Cucurbit Genetics Cooperative

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The **Cucurbit Genetics Cooperative** (CGC) was organized in 1977 to develop and advance the genetics of economically important cucurbits. Membership to CGC is voluntary and open to workers who have an interest in cucurbit genetics. Membership is on a biennial basis.

CGC Membership and Subscription Rates

Biennium	**Member**	**Library**
1992-93	$14.00 US*	$24.00 US

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

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Comments from the CGC Coordinating Committee

The Call for Papers for the 1993 Report (CGC Report No. 16) will be mailed in August 1992. Papers should be submitted to the respective Coordinating Committee members by 31 December 1992. The Report will be published by June 1993. As always, we are eager to hear from CGC members regarding our current activities and the future direction of CGC.

Gary W. Elmstrom (melon)
Mark G. Hutton (other genera)
J. Brent Loy (*Cucurbita* spp.)
Dennis T. Ray (watermelon)
Todd C. Wehner (cucumber)
Timothy J Ng, Chair

Comments from the CGC Gene List Committee

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

It is hoped that scientists will consult these lists as well as the rules of gene nomenclature for the Cucurbitaceae (see page 112) before selecting a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature were adopted in order to provide guidelines for the naming and symbolizing of genes previously reported and those while will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

Cucumber:	Todd C. Wehner
Melon:	Michel Pitrat
Watermelon:	Warren R. Henderson
Cucurbita spp.:	Mark G. Hutton & Richard W. Robinson
Other genera:	Richard W. Robinson

Comments from the CGC Gene Curators

CGC has appointed curators for the four major cultivated groups: cucumber, melon, watermelon and *Cucurbita* spp. A back-up Curator for the "Other genera" category is needed; anyone wishing to take on this responsibility should contact the Chair.

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

Cucumber:	Todd C. Wehner	Watermelon:	Gary W. Elmstrom
	Jack E. Staub		E. Glen Price
Melon:	J.D. McCreight		Billy B. Rhodes
	Michel Pitrat	*Cucurbita* spp.:	Mark G. Hutton
Other genera:	Richard W. Robinson		Richard W. Robinson

15th Annual CGC Business Meeting
23 July 1991 -- University Park, Pennsylvania USA

The 15th Annual Business Meeting of the Cucurbit Genetics Cooperative (CGC) was held on Tuesday, 23 July 1991, in the Willard Building of the Pennsylvania State University in conjunction with the 88th Annual meeting of the American Society for Horticultural Science (ASHS). Approximately 22 members were in attendance.

Minutes from the 14th Annual Business Meeting (Tucson, AZ) were approved. Mention was made of (1) the upcoming U.S. cucurbit meetings in Colorado and Kentucky, and (2) the 5th Eucarpia Cucurbitaceae Symposium scheduled for Poland in 1992.

CGC membership figures, summary statistics for the first 14 CGC Reports, and the 1990 CGC Financial Statement were presented. CGC Report No. 14 (1991) had not yet been mailed by the time of the Business Meeting because of a printer's error. [Editor's note: CGC Report No. 14 was subsequently mailed on 31 July 1991.] The Call for Papers for CGC Report No. 15 (1992) was scheduled to be mailed 31 August 1991, with submissions due by 31 December 1991.

The concept of a "core collection" for cucumber was proposed by Jack Staub and Todd Wehner; selection of accessions for the collection would be based upon morphological and biochemical (e.g. isozyme) traits. Within the core collection would be a "sampler" unit for investigators requesting access to a small but diverse germplasm set. The question was raised as to the current genetic purity of cucurbit plant introductions in the U.S. collection. Mark Widrlechner commented that most of the cucurbit collections at the North Central Plant Introduction Station (Ames, IA) were now in relatively good shape with the notable exception of melon. However, he also cautioned that the formal establishment of a core collection might not be currently possible because of tight budgets.

Wehner and Staub also proposed the establishment of a database for cucurbit cultivars structured similarly to the ASHS Vegetable Cultivar List maintained by Ed Tigchelaar. Jim McCreight volunteered that this topic would be taken up by the Cucurbit Crops Advisory Committee at their September meeting.

Mike Havey proposed that combined meetings of the commodity-specific cucurbit groups be scheduled on a biennial or quadrennial basis. There was general agreement that this was a worthwhile endeavor, and efforts would be made to schedule several groups together in 1992.

