbols and names were recently summarized (2) as *B* (*Bicolor*) fruit), *D*, (*Dark* green stem), *l* (*light* fruit color), *l-2* (*light* pigmentation on fruit-2), *St* (*Striped* fruit), *W* (*White* fruit), and *Y* (*Yellow* fruit color). However, this list is not complete, nor does it contain a full description of the effects and interactions of these genes. Some new data would also indicate that modification of the list is also necessary. The goal here is to present a revised list of genes affecting fruit exterior color in *C. pepo*, including a description of the effect(s) and interactions of each, and to review some of the literature, especially with respect to its synonymies and anomalies.

Revised gene list:

Preferred gene symbol	Synonym	Name
B		*Bicolor* fruit (13)
D	*R*	*Dark* peduncle, stem, and fruit (3, 7)
Ep-1		*Extender of precocious* yellow coloration-1 (15)
Ep-2		*Extender of precocious* yellow coloration-2 (15)
1-1, 1-1St	*C, St*	*light* fruit coloration-1 (7,13)
1-2		*light* fruit coloration-2 (7)
W		*White* fruit coloration (16)
Y		*Yellow* fruit coloration (16)

Description of effects and interactions:

***B*.** Preanthesis ovaries yellow or bicolor, yellow and green. Incompletely dominant to alternative allele represented as *b* in literature prior to 1981 and as *B*+ in literature since 1981. A third allele, represented as *B^{w}* (weak *B*) probably exists (13, 14) but proof with an allelism test has not been presented. When homozygous or when heterozygous in the presence of at least two *Ep* alleles (15), *B* is epistatic to *Y* (11). Interacts in complementary fashion with *L-2* to condition orange fruit flesh color (5), with *L-1* and *L-2* to condition intense yellow color of young (summer squash) fruit (7), and with *W* to produce cream (instead of white) mature fruit color (13). Pleiotropic, affecting foliar as well as fruit characteristics, with differing degrees of expression of the various effects occurring in different genetic backgrounds (14).

D. Plant stems dark, fruits and their peduncles dark from two weeks past anthesis; thus pleiotropic, affecting foliage and fruit. Alternative allele *d* for light stems, peduncles and fruits (3, 7). the *D* allele is epistatic to both *l-1* and *l-2* when either or both *l* genes are in homozygous recessive state (7). In half-mature fruit, *D* also is epistatic to *l-1st*. Originally (3), *D* was considered to condition dark stems only, and reported to be tightly linked to a fruit-color gene, *R* (*Reversal*, or non-persistent color, or *r* for recessive white, refs. 2, 3). The *R* symbol was subsequently (8) adopted based on the contention that *D* and *R* are linked but separate loci. However, results of later studies (5, 7 and H.S. Paris unpublished data) cast serious doubt on the case for separate linked loci. Due to the lack of firm evidence, *R* should presently be considered synonymous with *D*. Other genes affecting stem color probably exist.

Ep-1, Ep-2. Extend the precocious yellow coloration conditioned by *B* (15). Incompletely dominant to alternative alleles ep-1 and ep-2 and additive in action: two doses of any combination of Ep alleles result in completely yellow fruit when B is heterozygous and in extension to the adjacent peduncle, calyx, and/or corolla when B is homozygous. No known effect in genotype b/b (B+/B+) .

***l-1, l-1St, l-2*.** Young fruits lightly colored when either *l* is homozygous recessive. Complementary action of *L-1* and *L-2* results in fruit that are intensively colored throughout development (7). The gene designations *c* (3) and *l* (13) have been shown to be synonymous (8). Other results (H.S. Paris, unpublished) show that the originally designated *l* is in fact *l-1* and not *l-2*. Allele *l-1St* results in striped fruit and is recessive to *L-1* and dominant to *l-1*. Complementary action of *l-1St* and *L-2* results in striped young (summer squash) and mature fruits. The symbol *St* was originally suggested for the gene conditioning striping of 'Caserta' (10). Striping was considered by Shifriss (13) to be conditioned by an allele of *l* (*l-1*) but he did not present evidence in support of this contention, and therefore the symbol *St* was accepted (9). In the progeny (approximately 100) plants of the three-way cross, *l-1/l-1 L-1/L-2* x *(L-1/L-1 l-2/l-2*) x 'Caserta', *L-2/L-2*), only intense-colored and striped, and no light-colored individuals were obtained, showing that the striping of 'Caserta' is conditioned by an allele of *l-1,* or is very tightly linked to *l-1* (6). Therefore the symbol *l-1St* is now to be preferred. However, other genes conditioned striping undoubtedly occur at separate loci.

W and ***Y*.** *W* was originally (16) reported to be epistatic to *Y*. *W* is probably epistatic to some fruit color genes (8, 13) but not to *Y* (2).

In summary, eight genetic loci having an effect on fruit exterior color have been identified. four of these loci affect fruit hue exclusively, whereas the other four affect intensity as well as hue (7). There are at least several other genetic loci which affect fruit exterior color, and these await identification.

In many articles on cucurbit genetics, "+" (wild-type, normal) notation has been used, as proposed by Robinson et al. (9). Such use may be appropriate when the mutant allele would have a clear deleterious effect on wild forms, such as the effect of *B* on young, developing fruit (1). However, in most cases, genetic studies have been conducted in crosses among cultivars rather than among wild and cultivated forms. In these crosses, the defining of "normal" is difficult, and given the lack of knowledge of the genetics of the wild form, the assignment of the wild-type symbol is usually guesswork. This can and has resulted in the assignment of the "+" to the mutant form of a gene. For example, the use of the symbol *l*+ instead of *L* in the latest gene list (2) is premature at best and a mistake at worst: Wild *C. pepo* has alternating, brown, intense-colored and narrow, light colored stripes. Conceivably, *l-1St* or some other, as yet unidentified, allele and not *L-1* might be the wild type. Another example has occurred with regard to the locus responsible for the presence or absence of lignification of the fruit rind (phenyl ammonia lasses activity or inactivity, ref. 12). all *C. pepo* gourds have hard, lignified rinds. Mains (4) found that the hard rind of gourds was conditioned by a single dominant gene, and soft (not lignified) rind by its recessive allele. Robinson et al. (9) assigned the symbol *Hr* to this gene. Perhaps inevitably, the symbol *Hr*+ was subsequently (14) used to designate the recessive allele for soft rind!! Therefore, in crosses among cultivars, it would seem to be more prudent to use upper and lower case symbols for alternative alleles of the various identified loci, at least until such time as the wild-type alleles have been clearly identified.

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Relationship between the *B* Genes of Two *Cucurbita* Species, II

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The primary effect of a *B* gene is precocious depletion of chlorophyll in young fruits prior to anthesis (1). Genes conditioning this effect exist in both *C. pepo* and *C. maxima*. It is practically impossible to study the relationship between the *B* of *C. pepo* and the *B* of *C. maxima* by breeding experiments. This is because the two species are isolated by strong genetic barriers. The barriers were circumvented by transferring the *B* genes of these species to *C. moschata*. As a result, two different *B* lines of *C. moschata* were established: NJ-B and IL-B. NJ-B carries the *B* of *C. pepo* and IL-B carries the *B* of *C. maxima.* A preliminary study of inheritance involving NJ-B x IL-B was conducted at Rutgers University, New Brunswick, NJ, and the results raised some unexpected issues.

First, individual F_1 plants differed in their capacity to manifest a "midrib pattern" of chlorophyll depletion in leaf blades (Fig. 1, ref. 2). Neither NJ-B nor IL-B exhibits this pattern. Second, apart from a small proportion of albino (lethal) seedlings, most F_2 segregates were difficult to classify, casting some doubt on the validity of the data. The F_2 plants were grown without supplementary light during winter months in a greenhouse that was not well insulated from outside temperature fluctuations.

The difficulties experiences in classification were largely due to the fact that precocious depletion of chlorophyll in this F_2 can affect several or all aerial organs of a plant. Moreover, a particular organ may or may not be affected depending on the stage in plant development at which it is differentiated. The effect is also subject to variations in the environment. Under such circumstances each F_2 plant must be observed over a long period of time in order to critically assess its complex phenotype, a laborious task.

Nevertheless, the preliminary results suggested that the analysis of this cross might shed some light not only on the relationship between the two B genes but also on the genetic control of chlorophyll during plant development. And this thought gave the impetus to the present investigation.

Breeding materials. Two clones were available from the previous study. Clone NOMP was obtained from an F_1 plant (5356-1) that did not exhibit the midrib pattern, and clone MP was obtained from an F_1 plant (5356-14) that exhibited this pattern. The two clones were propagated vegetatively and grown to maturity for five years. During this period they behaved in a consistent manner: NOMP did not exhibit the pattern and MP exhibited it in winter but not in summer. For the present study, new F_1 seed was obtained from NJ-B x IL-B. But the BC_1 and F_2 seed was obtained through the use of NOMP and MP clones.

Environment. The seed of the parental inbreds, the new F_1, the BC_1 and the F_2 was sown in a greenhouse in Naples, Florida, on 12 September 1988, and the seedlings were transplanted to the field on 22 September. The greenhouse temperature exceeded 30°C during germination and early seedling growth. Cultural practices were similar to those commonly used by commercial growers in the area,. Field observations of individual plants continued until the end of November.

Results and Interpretation (consult Table 1). The first significant observation was the absence of albino (lethal) seedlings in any one of the breeding materials.

In the BC_1 (test 4), the proportion of plants with precociously pigmented fruits and precociously pigmented stems (phenotypic classes 4 + 5) to plants with precociously pigmented fruits and green stems (class 3) to plants with precociously pigmented bicolor fruits and green stems (class 2) does not disagree with a 2:1:1 ratio (97:46:49, the expected ratio being 96:48:48, P=0.90-0.95). In the F_2 (test 7), the proportion of plants with precociously pigmented fruits and precociously

pigmented stems (classes 4 + 5 + 6) to plants with precociously pigmented bicolor fruits and green stems (class 2) to plants with green fruits and green stems (class 1) does not disagree with a ratio of 12:1:2:1 (258:17:42:19, the expected ratio being 252:21:42:21, P =0.75-0.90).