US Cucurbit Crop Advisory Committee (CCAC) - 1991-92 Update

J.D. McCreight, USDA-ARS, Salinas, CA USA

On **4 November 1990**, CCAC met in Tucson, Arizona in conjunction with the American Society for Horticultural Science (ASHS). Entry of disease resistance data from cooperators into the U.S. Germplasm Resource Information Network (GRIN) was identified as the highest priority. These data must be submitted to and accepted by CCAC and must be accompanied by a description of methodologies and the rating scale. The curators will also evaluate and accept historical data, and data from foreign countries may also be evaluated and entered on a case-by-case basis.

The "Core Concept" and its applicability to the cucurbit collections was also discussed further; its purpose is to decrease the maintenance effort of germplasm collections without decreasing the diversity in the collection or in the evaluation of the collection. Elimination of duplicate accessions, i.e. the same variety or Plant Introduction (PI) under different designations, in the National Seed Storage Laboratory (NSSL) was discussed. Accessions in the Regional Plant Introduction Stations (RPIS) should, however, be backed-up at NSSL. Seed stocks were received from the Seed Savers Exchange, IBPGR and Asgrow Seed Company. Disposition of the IVT (The Netherlands) and Vavilov Institute (Russia) collections were discussed.

Two proposals were recommended for funding in FY1991: "Evaluation of the U.S. Plant Introduction Collection of Cucumber (*Cucumis sativus* L.) for Chilling Resistance" (T.C. Wehner and L. Smeets), and "Evaluation of the U.S. Plant Introduction Collection of *Cucumis melo* (melon) for Resistance to *Alternaria cucumerina* (Alternaria Leaf Blight)" (C.E. Thomas and E.L. Jourdain).

On **8 September 1991**, CCAC met in conjunction with the National Melon Research Group and Squash Breeders meetings in Rocky Ford, Colorado. The meetings were hosted by Hollar Seeds of Rocky Ford. A new computer system for GRIN was expected to be installed by Spring 1993 which will be faster, have more capacity, and include animal germplasm in accordance with the 1991 Farm Act. The "Core Concept" continues to receive attention for efficient germplasm storage and evaluation. The Alfalfa Crop Advisory Committee is the leading advocate/user/tester of the concept.

Confusion remained about the incorporation of commercial varieties and F1 hybrids in the National Plant Germplasm System (NPGS). Kathy Reitsma stated that only those varieties no longer available are included. Previously NPGS stated that all varieties would be included; Plant Variety Protection seems to confuse this issue. The De-Accession Sub-Committee under Jon Watterson's direction is working on a procedure for determining which cultivars in NSSL are duplicates. CCAC proposed to the ASHS Genetics and Germplasm Working Group

that seed samples be submitted to NSSL before final acceptance of HortScience cultivar/germplasm release notes.

Numerical scales for descriptors were not assigned in the CCAC Report. CCAC subcommittees should define classes this year; these need to be defined prior to entry of evaluation data. As GRIN conforms to IBPGR protocol, GRIN must conform to IBPGR scales for resistance traits where "1" is most resistant and "9" most susceptible. This conflicts with that used by many plant breeders who rate "1" most susceptible and "9" most resistant.

Many cucurbits have been increased via open-pollination. The Ames RPIS is trying to produce cage-increased seeds from original seeds, and strives for approximately 500 cages per year of melons. It was recommended that we attempt to obtain the IVT collection and contact the Vavilov Institute to exchange information and germplasm. Efforts to obtain germplasm from the People's Republic of China were encouraged.

Four germplasm evaluation proposals were received by CCAC for 1992 NPGS funding. Two were recommended for funding: (First Priority) "Evaluation of the U.S. Plant Introduction Collection of Melon (*Cucumis melo*) for Resistance to Gummy Stem Blight (*Didymella bryonia* Auersw.) Rehm" (M.A. Kyle and T.A. Zitter), and (Second Priority) "Evaluation of the U.S. Plant Introduction Collection of Squash (*Cucurbita* spp.) for resistance to Gummy Stem Blight (*Didymella bryonia* Auersw.) Rehm" (M.A. Kyle and T.A. Zitter). The two proposals not recommended for funding were: "Evaluation of *Cucumis* sp. Germplasm for Resistance to Zucchini Yellow Mosaic Virus" (J.D. Norton and G.E. Boyhan), and "Evaluation of the *Cucurbita* Germplasm for Susceptibility to the Silverleaf Disorder" (J.K. Brown, D.T. Ray and L. Wessel-Beaver).

The 1992 CCAC meeting will be in Raleigh, North Carolina, in conjunction with the Cucurbit Conference scheduled for 20-24 September. Claude Thomas proposed for discussion at these meetings the idea of having **Cucurbitaceae '94** (at a location other than Charleston). This would be five years after the Charleston meeting (**Cucurbitaceae '89**) and mid-way between the **EUCARPIA** meetings which are held every four years (1988, 1992 and 1996).

1992 Cucurbit Conference

Raleigh, North Carolina
19-23 September 1992

The 1992 Cucurbit Conference will be held in Raleigh, North Carolina, and hosted by North Carolina State University. Todd C. Wehner will be the Conference Chair, and Thomas Monaco will be coordinating the Cucurbit Field Day. The following groups are scheduled to meet as part of the conference:

Group	Dates	Contact Person
Pickling Cucumber Improvement Committee	21-22 September 1992	Jonathan R. Schultheis Dept. Horticultural Science North Carolina St. University Raleigh, NC 27695-7609 USA
National Cucumber Committee	20 September 1992	Michael J. Havey Department of Horticulture University of Wisconsin Madison, WI 53706 USA
Cucurbit Crop Advisory Comm.	23 September 1992	J.D. McCreight USDA-AR, 1636 E. Alisal St. Salinas, CA 93915 USA
National Melon Research Group	23 September 1992	J.D. McCreight USDA-AR, 1636 E. Alisal St. Salinas, CA 93915 USA
Cucurbit Genetics Cooperative	21 September 1992	Timothy J Ng 1122D Holzapfel Hall College Park, MD 20742-5611USA
Squash Breeders Group	23 September 1992	David Groff Asgrow Seed Co., Rt. 1, Box 1907 Tifton, GA 31794 USA
Watermelon Research Group	22 September 1992	Gary W. Elmstrom Univ. Fla., Central FL R&E Ctr 5336 University Avenue Leesburg, FL 34748 USA

One item for discussion at the 1992 Cucurbit Conference will be whether there should be a CUCURBITACEAE '94 conference. This would occur five years after the Charleston meeting (CUCURBITACEAE '89) and would be mid-way between the Eucarpia Cucurbitaceae Symposia, which are held every four years (e.g., 1988, 1992 and 1996).

Evaluation of the U. S. Cucumber Germplasm Collection for Tolerance to Soil Moisture Deficit

E. Van Wann, Research Geneticist

U. S. D. A. Agricultural Research Service, South Central Agricultural Research Laboratory, Lane, Oklahoma 74555

One of the objectives of the U. S. National Plant Germplasm System (NPGS) is to evaluate and develop an automated descriptive inventory of all accessions (plant introductions) in the system. The Germplasm Resources Information Network (GRIN) was established to receive and enter descriptive data into the automated system as it becomes available. The Crop Advisory Committee (CAC) responsible for each crop species develops a list of descriptors and provides oversight for much of the evaluation work. This evaluation of cucumber (*Cucumis sativus* L.) accessions (PIs) for tolerance to soil moisture deficit was supported in part by funds provided by the NPGS as approved by the CAC for cucurbit crops.

The U. S. cucumber germplasm collection consists of approximately 800 accessions. They have diverse origins, representing about 45 countries and most of the continents of the world (1, 3). Although the cucumber collection is small compared to other major crop species, it has been described as marginally adequate for economically important traits, such as disease resistance and tolerance to environmental stresses (2). Genetic diversity is critical to the development of improved cultivars. Also important to plant breeders is the availability of identified sources of genetic traits that may be desired in breeding programs. Stress tolerance, especially drought tolerance, has not been studied extensively in cucumber, and the amount of genetic diversity for that trait is unknown. This project was undertaken to determine the extent of genetic diversity for response to soil moisture deficit and to provide evaluation data to GRIN.

To date, 649 accessions from the cucumber collection that is maintained by the Regional Plant Introduction Station at Ames, Iowa have been evaluated for their response to soil moisture deficit. A segment of the collection was evaluated in 1989, 1990 and 1991; with 300 the first year, 200 the second and 149 the third year. The evaluations were made in field plantings using a randomized complete block design with 4 replications. Each plot consisted of 2 rows 1.5 m long spaced 0.4 m apart on beds 1.5 m wide. Plots were over-seeded and thinned to 20 plants per plot after seedling emergence.