The new results are compatible with the hypothesis that the two *B* genes are non-linked; that there exists a third gene; that the third gene is closely linked to the *B* of IL-B; that this linked gene activates the expression of *B* in stems; and that the bicolor fruited plants carried a single dose of *B*, donated exclusively by NJ-B, and three doses of *B*+ . This suggests that the effect of a single B of IL-B is stronger than that of a single *B* of NJ-B in extending precocious chlorophyll depletion over the entire fruit.

If the above hypothesis is basically correct, let B_1 represent the *B* of *C. pepo*, B_2 the *B* of *C. maxima* and *Ac-B* the activator of *B*. Then, the partial genotype of NJ-B is B_1* *Ac-B*+ / B_1 * *Ac-B*+ , B_2+ * *Ac-B*+ / B_2+ * *Ac-B*+ . And the partial genotype of the IL-B is B_1+ * *Ac-B*+ / B_1+ * *Ac-B*+, B_2 * *Ac-B* / B_2* *Ac-B*.

The effect of chlorophyll depletion on whole plants was more extensive and more severe in progenies obtained from the MP clone than in progenies obtained from the NOMP clone. This was particularly striking in the F_2. The difference between the two BC_1 progenies (test 3 vs test 2) was hardly perceptive to the observer in the field, and might not be biologically significant. On the other hand, the data in Table 1 do not reflect adequately the true magnitude of the difference between the two F_2 progenies (test 6 vs test 5). The reason for this is that class 6 consisted of a wide spectrum of phenotypes. At one end of the spectrum were essentially class 5 plants that exhibited the midrib pattern late in the season. At the other end of the spectrum were highly variegated, almost completely yellow, plants that were essentially semi-lethal. In test 5, the 6 plants of class 6 were initially recorded as class 5 individuals, but at the end of November their new leaves exhibited the midrib pattern and therefore these plants were reclassified under class 6. In test 6, at least 35 of the 55 class 6 plants were recorded as variegated, and 8 of the 35 were almost completely yellow or essentially semi-lethal. Genotypes of such individuals might appear as albino (lethal) seedlings under conditions of low temperature and low light intensity.

Variegated plants similar to those of class 6 were observed in *C. maxima* about 10 years ago (Shifriss, unpublished). These variegated plants were F_2 segregates of crosses between PI 165558, a *B*/*B* cultivar from India, and several North American cultivars, *B*/*B* and *B*+/*B*+. The stem of PI 165558 is precociously pigmented (indicating the presence of B_2 * *Ac-B*), whereas the stems of most North American *B*/*B* cultivars are green (indicating the presence of B_2 * *Ac-B*+). Since PI 165558 was the donor of *B* to IL-B (2), it must have actually donated B_2 * *Ac-B*. Perhaps the gene pool of *Cucurbita* carries some elements that extend the effect of B_2 * *Ac-B* over the entire plant.

Finally, two of the nine unclassified plants (test 7) were tentatively described as having precociously pigmented stems, green ovaries and green leaves. If the function of the linked gene, presently designated by symbol *Ac-B*, is not related to the effect of *B*, then this linked gene should be designated by a different symbol, e.g., *Cds*, for chlorophyll depletion in stems.

Table 1. Inheritance of precocious depletion of chlorophyll in a cross between two special lines of *C. moschata*. 1988 field data, Naples, Florida.

		Phenotypic classes[z]								
		1	**2**	**3**	**4**	**5**	**6**			
Test	**Breeding materials**	**GF GS GP GB**	**PDC-BiF GS GP GB**	**PDC-UF GS GP GB**	**PDC-UF PDC-S GP GB**	**PDC-UF PDC-S PDC-P GB**	**PDC-UF PDC-S PDC-P PDC-B**	**Number of classified plants**	**% plants of class 6**	**Number of unclassified plants**
-	P_1* NJ-B	0	0	12	0	0	0	12	0	0
-	B_2* IL-B	0	0	0	0	12	0	12	0	0
1[y]	F_1* P_1x P_2	0	0	0	0	18	0	18	0	0

2[x]	BC_1* F_1x P_1	0	20	23	49	1	0	102	0	0
3[w]	BC_1*F_1x P_1	0	20	23	38	9	0	90		
4	Total for BC_1	0	40	46	87	10	0	192	0	0
5[x]	F_2	1	16	9	54	36	6	122	4.9	3
6[w]	F_2	18	26	8	54	53	55	214	25.7	6
7	Total for F_2	19	42	17	108	89	61	336	18.2	9

[z] Key to phenotypic symbols: B = leaf blade; Bi = bicolor; F = fruit; G = green; P = petiole; PDC = precocious depletion of chlorophyll; U = uniformly pigmented, referring specifically to fruit. PDC may be associated with either white, tan, yellow or golden pigmentation.

[y] The F_1 hybrids of reciprocal crosses were indistinguishable phenotypically. None of the 18 plants exhibited the "midrib pattern" (see text). The petioles of the F_1 plants were less intensely pigmented and more variable than the petioles of P_2.

[x] This test was made through the use of an old F_1 clone (NOMP) that did not exhibit the "midrib pattern".

[w] This test was made through the use of an old F_1 (MP) that manifested the "midrib pattern".

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Cucurbit Genetics Cooperative Report 12:79-81 (article 33) 1989

Relationship between Gene *B* and Gene *Ses-B* in *Cucurbita pepo* L.

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Gene *B* conditions precocious depletion of chlorophyll. And the loss of chlorophyll is often associated with precocious yellow pigmentation. The primary target of *B* is the fruit (2). But *B* can also affect other potentially photosynthetic organs, depending on the genetic background and the environment. The "genetic background" is represented by specific nuclear elements. For example, some environmental conditions, the presence of gene *Ses-B+* allows the expression of *B* in leaf blades early in plant development. In contrast, gene *Ses-B* selectively suppresses the expression of *B* in leaf blades under a wide range of environmental conditions (3).

The influence of the environment is illustrated in the following. When seed of 'Jersey Golden Acorn' (JGA), *B/B Ses-B+/Ses-B+*, is sown in May in New Brunswick, NJ, the first true leaves are often completely yellow. Similarly, the first true leaves are often completely yellow when seed is sown late in November under greenhouse conditions in New Brunswick. But when the seed is sown in September in Naples, Florida, the first true leaves are completely green. It is assumed that relatively low temperatures or low light intensities trigger the effect of *Ses-B+*. However, the precise temperature and light conditions necessary to elicit the *Ses-B+* effect have not been determined. Moreover, the role of other non-genetic factors cannot yet be excluded.

There are marked variations in sensitivity of *B/B* lines to environmentally-induced leaf yellowing, a fact that alludes to a more complex genetic basis for this trait. But even a single *B/B* line, such as JGA, can manifest leaf yellowing in different ways. Examples: (a) Incomplete penetrance and variable expressivity, based on the phenotype of the first true leaf. (b) 100% penetrance and high expressivity, based on the first true leaf, followed by 1 to 3 partially yellow leaves, and then a switch to completely green leaves. (c) The first 3 to 6 leaves are yellow or partially yellow, followed by a distinct variegated phase in which chlorophyll depletion is largely confined to leaf veins, and then a switch to completely green leaves. (d) A prolonged phase of 10 to 30 yellow or partially yellow leaves followed by a switch to green.

Nevertheless, there is little doubt that in some crosses the inheritance of sensitivity is monogenic. It is speculated that *Ses-B+* and *Ses-B* are special regulators of *B*. In order to study the physical relationship between *B* and these regulators by breeding experiments two requirements must be met. First, the parental lines must carry alternative alleles. If JGA is to be used as a *B/B* parent that carries *Ses-B+*, it is necessary to find a *B+/B+* parent that carries *Ses-B/Ses-B*. Second, one must find an environment in which JGA predictably manifests 100% penetrance and high expressivity of leaf yellowing. Otherwise, it would be extremely difficult, if not impossible, to critically classify segregating generations.

'Sweet Dumpling' (SD), a *B+/B+* cultivar, was found to carry a strong *Ses-B* (Shifriss 1982, unpublished). This finding fulfilled the first of the above two requirements. As a result, seed was produced of F_1, BC_1 and F_2, using JGA and SD as parents. At the same time, attempts were made (through the use of growth chambers as well as greenhouse and field facilities) to find an environment that elicits the full effect of *Ses-B+*. These attempts were largely unsuccessful. In four experiments, JGA manifested incomplete penetrance and variable expressivity of leaf yellowing.

But there was one limited test in which JGA manifested 100% penetrance and high expressivity of leaf yellowing. This test included the parents and the F_2. The F_1 and the BC_1 seedlings were lost by accident. The seed was sown in November of 1983 and the plants were grown for five months under uncontrolled greenhouse conditions at Rutgers University in New Brunswick. In a subsequent sowing, the parents and the F_1 were grown during the summer of 1984, and their fruits are illustrated in Figure 1.

The data are presented in Tables 1, 2, and 3. The key for grades of yellowing in the first true leaves (Table 1) is as follows: 1 = completely green or green with 1 to 2 tiny yellow spots; 5 = yellowing extends over 3/4 of the leaf surface, and 2 to 4 =

intermediate grades between 1 and 5. Yellowing appears to reflect a diffused phenomenon rather than an extension of spotting. It is well established that *B+/B+* plants can exhibit yellow spotting under some environmental conditions (1).

The data in Tables 1, 2, and 3 suggest that *B* and *Ses-B* are non-linked.

Table 1. Limited data on the inheritance of precocious yellow pigmentation.