Soil moisture was maintained by irrigation so that soil tensiometer readings did not exceed 20 kPa until plants began to flower. By withholding irrigation at that time, the soil tensiometers were allowed to reach approximately 55 kPa. During the period in which the tensiometers were between 35 and 55 kPa, plots were rated visually on a scale of 1 to 9. The scale was established based on the percentage of leaves in a plot that showed wilting, where 1 = 100 % wilting, 3 = 75 %, 5 = 50 %, 7 = 25 %, and 9 = 0 % of the leaves wilted. The interim values were used when the percentage of wilted leaves was judged to be between the 25 % intervals.

In each of the three segments of the germplasm collection, we found a full range of responses to the moisture deficit. The frequency distribution of individual accessions showed a bell-shaped curve (Fig. 1). The similarities of the three frequency distributions indicated that evaluations could be compared across segments (years).

The most tolerant accessions in each segment of the germplasm collection are listed in Table 1. In 1989, 4 accessions had a mean rating of 7.7 and above (2 % of the population) which was considered to have strong tolerance to soil moisture deficit. In 1990 and 1991, the frequency of tolerant accessions was 4.0 and 4.7 %, respectively. In each of the tests, there were accessions at

the extreme end of the rating scale that showed high susceptibility to wilting when subjected to soil moisture deficit. Those accessions amounted to approximately 20 % of all the accessions tested.

Some of the accessions that were rated highly tolerant had unique plant and leaf characteristics. Two had a short internode (dwarf) plant type and another had upright, glabrous leaves. However, others had normal plant and foliage characteristics typical of most commercial cultivars.

In a separate study, the visual rating technique was compared to several other parameters of cucumber plant response to soil moisture deficit (Wann and Staub, unpublished). Visual rating was shown to be a reliable technique for evaluating cucumber plant response to soil moisture deficit. In that study, the Scheduler Plant Stress Monitor (The Carborundum Company, Solon, OH 44139) was used to determine the crop water stress index (CWSI). There was a significant negative correlation ($r = -0.59$, $P = 0.01$) between the visual ratings and the CWSI. However, neither rating system would be expected to distinguish among plant mechanisms that might account for their tolerance to a soil moisture deficit.

Table 1. Cucumber accessions with a mean stress rating above 7.5 on a scale of 1 to 9, where 1 = severe stress (100% of leaves wilted) and 9 = no visible stress (none of the leaves wilted).

PI accession	Origin	Visual rating[z]
1989 test		
200815	Burma	7.7
263079	Soviet Union	9.0
308915	Soviet Union	9.0
308916	Soviet Union	9.0
344445	Iran	8.1
422181	Czechoslovakia	7.7
1990 test		
164433	India	9.0
211962	Iran	7.6
279468	Japan	7.6
292012	Israel	8.0
344438	Iran	8.1
426629	Pakistan	8.5
525075	Mauritius	8.1
1991 test		
169392	Turkey	7.7
169395	Turkey	8.1
176519	Turkey	7.8
204568	Turkey	7.8
211984	Iran	7.7
211985	Iran	7.9
249550	Iran	8.0

[z]Mean of 4 observations taken from each of 4 replications.

Literature Cited

1. Knerr, L. D., J. E. Staub, D. J. Holder and B. P. May. 1989. Genetic diversity in *Cucumis sativus* L. assessed by variation at 18 allozyme coding loci. Theor. Appl. Genet. 78: 119-128.

2. Staub, J. E., H. Bachzynska, D. van Kleinwee, M. Palmer, E. Lakowska and A. Dijkhuizen. 1989. Evaluation of cucumber germplasm for six pathogens. Proc. Cucurbitaceae 89: 149-153. Charleston, SC.

3. Staub, J. E. and Alina Krasowska. 1990. Screening of the U. S. cucumber germplasm collection for heat tolerance. Cucurbit Genet. Coop. Rpt. 13: 4-7.

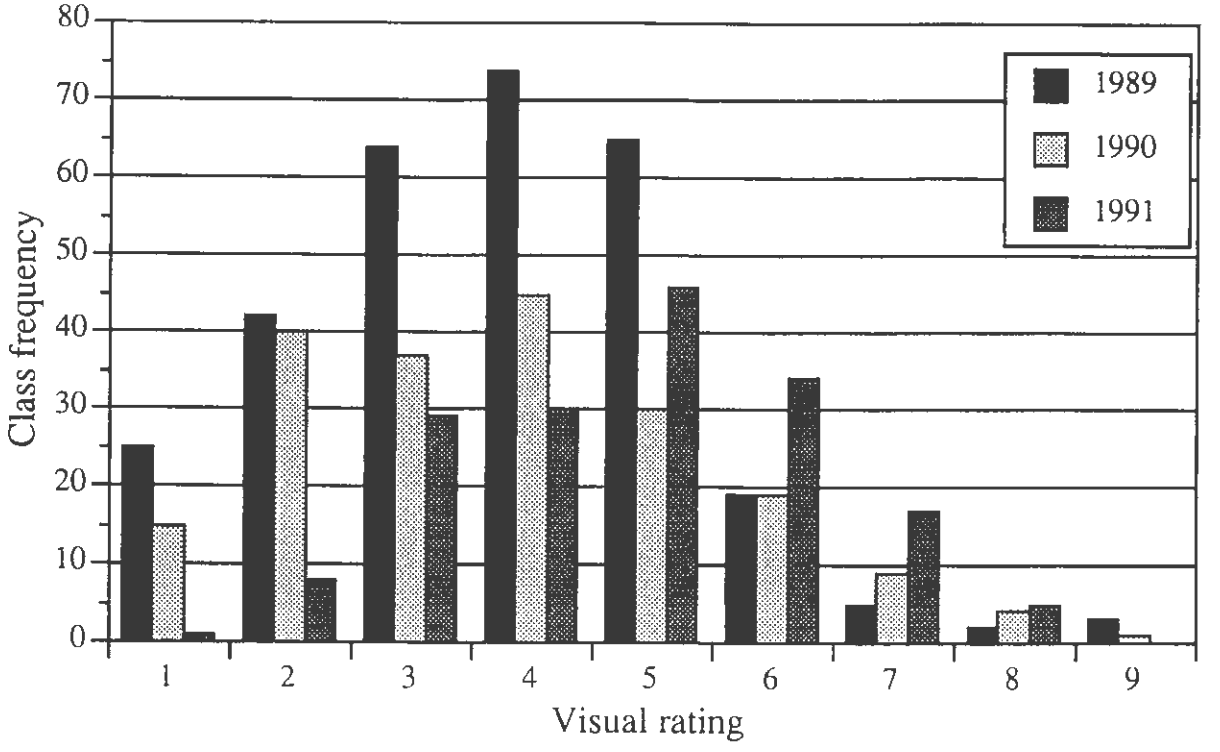

Fig. 1. Frequency distribution of cucumber accessions based on visual rating of plant response to imposed soil moisture deficit. Ratings were made on a scale of 1 to 9 where 1 = severe stress (100% of leaves wilted) and 9 = no visible stress response. Tests were run in 3 years: 1989 (300 accessions), 1990 (200 accessions) and 1991 (149 accessions).

Phylogenetic Relationships Among Several African *Cucumis* Species: A Working Evolutionary Hypothesis

J. E. Staub and L. D. Knerr
Vegetable Crops Research, USDA/ARS, Department of Horticulture, University of Wisconsin, Madison, WI 53706

Phylogenetic models of the genus *Cucumis* have been constructed based on variation in morphology and geography, cucurbitacins, flavonoids, cytological studies and chromosome banding patterns, cross-compatibility, isozymes, and nuclear DNA. The genus *Cucumis* has been partitioned into two subgenera (3): *Cucumis* (x=7) and *Melo* (X=12). The majority of the *Cucumis* species have been placed in the subgenus *Melo* which are indigenous mainly to Africa. Although it has been implied that the African *Cucumis* species arose from a common ancestor, phylogenetic relationships among three groups (annual, perennial diploid monoecious, and polyploid dioecious and monoecious) of African *Cucumis* species are not conclusive.