	Number of seedlings that exhibited different grades of yellowing in the first true leaf								
Breeding materials	**1**	**2**	**3**	**4**	**1-4**	**5**	**Total**	**X^2 (13:3)[z]**	**P**
P_1, JGA	0	0	0	0	0	10	10	--	--
P_2 SD	10	0	0	0	10	0	10	--	--
F_2	90	2	36	36	164	32	198	0.32	0.50-0.75

[z] Testing 164:34

Table 2. Classification of the 90 plants (grade 1, table 1) based on fruit color at later stages of development.

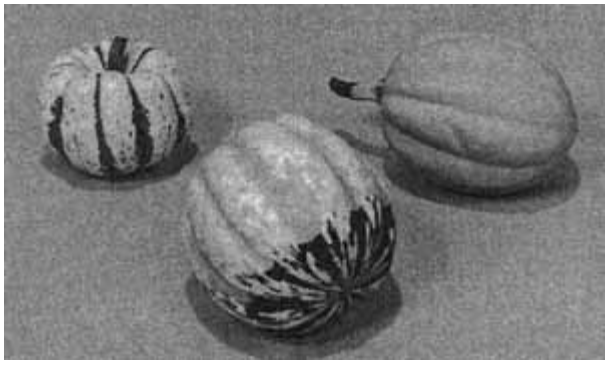
Number of plants that produced:					
green fruits	**bicolor fruits**	**yellow fruits**	**Total**	**X^2 (4:2:1)**	**P**
53	232	14	90	0.44	0.75-0.90

Table 3. Classification of the entire F_2 based on data in Tables 1 and 2.

Number of seedlings of:					
grades 1 to 4 that at later stages produced bicolor or yellow fruits	**grade 5 that at later stages produced bicolor or yellow fruits**	**grade 1 that at later stages produced green fruits exclusively**	**Total**	**X^2 (9:3:4)**	**P**
111[z]	34	53	198	0.51	0.75-0.90

[z] This number was obtained by subtracting 87 (34 + 53) from 198.

Figure 1. Upper left, SD; upper right, JGA; bottom, F_1



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Control of Chlorophyll During Plant Development: Hypothesis

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The term "control" in the title pertains to a series of steps that transforms proplastids into chloroplasts, the organelles of chlorophyll synthesis. These steps occur in competent cells that are exposed to light. According to present hypothesis there are two systems of control: one at the organelle level and another at the organismal level. The organelle system controls the steps that lead to normal chloroplasts under favorable intracellular conditions. But intracellular conditions potentially vary indifferent organs and at different stages during plant development. Furthermore, these conditions are affected by fluctuations in the external environment. The organismal system, acting as a buffer to each variations, tends to maintain favorable internal conditions for effective control by the organelle system. The focus here is on the organismal system.

The control at the organismal level is perceived as a homeostat of plastid transformation (HPT). The term "homeostat" is derived from the concept of homeostasis. The HPT consists of different nuclear genes that act in a selective manner, singly or in combination, as homeostatic regulators. Thus, the capacity of competent cells to transform proplastids into chloroplasts in different organs and at different developmental stages is sustained by these regulators. Some mutants of these regulators adversely affect or completely block the course of plastid transformation.

In a broader sense, HPT enables higher plants to carry on photosynthesis persistently and efficiently throughout life, assuming normal fluctuations in the external environment. HPT probably played a role in the evolution of higher organisms. This is because persistent production of photosynthates during plant development was advantageous not only to the producers, the autotrophs, but also to their animal predators, the heterotrophs.

The above hypothesis originated from studies of precocious depletion of chlorophyll in *Cucurbita*. The supporting evidence is based on the identification of two groups of genes that are unique to their specific effects.

The first group targets specific organs selectively. This group consists of gene *B* and its selective activators and selective suppressors. *B* is a major nuclear element that brings about precocious depletion of chlorophyll in fruits in all known genetic backgrounds. But *B* can be expressed or suppressed in other organs (e.g., leaf blades, stems) depending on the presence of selective activators such as *Ses-B+* and *Ac-B* or selective suppressors, such as *Ses-B* and *Ac-B+*. These findings suggested that the action of *B*, *B+*, *Ses-B+*, *Ac-B* and *Ac-B+* is organ-specific, and that *B+*, *Ses-B*, and *Ac-B+* are effective homeostatic regulators. The information on the behavior of gene *B* has been published, but see also the two preceding articles in present issue of CGC Report.

The second group of genes targets leaf blades at a particular time during plant development. Usually the first five to seven sequential leaves on the main stem are not affected (Shifriss, unpublished). A similar manifestation is exhibited by certain cultivars of *Amaranthus tricolor* (e.g., 'Illumination') except that in these cultivars the entire shoot tip is affected sometime during development. As a result, the upper portion of an affected plant is completely devoid of chlorophyll. Separate progenies obtained from self-pollination of the upper and lower portions of such a plant behave developmentally in identical manner.

The time and extent of gene expression in both groups are highly affected by non-genetic fluctuations. This is particularly true for heterozygotes.

The hypothesis of homeostatic regulators can be tested. First, consider the future synthesis of two isogenic *B+* inbreds; one carrying *Ses-B* and another, *Ses-B+*. These inbreds will appear indistinguishable phenotypically. However, when tested for photosynthetic activity in diverse environments the difference between them will become evident. Either the *Ses-B* inbred will be consistently superior over the *Ses-B+* inbred or each will be superior in a different ecological niche. Second molecular analysis will demonstrate that the DNA sequences of some of the homeostatic regulators in *Cucurbita* are shared by many

distantly related species of higher plants, and that these sequences influence the potential of crop yield.

While light triggers the process of plastid transformation, the evidence in *Cucurbita* and other taxa suggests the existence of an hierarchy of regulators that sustains this process during development. any alternative to the HPT hypothesis should offer a more convincing interpretation for the kinds of specificity as well as for the widespread distribution of such genes as *Ses-B* and *Ses-B*+ among the *B*+ cultivars of *Cucurbita*.

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Determination of Molecular Weight of Chloroplast DNA of *Cucurbita pepo* L. using Different Restriction Enzymes

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Chloroplasts contain their own complement of DNA as well as protein synthesis apparatus. The chloroplast DNA (CpDNA) exists as covalently closed circular molecular molecules, ranging in size from 120 to 180 kilobase pairs (kbp) in flowering plant species (4). Chloroplasts, however, are not autonomous: the biogenesis of chloroplasts requires the coordinative expression of both specific nuclear genes and chloroplast genes.

In order to understand mechanisms that control the expression of nuclear and chloroplast genes, one prerequisite is the ability of physically purify the chloroplast DNA and to know genetic organization of the chloroplast DNA. The first introductory study for estimating molecular weight among members of Cucurbitaceae was conducted by Juvik and Palmer (3). However, only the ranges and numbers of fragments produced by different restriction endonucleases were reported. In this report, a rapid method of restriction enzyme analysis of the squash cpDNA is described in some detail and the size of *C. pepo* chloroplast genome is estimated.

Chloroplast Isolation. Squash (*Cucurbita pepo* L.) chloroplasts were extracted from young leaves according to the protocol of Gounaris et. al (1) with following modifications. The crude extraction of chloroplasts was resuspended in homogenized buffer and collected by centrifugation at 1500 X g for 15 min. instead of using discontinuous sucrose gradient centrifugation to purify chloroplasts (2, 3), a continuous sucrose gradient was used to remove contaminating nuclear DNA. The resuspended pellet was loaded onto a 30 to 60% w/v gradient of sucrose, and spun at 100,000 X g in a SW-27 rotor at 4°C for 1 hr. The chloroplast bands were collected, diluted with an equal volume of TE buffer, and centrifuged at 2,500 X g for 5 min.

Isolation of cpDNA. The chloroplast pellet was resuspended in 5 ml of the homogenization buffer,m to which 1/10 volumes of 1 mg/ml RNAse A and 2 ml of 10% s/v sodium sarkosinate were added. The suspension was incubated at room temperature for 30 min. for chloroplast lysis. The DNA sample was extracted with an equal volume of buffer-saturated phenol, three times with 4 ml of phenol and 2 ml of chloroform, and twice with water-saturated n-butanol. DNA was precipitated at -70°C for 1 hr by adding 1 ml of 7.5 M ammonium acetate and 2.5 volumes of absolute ethanol. The precipitated DNA pellet was washed with 70% ethanol, dried under nitrogen gas, and dissolved in TE buffer and stored at -20°C.

Digestion of cpDNA with restriction endonucleases. The chloroplast DNA were digested with selected restriction endonucleases under the conditions recommended by the suppliers. Restriction fragments of plastid DNA were separated by electrophoresis in 0.5-1.7% agarose gels, depending on the size of fragments.

Table 1. Numbers, sizes (in Kbp) and stoichiometries (brackets) of squash cpDNA restriction fragments generated by different endonuclease restriction enzymes.

	Restriction enzyme				
Fragment nos.	**Sal**	**Pvu II**	**Bgl I**	**Sac II**	**Pst I**
1	47.7 (2x)	57.3	47.5	29 (2x)	29.9 (2x)
2	26.3	28.6	35.3	25.4	25.2
3	21.1	19.5	22.8	20	21.0

4	18.8	16.2	21.1	5	13.6
5	2.4 (2x)	14.2	11.1	16.6	11.9
6	--	10.5	7.4	15.3	10.0
7	--	7.9	6.5 (2x)	12.8	8.4
8	--	6.1 (2x)	4.3 (2x)	10.7	6.1
9	--	--	--	5.4	4.3 (2x)
10	--	--	--	1.6	1.4
11	--	--	--	--	0.6
Total	166.4	166.4	166.7	166.2	166.5

The previously reported method for cpDNA isolation is very time-consuming and tedious (2, 3). The proposed method was modified to avoid the pronase treatment and CsCl density centrifugation, which are replaced with phenol and phenol/chloroform treatment (1).