Of the four groups which comprise the subgenus *Melo* (3), Group 2 has recently been further subdivided into 2 subgroups using meiotic and crossability analysis, electrofusing of peroxidases, cucurbitacins, flavonoids, and geographic distribution (6). The *Myriocarpus* subgroup consists of *C. africanus* Lin. f., *C. heptadactylus* Naud.(4x), *C. myriocarpus* Naud. ssp. *leptodermis* (Schweick.) Jeffrey and Halliday, and ssp. *myriocarpus*. The *Anguria* subgroup consists of *C. aculeatus* Cogn. (4x), *C. anguria* var. *anguria* L.,*C. anguria* var. *longipes* Meeuse (1), *C. dipsaceus* Ehrenb. ex Spach, *C. ficifolius* A. Rich. (2x and 4x), *C. figarei* Deille (6x), *C. prophetarum* L., and *C. zeyheri* Sond. (2x and 4x).

We have used isozyme analysis to describe biochemical relationships among the wild cross-compatible African diploid species of *Cucumis* (8). We proposed that phylogenetic affinities exist between *C. anguria* var. *anguria, C. africanus*, and *C. dipsaceus*, and that the relationships among *C. anguria* var. *longipes*, *C. metuliferus*, and *C. myriocarpus* are closer than had previously been proposed. In contrast, *C. myriocarpus* is more differentiated from *C. anguria* var. *anguria* than either *C. africanus* or *C. dipsaceus* (1, 8). Singh and Yadava (7) have argued that *C. myriocarpus* is genetically separated from *C. dipsaceus*.

Recently, we have analyzed electrophoretic variation of seven African species within *Cucumis*, subgenus *Melo*, subgroup *Myriocarpus* [4x *C. aculeatus*, *C. anguria* var. *anguria*, *C. dipsaceus*, *C. ficifolius*, *C. myriocarpus* ssp. *leptodermis* Schweick., *C. prophetarum*, and 2x and 4x *C. zeyheri* Sond.], and 2x *C. sativus* L. of subgenus *Cucumis* (9). Analysis of allelic frequencies among 14 polymorphic loci indicated that *C. ficifolius*, *C. anguria* var. *anguria*, and 2x and 4x *C. zeyheri* have biochemical affinities and could be distinguished from *C. aculeatus* and *C. dipsaceus* which were similar. Within the first group, *C. myriocarpus* ssp. *leptodermis* and one *C. prophetarum* accession formed a well-defined group. *C. sativus* was dissimilar from all other species studied.

The wild African species of *Cucumis* are undoubtedly linked by common evolutionary episodes. Accurate phylogenetic construction requires synthesis of relevant data and the prudent refinement of existing hypotheses. A systematic survey of all available data was conducted to develop a testable evolutionary hypothesis which might then lead to a more definitive phylogenetic construction of the wild African species (Fig. 1). From this synthesis it is hypothesized that four annual (*C. africanus*, *C. anguria*, *C. dipsaceus*, *C. myriocarpus*) and four perennial (*C. aculeatus*, *C. ficifolius*, *C. figarei*, *C. prophetarum*) species are derived from a common ancestor (Fig. 2). *C. zeyheri* has common affinities to two distinct species groups which are reproductively isolated. *C. zeyheri* could be either a potential, albeit distant, progenitor species of those two groups or a derivative of a tetraploid hybrid between the two groups. Ancestral forms of

4x *C. aculeatus* or *C. dipsaceus* may also have provided a secondary bridge for gene transfer during the evolution of those two groups.

There are several corollaries to that hypothesis which could be proffered (Fig. 1, 2). For instance, since F_1 hybrids between *C. anguria* and *C. prophetarum*, and between *C. zeyheri* and *C. prophetarum* are moderately to sparingly cross-fertile (4), it seems reasonable to hypothesize that the perennial *C. prophetarum* may have evolved from the annual *C. anguria* or perennial *C. zeyheri*. Likewise, our results and those of Dane (2) and Nijs and Visser (4) suggest that *C. ficifolius* is more reproductively isolated than *C. zeyheri* from all annual species except *C. dipsaceus*. *C. ficifolius* may therefore have initiated speciation earlier and/or contributed to the evolution of *C. dipsaceus*.

Those and other corollaries of the above working hypothesis can be tested using molecular techniques. In the study of Perl-Treves and Galun (5) the degree of homoplasy, the limited number of accessions used, and the number of "less characterized" and back or parallel "point mutations" did not allow them to clearly establish relationships among African species which formed a compact group. A larger number of accessions and endonucleases could be utilized to construct a rooted parsimonious tree. The working hypothesis presented above could then be tested using specific outgroups to make synaptomorphic comparisons.