The length of the restriction fragments was easily determined by calibrating the gel. This was done by running Lambda DNA digested with Hind III and Zho I in another slot of the same gel. The molecular weights of fragments larger than 30 kpb were estimated as the sum of subfragments derived from second digestion. For the five enzymes reported, the size of *C. pepo* DNA is estimated at 166 Kbp (Table 1) and work is in progress to prepare a detailed restriction enzyme map for *C. pepo* cpDNA.

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Taxonomic Position of Round Melon (*Praecitrullus fistulosus*)

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Round melon or 'tinda' is an Asian cucurbit having a chromosome number of x=12. This taxon was earlier considered as a botanical variety of watermelon, *Citrullus lanatus* (x=11). Pangalo (8), however, identified distinct morphological and cytological differences between *C. vulgaris* var. *fistulosus*(tinda) and *C. lanatus* (syn. *C. vulgaris*). There is now general agreement among botanists and cytologists in that round melon requires a separate taxonomic status from watermelon. Khoshoo and Vij (6) and Trivedi and Roy (12) suggested a separate species status for round melon in the genus *Citrullus*. However, many other scientists are of the opinion that round melon should be put in a different genus, separate from *Citrullus* (2, 4, 7, 11). Shimotsuma (10) was of the opinion that round melon with x=12 should be placed in the genus *Cucumis*, along with *C. melo* whose chromosome number is also 12. However, histological studies by Fursa (3) and analysis of leaf phenolics by Kaur et al. (5) brought out distinct differences between the two taxa.

Tinda is not crossable with either watermelon or muskmelon, but isozymes provided additional evidence for comparison of the two species. Round melon was compared with watermelon and muskmelon for two enzyme systems, peroxidase (PRX) and glutamate oxaloacetate transaminase (GOT). Polyacrylamide gel electrophoresis was carried out at 5°C, using vertical slab gels and a constant current of 40 mA per slab. The gel buffer for all analyses was pH 9.0 tris-chloride, and the electrode buffer was pH 8.3 tris-glycine. Bromophenol blue (0.2%) in imidazole buffer (pH 7.0) was used as a tracer dye, and relative mobility (Rm) was calculated. Peroxidase analyses were made on roots and hypocotyls of 4-5 week old seedlings, with gel concentration of 7% acrylamide and staining adopted from Conklin and Smith (1). Glutamate oxaloacetate transaminase analyses were made on 3-4 day old seedlings, with 9.5 acrylamide gel concentration and staining technique adopted from Shaw and Koen (9).

Seven peroxidase isozymes were found (Fig. 1) at Rm 0.01, 0.04, 0.11, 0.15, 0.44, 0.47, 0.76), different in electrophoretic mobility from the six isozymes found in *Citrullus lanatus* (Rm=0.07, 0.12, 0.19, 0.43, 0.54, 0.57) and the eight isozymes of *Cucumis melo* (Rm=0.05, 0.15, 0.443, 0.48, 0.52, 0.56, 0.61, 0.73). In the GOT zymogram, the three isozymes of *Praecitrullus* (Rm=0.13, 0.26, 0.30) were different from the two found in *Citrullus lanatus* (Rm=0.22, 0.25) and the four isozymes found in *Cucumis melo* (Rm=0.17, 0.23, 0.34, 0.38).

Thus, it was found that there was no similarity of *Praecitrullus* with *Citrullus lanatus* or *Cucumis melo* for PRX or GOT, although Zamir et al. (13) noticed similarity between *C. lanatus* and *C. colocynthis* for GOT and PRX zymograms. The present study substantiates Pangalo's classification of round melon in a genus separate from that of watermelon.

Comparing *Praecitrullus* with *Cucumis melo*, it was found that the two species did not have any PRX or GOT isozymes in common. The isozyme at GOT_4 which was present in the 12 *Cucumis* species analyzed was absent in *Praecitrullus*. Thus, the present study disputes the argument of Shimotsuma (10) that round melon should be placed in the genus *Cucumis*. The indian round melon or 'tinda' is unrelated to and different from muskmelon and watermelon. The present study supports Pangalo's classification of 'tinda' under a new genus, *'Praecitrullus'*.

Figure 1. Peroxidase and GOT zymograms of *Citrullus lanatus* (50), *Praecitrullus fistulosus* (52) and *Cucumis melo* (8).

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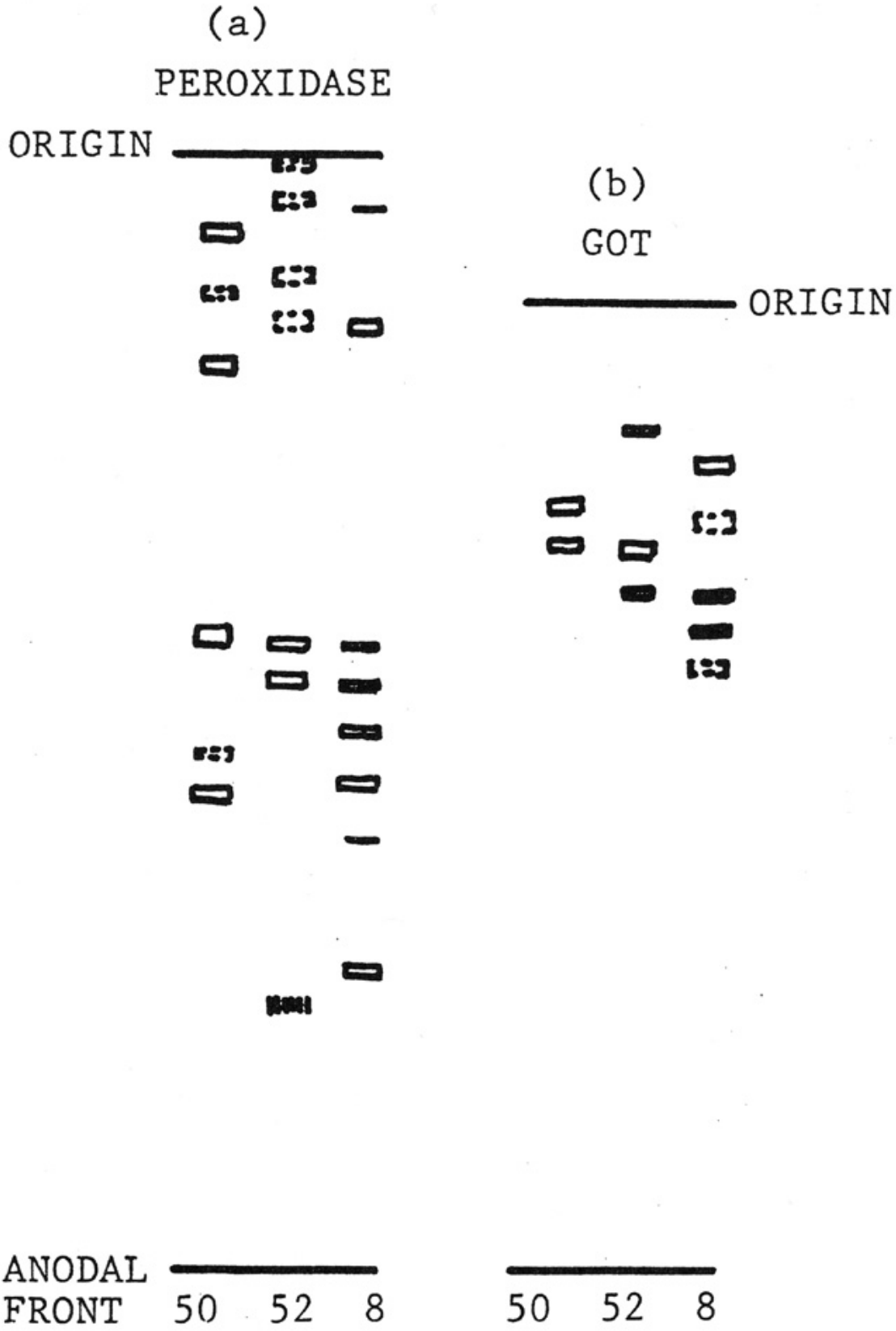
(a)
PEROXIDASE
ORIGIN
(b)
GOT
ORIGIN
ANODAL
FRONT
50
52
8
50
52
8

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Allozyme Studies in the Benincaseae

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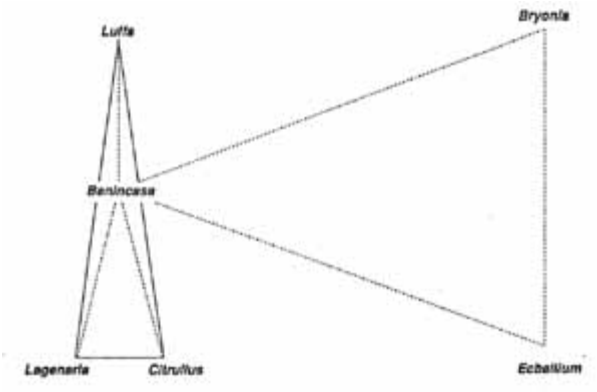
We employed starch gel electrophoresis to evaluate allozyme activity and variation in six genera in the tribe Benincaseae (Cucurbitaceae). Germplasm accessions of the domesticated species, *Benincasa hispida* (Thunb.) Cogn. (winter-melon), *Citrullus lanatus* (Thunb.) Mats. & Nakai (watermelon), *Lagenaria siceraria* (Mol.) Standley (bottle gourd), *Luffa acutangula* (L.) Roxb. (ridged loofah) and *Luffa cylindrica* (L.) M.J. Roem. (smooth loofah), were obtained from commercial and private sources. Five and fifteen different cultivars of *B. hispida* and *Lagenaria siceraria*, respectively, were included in our experiments. The winter-melon cultivars represented the major morphological groupings in the species (2). One bottle gourd cultivar came directly from Niger, Africa, three were from Mexico, and three were from Taiwan. Germplasm representing wild *Bryonia dioica* Jacq. (bryony), *Citrullus colocynthis* (L.) Schrad., and *Ecballium elaterium* (L.) A. Richard (squirting-cucumber) was procured from the Botanical Gardens at Caen and Bordeaux, France. Self pollinations of *C. lanatus, Lagenaria siceraria,* and both species of *Luffa* added genetic interpretation of enzyme banding patterns.

Cotyledons of young seedlings provided the electrophoretic sample. We assayed over 40 enzymes using a variety of gel buffer systems. Reasonable scoring was possible for about half of those, including aspartate aminotransferase (AAT), aconitase (ACO), acid phosphatase (ACP), adenylate kinase (ADK), catechol oxidase (CO), glutamate dehydrogenase (GDH), glucose-6-phosphate isomerase (GPI) glycerate dehydrogenase (GD2), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 'malic' enzyme (ME), menadione reductase (MNR), mannose-6-phosphate isomerase (MPI), peptidase (PEP), phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKDH). Germination difficulties prevented the inclusion of the wild species in the assays of AAT, CO, MNR, MPI, and PEP.

Most species displayed relatively little genetic variation. In spite of morphological diversity, allozyme variation in the winter-melon was limited to ADK, MDH, ME, and SKDH. Polymorphism within and among cultivars of the bottle gourd was detected in ACO, ADK, G6PDH, LAP, ME, PGD, and SKDH. When African and Oriental accessions differed genetically, Mexican cultivars often exhibited both sets of alleles. Although little variation was observed in species of loofah, variation between them was detected in approximately 70% of the scorable enzyme systems. Allozyme variation within and between species of *Citrullus* was similar to that found in a previous study (1). In our study, ACO nd GDH were additional variable enzymes. Bryony appeared to be the most genetically diverse species; polymorphism was detected in ACO, GDH, GIP, G2D, G3PDH, IDH, MDH, PGM, and SKDH. Variation in the squirting-cucumber could not be properly assessed since few individuals were tested.

Limited variability within species and similarity in band migration among genera provided reasonable justification for attempting generic comparisons. Enzyme systems in which bands from different genera comigrated and homology was assumed included AAT, ADK, CO, GIP, G2D, G3PDH, MDH, ME, MNR, MPI, PEP, PGD, AND PGM. Figure 1 represents our interpretation of genetic relationships as revealed by these generic comparisons. Shorter lines represent a larger proportion of shared allozyme alleles. *Bryonia* and *Ecballium* are compared to each other and to the remaining group of genera as a whole.

Figure 1. Allozyme relationships among genera in the Benincaseae.



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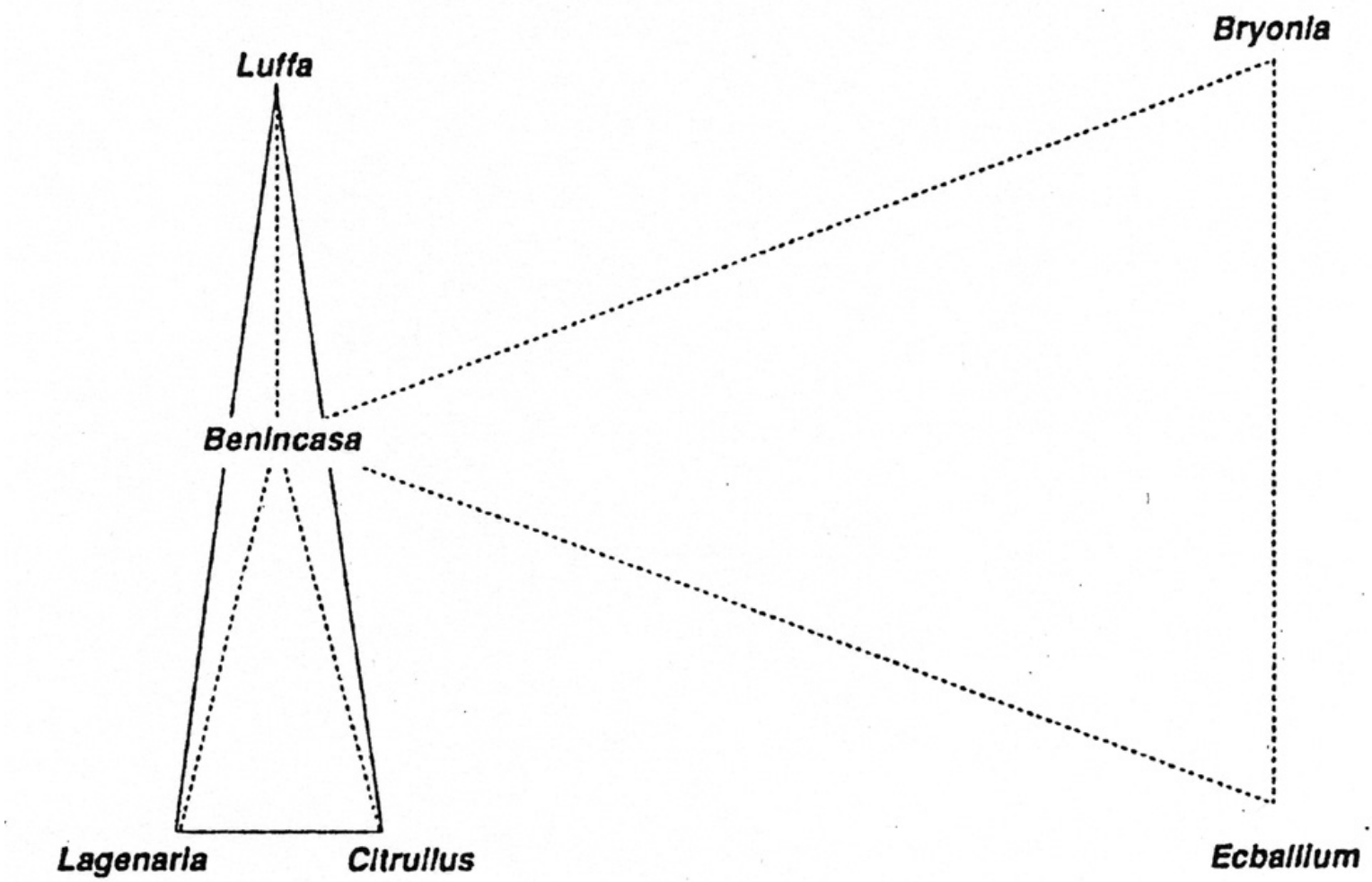
Bryonia
Luffa
Benincasa
Lagenaria
Citrullus
Ecballium

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Gene List for Cucumber

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Lists of the known genes for the Cucurbitaceae have been published previously in HortScience and the report of the Cucurbit Genetics Cooperative. However, in the interest of updating and collecting the information on cucumber in one place, following is a complete list of the 105 known genes for *Cucumis sativus* L.

Gene symbol			
Preferred	**Synonym**	**Character**	**References**
a		*androecious*. Produces primarily staminate flowers if recessive for *F*. *A* from MSU 713-5 and Gy 14A; *a* from An-11 and An-314, 2 selections from 'E-e-szan' of China.	45
ap		*apetalous*. Male sterile. Anthers become sepal-like. *Ap from 'Butchers Disease Resisting'; ap* from 'Butchers Disease Resisting Mutant.	29
Ar		*Anthracnose resistance*. One of several genes for resistance to *Colletotrichum lagenarium*. *Ar* from PI 175111, PI 175120, PI 179676, PI 183308, PI 183445; *ar* from 'Palmetto' and 'Santee'.	9
B		*Black* or brown spines. Dominant to white spines on fruit. *B* from 'Richard's Invincible', 'Nezhin', 'Muron', and 'Everyday'; *b* from 'White Spine, 'Vyaznikov', 'Berlizov', and 'Vickery'.	15, 31, 33, 36, 86, 87, 95, 103
B-2		*Black spine-2*. Interacts with *B* to produce F_2 of 15 black : 1 white spine. *B-2* from Wisc. 9362; *b-2* from PI 212333 and 'Pixie'.	79
B-3		*Black spines-3*. Interacts with *B-4* to produce F_2 of 9 black:7 white spine. *B-3* from LJ90430, *b-3* from MSU 41.	17
B-4		*Black spines-4*. Interacts conversely of *B-3*. *B-4* from LJ 90430; *b-4* from MSU 41.	17
bi		*bitterfree*. All plant parts lacking cucurbitacins. *bitterfree. Bi* from a Dutch variety; *bi* from an 'Improved Long Green' selection.	6
bl	*t*	*blind*. Terminal bud lacking after temperature shock. *Bl* from 'Perseus' and inbred BDR; *bl* from 'Hunderup' and inbred HP3.	12
bla		*blunt leaf.* Leaves have obtuse apices and reduced lobing and serration. *bla* from a mutant of 'Wisc. SMR-18'.	66
Bt		*Bitter fruit*. Fruit with extreme bitter flavor. *Bt* from PI 173889 (Wild Hanzil Medicinal Cucumber); *bt* from 'Model', 'National' and 'Long Green'.	8
bu		*bush*. Shortened internodes. *Bu* from 'Wisc. SMR 12' and others; *bu* from 'KapAhk 1.	64
Bw		*Bacterial wilt resistance*. Resistance to *Erwinia tracheiphila*. *Bw* from PI 200818; *bw* from 'Marketer'.	56, 73
c		*cream mature fruit color.* Interaction with *R* is evident in the F_2 ratio of 9 red (*R* +) : 3 orange (*R c*) : 3 yellow (++) : 1 cream (+ *c*).	33
Cca		*Corynespora cassicola* resistance. Resistance to target leaf spot; dominant to susceptibility. *Cca* from Royal Sluis Hybrid 72502; *cca* from GY 3.	3