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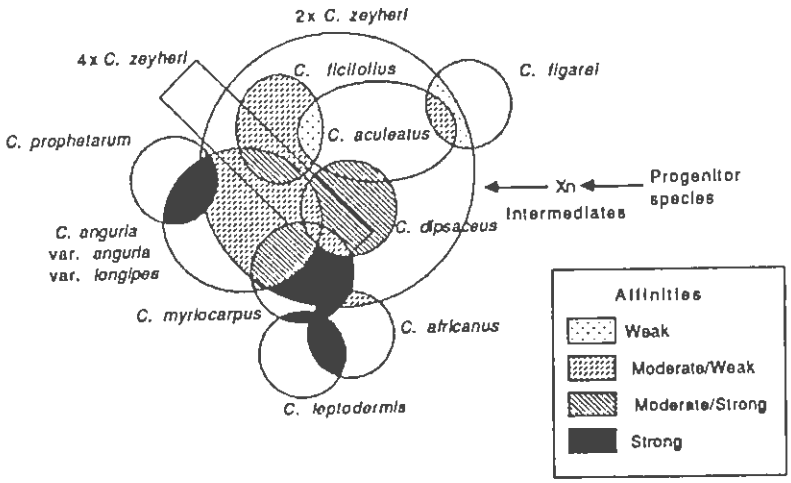

Figure 1. A composite diagram of cytogenetic, biochemical and cross-compatibility relationships among eight African *Cucumis* species. Weak affinities (a synthesis of all criteria) of *C. zeyheri* 4x with other species is represented by a rectangle.

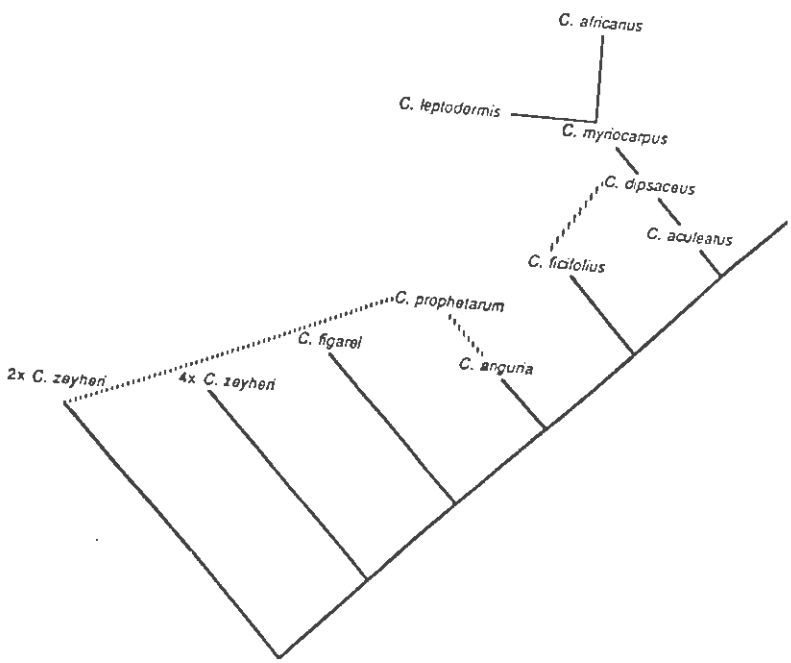

Figure 2. A working hypothesis of the evolutionary relationships among ten African *Cucumis* species. Dashed lines indicate possible gene flow between species.

Cucumber Germplasm Resources in Southwest China

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The majority of Chinese cucumber cultivars of the outdoor type have been grown for fresh consumption in China for well over 2000 years. Only in recent history were the greenhouse and pickling types introduced into China. In a brief summary of intraspecific variation in economically important Chinese cucurbits (6), Chinese cucumber cultivars were divided into three types (horticultural groups): North China type (NC), South China type (SC), and Southwest China type (SW). The NC and SC types have long been recognized by Chinese horticulturists in China (1, 6) and Japan (3, 4). It was not until 1989 that the SW type was recognized.

There are diverse cucumber cultivars native to southwest China. Recent investigations in Sichuan (2), Yunnan (Zhang, Y. H., pers. comm.), and Guizhou (Z. Y. Guo, personal communication) suggested that all southwest local cultivars belong to the SC type. However, my field surveys and culture experiments indicate that some of the local cultivars in the southwest are distinct from the SC type with respect to fruit traits and ecological requirements. Plants of these local cultivars are characterized by their large short fruits, vigor, and long growing period (ca. 200 days). They are grown in mountain regions: Yaan and Shanzia Regions in Sichuan, Anlong and Tongxing in Guizhou, and Dehong, Zhaotong, and Zishuanbanna in Yunnan. The SW type is composed of those cultivars.