Ccu		*Cladosporium cucumerinum resistance*. Resistance to scab.*Ccu* from line 127.31, a selfed progeny of 'Longfellow'; *ccu* from 'Davis Perfect' and other selections.	2, 4, 5, 7
cd		*chlorophyll deficient*. Seedling normal at first, then becoming light green; lethal unless grafted. *Cd* from normal progeny of the backcross of MSU 71305 x 'Midget' to 'Midget'; *cd* from a mutant selection of the same source.	11
cl		*closed flower*. Flowers do not open; male sterile.(non-fertile pollen). *cl* from a Korean line.	30
cla		*Colletotrichum lagenarium resistance*. Resistance to race 1 of anthracnose; recessive to susceptibility. *Cla* from 'Wisc. SMR 18'; *cla* from SC 19B.	3
Cm		*Corynespora melonis* resistance. Resistance to *C. melonis*; dominant to susceptibility. *Cm* from 'Spotvrie'; *cm* from 'Esvier'	89
Cmv		*Cucumber mosaic virus resistance*. One of several genes for resistance to CMV *Cmv* from 'Wisc. SMR 12', their resistance from 'Chinese Long' except 'Wisc. SMR 15' which also gets it from 'Tokyo Long Green'. *cmv* from 'National Pickling' and Wisc SR 6.	84, 93
co		*green corolla*. Green petals which turn white with age and enlarged reproductive organs; female sterile. *co* is from a selection of 'Extra Early Prolific'.	18, 32
cor-1		*cordate leaves-1*. Leaves are cordate, cor-1 from 'Nezhinskii'.	28
cor-2	*cor*	*cordate leaves-2*. Leaves are nearly round with revolute margins and no serration. Insect pollination is hindered by short calyx segments which tightly clasp the corolla preventing full opening. *cor-2* from an induced mutant of 'Lemon'.	67
cp		*compact*. Reduced internode length, poorly developed tendrils, small flowers. *Cp* from 'Chipper', Gy 3, 'Poinsett', 'Tablegreen 65' and PG 57; *cp* from PI308916.	39
cr		*crinkled* leaf. Leaves and seed crinkled.	57
cs		*carpel splitting*. Fruits develop deep longitudinal splits. *Cs* from Gy 14A; *cs* from TAMU 1043 and TAMU 7210 which are second and fifth generation selections of MSU 3249 x SC 25.	13, 60
D	*g*	*Dull fruit skin*. Dull skin of American cultivars, dominant to glossy skin of most European cultivars. *D* from 'Vickery', 'Nezhin'; *d* from 'Everyday' and 'Galakhov'.	62, 86, 87
de	*l*	*determinate habit*. Short vine with stem terminating in flowers; modified by *In-de* and other genes; degree of dominance depends on gene background.*De* from 'Stono', 'Straight Eight', 'SMR 58', MR 17, MR 25, 'Palmetto', 'Napa', 'Highmoor', 'Burpee's Extra Early', 'Ashley', 'SMR 17', CU 54-467, CU 55-610, CU 56-388, 'Marketeer' and 'Tokyo'; *de* from Penn 76.60G, Minn 158.60, 'Hardin's PG 57', 'Hardin's Tree Cucumber' and S_2-1 (an inbred selection from line 541).	20, 26, 33, 56
df		*delayed flowering*. Flowering delayed by long photoperiod; associated with seed dormancy. *Df* from 'Marketer', Wisc. 1606, Wisc. 1609 and Wisc 1548; *df* from 'Baroda' (PI 212896) and PI 215589 (*C. hardwickii*).	19, 83
di		*diabrotica resistance*. Resistance to the spotted and banded cucumber beetle. *di* from 'Eversweet'.	14
dl		*delayed growth*. Reduced growth rate; shortening of hypocotyl and first internodes. *Dl* from 'Marketer', 'Marketmore' and 'Tablegreen'; *dl* from 'Dwarf Marketmore' and 'Dwarf Tablegreen' both deriving dwarfness from 'Hardin's PG 57'.	49
dm	*P*	*downy mildew resistance*. One of several genes for resistance to *Pseudoperonospora cubensis*. Dm from Sluis & Groot Line 4285; dm from 'Poinsett'.	36, 85, 90
dvl	*dl*	*divided leaf*. True leaves are partly or fully divided, often resulting in compound leaves with 2 to 5 leaflets and having incised corollas. *Dvl* from 'Levo; *dvl* from lot 318 and 319.	55
dw		*dwarf*. Short internodes. dw from an induced mutant of 'Lemon'.	70

Es-1		*Empty chambers-l*. Carpels of fruits separated from each other, leaving a small to large cavity in the seed cell. *Es-1* from 002-75; *es-1* from Gy 30-75	48
Es-2		*Empty chambers-2*. Carpels of fruits separated from each other, leaving a small to large cavity in the seed cell. *Es-2* from PP-2-75; *es-2* from Gy-30-75.	48
F	*Acr* *acr*^{F}, *D, st*	*Female*. High degree of female sex expression; interacts with *a* and *M*; strongly modified by environment and gene background. F and f are from the variety 'Japanese'.	25, 42, 43, 62, 82, 87
fa		*fasciated*. Plants have flat stems, short internodes, and rugose leaves. fa was from a selection of 'White Lemon'.	65, 81
Fba		*Flower bud abortion*. Preanthesis abortion of floral buds, ranging from 10 to 100%. *Fba* from MSU 713-5; *fba* from MSU 0612.	50
Foc		*Fusarium*oxy*sporum f. sp. cucumerinum resistance*. Resistance to fusarium wilt; dominant to susceptibility. *Foc* from Wisc. 248; *foc* from 'Shimshon'	52
g		*golden* leaves. Golden color of lower leaves. *G* and *g* are both from different selections of 'Nezhin'.	87
gb	*n*	*gooseberry fruit*. Small, oval shaped fruit. *Gb* from 'Nehzin'; *gb* from the 'Klin mutant'.	87
gc		*golden cotyledon*. Butter colored cotyledons; seedlings die after 6 to 7 days. *Gc* from 'Burpless Hybrid'; *gc* from a mutant of 'Burpless Hybrid'.	97
gi		*ginko*. Leaves reduced and distorted, resembling leaves of *Gingko*; male and female sterile. Complicated background: It was in a segregating population whose immediate ancestors were offspring of crosses and BC's involving 'National', 'Chinese Long', 'Tokyo Long Green', 'Vickery', 'Early Russian', 'Ohio 31' and an unnamed white spine slicer.	37
gl		*glabrous*. Foliage lacking trichomes; fruit without spines. *Gl* from 'Mayak 422' and 'Odnostebelnyi'; *gl* from NCSU 75 and M834-6.	35, 69
glb		*glabrate*. Stem and petioles glabrous, laminae slightly pubescent. *Glb* from a mutant of 'Burpless Hybrid'; *glb* from 'Burpless Hybrid'.	100
gy	*g*	*gynoecious*. Recessive gene for high degree of female sex expression. *Gy* and *gy* are both found in different selections (S_{10}) made from 'Borszagowski'.	47
H		*Heavy netting of fruit*. Dominant to no netting and completely linked or pleiotropic with black spines (*B*) and red mature fruit color (*R*).	33, 87
I		*Intensifier*of *P*. Modifies effect of *P* on fruit warts in *Cucumis sativus* var. *tuberculatus*.	87
In-de	*In*(*de*)	*Intensifier*of *de*. Reduces internode length and branching of *de* plants. *In-de* and *in-de* are from different selections (S_5-1 & S_5-6, respectively) from a determinant inbred S_2-1 which is a selection of line 541.	26
In-F	*F*	*intensifier of female sex expression*. Increases degree of female expression of *F* plants. *In-F* from monoecious line 18-l; *in-F* from MSU 713-5.	44
l		*locule number*. Many fruit locules and pentamerous androecium, 5 locules recessive to the normal number of 3.	103
1h		*long hypocotyl.* As much as a 3 fold increase in hypocotyl length. *Lh* from MSU 713-5; *lh* from a 'Lemon' mutant.	72
ll		little leaf. Normal sized fruits on plants with miniature leaves and smaller stems. *Ll* from Wisc 2757; *ll* from 'Little John.	27, 94
ls		*light sensitive*. Pale and smaller cotyledons, lethal at high light intensity. *Ls* from 'Burpless Hybrid'; *ls* from a mutant of 'Burpless Hybrid'.	99
m	*a, g,*	*andromonoecious*. Plants are andromonoecious if *(m* +); monoecious if (++); gynoecious if (+ *F*); and hermaphroditic if (*m F*). *M* from 'Chicago Pickling' and	74, 82, 87, 91, 103

		'Long Green'; *m* from 'Lemon'.	
m-2	*h*	*andromonoecious-2*. Bisexual flowers with normal ovaries.	34, 47
mp	pf^{+}, pf^{d}, pf^{P}	*multi-pistillate*. Several pistillate flowers per node, recessive to single pistillate flowers per node, recessive to single pistillate flower per node. *Mp* from Gy 14A and CU 551F; *mp* from MSU 604G and MSU 598G.	24, 51
Mp-2		*Multi-pistillate* Several pistillate flowers per node. Single dominant gene with several minor modifiers. *Mp-2 from MSU 3091-1;* mp-2 *from Gy 3.*	88
ms-1		*male sterile-1*. Male flowers abort before anthesis, partially female sterile. *ms-1* from 'Black Diamond' and 'A&C'.	71, 81
ms-2		*male sterile-2*. Male sterile; pollen abortion occurs after first mitotic division of the pollen grain nucleus. *Ms-2* from 'Burpless Hybrid'; *ms-2* from a mutant of 'Burpless Hybrid'..	98
n		*negative geotropic peduncle response*. Pistillate flowers upright; recessive to pendent position of most cultivars.	58
ns		*numerous spines*. Few spines on the fruit is dominant to many. *Ns* from 'Spartan Salad', 'Wisc. 'SMR-18' and 'Gy 2 *cp cp*' *ss* from 'Wisc 2757.'	22, 23
O	*y*	*Orange-yellow corolla.* Orange-yellow dominant to light yellow. *O* and *o* are both from 'Nezhin'.	87
opp		*opposite leaf arrangement*. Opposite leaf arrangement is recessive to alternate and has incomplete penetrance. *opp* from 'Lemon'.	68
P		*Prominent tubercles*. Prominent on yellow rind of *Cucumis sativus* var. *tuberculatus*. Incompletely dominant to brown rind without tubercles. *P* from 'Klin'; *p* from 'Nezhin'.	87
Pc	*P*	*Parthenocarpy*. Sets fruit without pollination.*Pc* from 'Spotvrie'; *pc* from MSU 713-205.	59, 61, 96
pl		*pale lethal*. Slightly smaller pale green cotyledons; lethal after 6 to 7 days. *Pl* from 'Burpless Hybrid'; *pl* from a mutant of 'Burpless Hybrid'..	100
pm-1		*powdery mildew resistance-1*. Resistance to *Sphaerotheca fuliginea*. *pm-1* from 'Natsufushinari'.	31, 40, 80
pm-2		*powdery mildew resistance-2*. Resistance to *Sphaerotheca fuliginea*. *pm-2* from 'Natsufushinari'.	31, 40, 80
pm-3		*powdery mildew resistance-3*. Resistance to *Sphaerotheca fuliginea*. *pm-3* found in PI 200815 and the PI 200818.	40, 80
pm-h	*s, pm*	*powdery mildew resistance expressed by the hypocotyl.* Resistance to powdery mildew as noted by no fungal symptoms appearing on seedling cotyledons is recessive to susceptibility*. Pm-h* from 'Wisc. SMR-18'; *pm-h* from Gy 2 cp cp', 'Spartan Salad' and 'Wisc. 2757'.	22, 80
pr		*protruding* ovary. Exerted carpels.	103
ps1	*p1*	*pseudomonas lachrymans* resistance. resistance to pseudomonas lachrymans is recessive. *Psl* from 'National Pickling' and 'Wisc. SMR 18'; *psl* from MSU 9402 and Gy 14A.	1
R		*Red mature fruit*. Interacts with *c*; linked or pleiotropic with *B* and *H*.	33
rc		*revolute cotyledon*. Cotyledons short, narrow, and cupped downwards; enlarged perianth.*Rc* from 'Burpless Hybrid'; *rc* from 'Burpless Hybrid' mutant.	102
ro		*rosette*; short internodes muskmelon-like leaves. *Ro* from 'Megurk', the result of a cross involving a mix of cucumber and muskmelon pollen.	76
s	*f,a*	*spine* size *and frequency*. Many small fruit spines, characteristic of European cultivars is recessive to the few large spines of most American cultivars. *S* from 'Vickery', 'Vyaznikov' and 'Berlizov'; *s* from 'Everyday', 'Nezhin' and 'Muron'.	13, 62, 86, 87

s-2		*spine-2*. Acts in duplicate recessive epistatic fashion with *s-3* to produce many small spines on the fruit. *S-2* from Gy 14; *s-2* from TAMU 72210.	13
s-3		*spine-3*. Acts in duplicate recessive epistatic fashion with *s-2* to produce many small spines on the fruit. *S-3* from Gy 14; *s-3* from TAMU 7220.	13
sa		*salt tolerance*. Tolerance to high salt levels is attributable to a major gene in the homozygous recessive state and may be modified by several minor genes. *Sa* from PI 177361; *sa* from PI 192940.	38
sc	*cm*	*stunted cotyledons*. Small concavely curved cotyledons; stunted plants with cupped leaves; abnormal flowers. Wisc. 9594 and Wisc. 9597 were used as heterozygous parents.	77, 78
Sd		*Sulfur dioxide resistance*. Less than 20% leaf damage in growth chamber. *Sd* from 'National Pickling'; *sd* from 'Chipper'.	10
sp		*short petiole*. Leaf petioles of first nodes 20% the length of normal. *sp* from the Russian mutant line 1753.	53
ss		*small spines*. Large, coarse fruit spines is dominant to small, fine fruit spines. *S*s from 'Spartan Salad', 'Wisc. SMR-18' and 'Gy 2 *cp cp*; *ss* from 'Wisc 2757.	22, 23
T		*Tall plant*. Tall height incompletely dominant to short height.	33
td		*tendrilless*. Tendrils lacking; associated with misshaped ovaries and brittle leaves. *Td* from 'Model' and Sc 8M ('Pixie'); *td* from a mutant of 'Southern Pickler'.	75
te		*tender skin of fruit*. Thin, tender skin of European cultivars; recessive to the thick, tough skin of most American cultivars. *Te* from 'Vickery'; *te* from 'Everyday'.	62, 86
Tr		*Trimonoecious*. Producing male, bisexual, and female flowers in this sequence during plant development. *Tr* from Tr-12, a selection of a Japanese variety belonging to the Fushinari group; *tr* fromH-7-25, MOA-309, MOA-3-3 and AH-311-3.	46
Tu		*Tuberculate fruit*. Warty fruit, characteristic of American cultivars is dominant to the smooth, nonwarty fruits of most European cultivars. *Tu* from 'White Spine' and 'Vickery'; *tu* from 'Richard's Invincible' and 'Everyday'.	86, 95
u	*M*	*uniform immature fruit color*. Uniform color of European cultivars such as 'Everyday' recessive to the mottled or stippled color of most American cultivars. *U* from 'Vickery'; *u* from 'Everyday'.	5, 59
ul		*umbrella leaf*.Leaf margins turn down at low relative humidity making leaves look cupped. Source of *ul* unknown.	54
v		*virescent*. Yellow leaves becoming green.	62, 87
vvi		*variegated virescent*. Yellow cotyledons, becoming green; variegated leaves.	2
w		*white immature fruit color*. White is recessive to green .W from 'Vaughn', 'Clark's Special', 'Florida Pickle' and 'National Pickling'; *w* from 'Bangalore'.	15
wf	*w*	*white flesh*. Intense white flesh color; recessive to dingy white; acts with *yf* to produce F_2 of 12 white: (++ and + *wf*): 3 yellow (*yf* +): 1 orange (*yf wf*). *Wf* from EG and G6, each being dingy white (++); *wf* from NPI which is orange (*yf wf*).	29
Wmv		*Watermelon mosaic virus resistance*. Resistance to strain 2 of watermelon mosaic virus. *Wmv* from 'Kyoto 3 Feet'; *wmv* from 'Bet-Alfa'.	16
wmv 1-1		*watermelon mosaic virus-1 resistance*. Resistance to strain 1 of watermelon mosaic virus by limited systemic translocation; lower leaves may show severe symptoms. *Wmv-1-1* from Wisc. 2757; *wmv-1-1* from 'Surinam'.	92
yc-1		*yellow cotyledons-1*. Cotyledons yellow at first, later turning green. *Yc-1* from Ohio M.R. No. 25; *yc-1* from a mutant of Ohio M.R. No. 25.	1
yc-2		*yellow cotyledons-2*. Virescent cotyledons. *Yc-2* from 'Burpless Hybrid'; *yc-2* from a mutant of 'Burpless Hybrid'.	101. 102

yf	*v*	*yellow flesh*. Interacts with yf to produce F_2 of 12 white (++ and + *wf*) : 3 yellow (*yf* +) : 1 orange (*yf wf*). *Yf* from 'Natsufushinari' which has an intense white flesh (*Yf wf*); *yf* from PI 200815 which has a yellow flesh (*yf Wf*).	41
yg	*gr*	*yellow-green immature fruit color*. Recessive to dark green and epistatic to light green.	103
yp		*yellow plant.* Light yellow green foliage; slow growth.	2
zymv		*zucchini yellows mosaic virus.* Inheritance is incomplete. Believed to be inherited in a recessive fashion with the source of resistance being 'TMG-1'.	63

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4. British Library, Document Supply Center Serial Acquisitions, Boston Spa, Wetherby, West Yorkshire LS23BQ,

England
5. Central Library of Agricultural Science P.O. Box 12, Rehovot, 76 100, Israel.
6. Del Monte Corp. P.O. Box 36, San Leandro, CA 94577.
7. DNA Plant Technology, Inc. Attn: Nergish Karanja, Librarian, 2611 Branch Pike, Cinnaminson, NJ 08077.
8. Estacion Experimental Menioza Casilla de Correo 3, 5507 Lujan de Cuyo, Mendoza, Republica Argentina.
9. Estacion Experimental Santiago del Estero Casilla de Correo 268, 4200 Santiago del Estero, Republica Argentina.
10. I.N.R.A. Regie Centre Avignon Domain St. Paul, Montfavet, France.
11. Institut Za Povrtarsvo Palanka Karadjordjeva 71, 11420 Smederevska Palanka, Yugoslavia.
12. Institute National De La Rech, Agron. Laboratoire D'Amelioration des Plantes, University de Paris-Sud - Bat. 360, Centre D'Orway, F, 91405 Orsay Cedex, France.
13. Institute Za Ratarstvo 1 Povrtarstvo-Biblioteka, M. Gorkog 30, 21-000 Novi Sad, Yugoslavia.
14. J.E. Ohlsens Enke A/S Roskildevej 325A, DK-2630, Tastrup, Denmark.
15. National Vegetable Research Station Attn: The Librarian, Wellesbourne, Warwic CV35 9EF, England.
16. New York State Agricultural Experiment Station Library Jordan Hall, Geneva, NY 14456.
17. Robson Seed Farms One Seneca Circle, Hall, NY 14463.
18. Sakata SEed America Research Station, P.O. Box 6007, Salinas, CA 93912.
19. Servico de Investigacion Agraria Library, Departmento de Agricultura, Montanana, 176, Zaragoza, Spain.
20. Swets North America P.O. Box 517, Berwyn, PA 19312.
21. Taiwan Agricultural Research Institute 189 Chung-cheng Road, Wan-Feng, Wu-feng, Taichung, Taiwan, Republic of China.
22. Universita degli Studi di Bari Dipartiento di Patologia Vegetale, Via G. Amendola, 165.A, 70126 Bari, Italy.
23. University of California, Davis. The Library, Davis, CA 95616.

Geographical Distribution of CGC Members in the United States

- **Alabama**
  - O.L. Chambliss
  - Fenny Dane
  - J.D. Norton
  - Gowind C. Sharma
- **Arizona**
  - Dennis Ray
- **California**
  - Bruce Balgooyen
  - Warren S. Barham
  - G.W. Bohn
  - N.C. Chen
  - Paul Cjhung
  - J.W. DeVerna
  - James C. Hollar
  - Krystyna M. Ladd
  - Alex Lee
  - J.D. McCreight
  - Brian J. Moraghan
  - Ken Owens
  - Lawrence Piece
  - Vicki Pierce
  - Kathleen S. Rigert
  - R.H. Schroeder
  - Joseph Stern
  - Paul Thomas
  - Jon Waterson
  - T.W. Whitaker
  - Colen Wyatt
- **Colorado**
  - Larry A. Hollar

- **Florida**
  - Howard Adams
  - Rosa Dumlao
  - Nick Eigsti
  - Gary Elmstrom
  - Dorothy A. Eyberg
  - Tom V. Williams
- **Hawaii**
  - Terry T. Sekioka
- **Idaho**
  - Steven L. Love
  - Gary Whiteaker
  - Paul Yorty
- **Illinois**
  - John Juvik
  - Weston Msikita
  - Douglas M. Scheirer
  - Robert M. Skirvin
- **Indiana**
  - Orie J. Eigsti
  - Dae-Geun Oh
- **Iowa**
  - Glenn Drowns
- **Kansas**
  - Laura C. Merrick
  - John Navazio
- **Maryland**
  - Joseph H. Kirkbride, Jr.
  - Wayne A. Mackey
  - Marisa Maiero
  - Timothy J. Ng
- **Michigan**
  - L.R. Baker
  - Richard Bowman
  - Rebecca Grumet
- **Nebraska**
  - Dermot P. Coyne
- **New Hapshire**
  - J. Brent Loy
- **New Jersey**
  - David Groff
  - Mark Hutton
  - Oved Shifriss
  - Jim Snyder
- **New York**
  - T.C. Andres
  - Richard McArdle
  - H.M. Munger
  - Martha A. Mutschler
  - Rosario Provvidenti
  - R.W. Robinson
- **North Carolina**
  - W.R. Henderson
  - Todd C. Wehner
- **Ohio**
  - Carol A. Laymon
  - C. Ramachandran

    - Greg Tolla
- **Oklahoma**
    - E. Glen Price
- **Oregon**
    - August C. Gabert
- **Pennsylvania**
    - Charles Boyer
    - David Unander
- **Puerto Rico**
    - Linda Wessel-Beaver
- **South Carolina**
    - Jeffrey W. Adelberg
    - George Fassuliotis
    - Brenta A. Murdock
    - Billy B. Rhodes
    - Claude E. Thomas
- **Texas**
    - Edward Cox
    - Joseph O. Kuti
- **Wisconsin**
    - Linda Cruibaugh
    - W.H. Gabelman
    - Michael J. Havey
    - Andreas Katsiotis
    - R.L. Lower
    - Mary Jean Palmer
    - Philipp W. Simon
    - Jack E. Staubb
    - Gary Taurick

CGC Members in Countries Other Than the United States

- **Argentina**
    - RODRIGUEZ, Jose Pablo
- **Australia**
    - HERRINGTON, Mark
    - McGRATH, D.J.
    - PRYKE, Peter I.
- **Austria**
    - BAUMGARTHER, Oswald
- **Brazil**
    - MALUF, Wilson Roberto
    - NAGAI, Hiroshi
    - TASAKI. Seikoh
- **Bulgaria**
    - ALEXANDROVA, Maria
- **Canada**
    - DECKER WALTERS, Deena
- **China, P.R.**
    - LIN, Depei
    - MING, Wang
    - CHINA, REPUBLIC OF
    - CHEN, Fure-Chyi
    - HUNG, Lih
    - YEH, Shyi-Dong
- **Columbia**

- JARAMILLO-VASQUEZ, Juan
- **Egypt**
  - HASSAN, Mohamed Nabil
- **England**
  - POOSTCHI, Iraj
- **France**
  - CHARBONNET, Daniel
  - COMBAT, Bruno
  - DUPUY, G..
  - GABILLARD, D.
  - GAUTIER, Graines
  - GONON, Yves.
  - IGNART, Frederic
  - LAFOND, M.D.
  - PITRAT, Michel
  - RISSER, Georgette
  - SOCKELL, M.
- **Germany**
  - SCHNOCK, Matin g.
  - WEICHMANN, G.
- **Greece**
  - VAKALOUNAKIS, Demetrios J.
- **Hungary**
  - MILOTAY, Peter
- **India**
  - SESHADRI, V. S.
- **Israel**
  - COHEN, Yiga
  - GALUN, Esra
  - HERMAN, Ran
  - KARCHI, Zvi
  - NECHAMA, Shulamit
  - NIEGO, Shlomo
  - PARIS, Harry
  - VENTURA, Yaacov
  - ZUTA, Zeev
- **Italy**
  - CRINO', Paola
- **Japan**
  - FUJIEDA, Kunimitsu
  - HAGIHARA, Toshitsugu
  - HIRABAYSHI, Tetsuo
  - IGARSHI, Isamu
  - IIDA, Akira
  - ITO, Kimio
  - KANNO, Tsuguo
  - KUGINUKI, Yasuhisa
  - MOCHIZUCKI, Tatsuya
  - OIZUMI, Toshikatsu
  - ORIDATE, Toshiroh
  - SHIGA, Toshio
  - YAMANAKA, Hisako
  - YUTAKA, Tabei
- **Korea**
  - KWACK, Soo Nyeon
  - OM, Y. H.
  - PARK, Hyo Guen

- **Mexico**
  - ORTEGA, Sergio Garza
- **The Netherlands**
  - van AREND, WIM
  - van BLOKLAND, G.D.
  - BOORSMA, P.A.
  - CUSTERS, J. B. M.
  - de GROOT, Ir. E. de
  - RUITER, Ir. A. C. de
  - JARL, Carin I.
  - KLAPWIJK, Ad. A.
  - VERHOFF, Ruud
- **Peru**
  - CAREY, Edward E.
  - HOLLE, Miguel
- **Poland**
  - MACKIEWICZ, Henryk O.
  - NIEMIROWICZ-SZCZYTT, Katarzyna
- **Portugal**
  - MONTEIRO, Antonio
- **Romania**
  - POLI, Virgil
- **Spain**
  - AYUSO, Ma Cruz
  - CUARTERO, J.
  - GOMEZ-GUILLAMON, Maria Luisa
  - van LEEUWEN, Loes
  - MILLER, Chris
  - NUEZ, Fernando
  - RODRIGUEZ, Pilar C.
  - ROIG, Luis A.
  - FITO, Semillas, S.A.
- **Sweden**
  - LUNDIN, Marianne
- **Thailand**
  - MANEESINTHU, Likhit
- **Tunisia**
  - JEBARI, Hager
- **United Arab Emirates**
  - HASSAN, Ahmed A.
- **Zimbabwe**
  - MUTANGADURA, Tandai

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Covenant and By-Laws of the Cucurbit Genetics Cooperative

Article I. Organization and Purposes

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

Article II. Membership and Dues

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 Coordination 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 ho fail to pay their current biennial dues within the first six months of the biennium are dropped from active membership. Such members ma 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 spokesman of the CGC, as well as its Secretary and Treasurer.

Approvals: W. Bemis; J.D. Norton; R.W. Robinson; W.R. Henderson; M.L. Robbins; R.L. Lower

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 or 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. Bemis; J.D. Norton; R.W. Robinson; W.R. Henderson; M.L. Robbins; R.L. Lower

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 a 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 t he Annual Meeting. Other business of the Annual Meeting may include topics 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 purpose.
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:

- lend any part of its income or corpus without the receipt of adequate security and a reasonable rate of interest to;
- pay any compensation in excess of a reasonable allowance for salaries or other compensation for personal services rendered to;
- make any part of its services available on a preferential basis to;
- make any purchase of securities or any other property, for more than adequate consideration in money's worth; or
- engage in any other transactions which result in a substantial diversion of income or corpus to any officer, member of the Coordinating Committee, or substantial contributor to the CGC.

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

Article X. Distribution on Dissolution

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

Cucurbit Genetics Cooperative Report 12:124 (financial statement) 1989

Financial Statement

Cucurbit Genetics Cooperative

31 December 1988

Item		**Amount**
Balance on 31 December 1987		$2,190.78
Receipts		
- Dues and back issues	$2,924.00	
- Interest	$170.96	
- Total		$3,094.96
Expenditures		
- Report No. 11 [z]	$1,301.32	
- Membership invoices [z]	$65.73	
- Report No. 12 (call for papers) [z]	$78.15	
- Mailing back issue inventory from California to Maryland	$105.17	
- Back issue orders (envelopes and postage)	$95.93	
- Total		-$1,646.30
Balance on 31 December 1988		$3,639.44

[z] Publishing and mailing.