Table 1 compares the morphology and ecological requirements of the SW, NC and SC types, and *Cucumis sativus* var. *hardwickii*. The SW type is closely allied with the SC type and the *C. sativus* var. *hardwickii*, and distinct from the NC type (Table 1). To date, phylogenies of Chinese cucumber cultivars are unknown. I propose that the SC and SW cultivars either were domesticated from the native wild type (*C. sativus* var. *hardwickii*) in southwest China, or were brought into China from India at prehistoric time via the ancient southwest Silk Road (Yang unpublished). The NC cultivars were introduced, via the Silk Road from middle Asia during the Han Dynasty (2nd century BC; see reference 6 and reference therein).

Compared to north China, little attention has been given to the southwest with respect to breeding and germplasm research of cucumber cultivars (1). From the mid 1980s, NC cultivars and F1 hybrids began replacing the indigenous cultivars in the southwestern provinces. Subsequently, valuable local cultigens in the southwest are disappearing. Southwest China, which is bounded by the Himalayas, has been suggested as the original center of domestication of the cucumber (see reference 5 and references therein). Although it still remains unclear of the exact report, it has been suggested that southwest Yunnan should be included as a center for domestication of cucumber (5). Recently, *C. sativus* var. *hardwickii* was observed in southwest China (Chen, J., pers. comm.). Southwestern China should be of interest for future research by cucumber breeders and taxonomists.

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Table 1. Ecological requirements and morphology of Chinese cucumber types.

Trait	NC	SC	SW	hardwickii
Photoperiod	day neutral	short days induce pistillate flowering	short days induce pistillate flowering	short days induce pistillate flowering
Ecological requirements	Temperate, dry tolerance	Temperate, humid	warm, humid	
Days to 1st harvest	50-60	50-70	120	-
Growth season	all	spring to summer	spring to fall	spring to fall
Plant growth	less vigorous	vigorous	vigorous	-
Trellis support	needed	unneeded	unneeded	unneeded
Stem	small	large	large	-
Leaves	small	large	very large	-
Branch number	2-5	2-10	>10	>10
Node of 1st pistillate flower	5-7	5-10	30	>30
Flower location	main vine	main vine	main and branch	main and branch
Fruit length	40-60 cm	15-30 cm	10-40 cm	-
Fruit diameter	4-7 cm	6-10 cm	10-20 cm	-
L:D ratio	8-15	3-8	1-4	1-4
Fruit wt.	150-400 g	150-500 g	1500-3000g	30 g?
Fruit ridges?	yes	not obvious	no	-
Fruit skin	thin	thick	thick	thick
Fruit warts	many	some	rare	no
Fruit spines	many, white	some, black	rare, black	no
Immature fruit color	dark green	light green	light green/white	light green
Mature fruit color	yellow	yellow, brown	brown or white	-
Fruit color uniformity	Uniform	mottled	mottled	mottled
Mature fruit netting	Rare	Numerous	Numerous	-
Seed length	0.8-1.0 cm	0.9-1.0 cm	1.2-1.4 cm	-
Seed width	0.3-0.4 cm	0.3-0.4 cm	0.3-0.4 cm	-
100-seed wt.	2-2.5g	2-2.4 g	4.0 g	-
Typical cultivars	BeiJing Cigua, Changchun Mici, Lingyan Daci	Wuhan Qingyudan, Di Huanggua, Hanzhou Qinpi	Zhaotong Da Huang Gua, Yaan Man Huanggua, Anlong Da Huanggua, Xishuangbanna Gourd	-

Survey of Cucumber Cultivars Grown in North Carolina

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A survey of North Carolina cucumber growers was administered via mail in January and February, 1991 for the 1990 crop of pickling and fresh-market types. The survey encompassed 15 counties, comprising over 90% of the North Carolina production area. Of the 935 cucumber growers that received questionnaires, 404 or 43% responded. Those who responded to the questionnaire reported that they had planted 3876 ha (9574 A) of cucumbers in the spring and 1657 ha (4092 A) in the fall. Total reported cucumber production area accounted for 5533 ha (13,666 A), 4338 ha (10,716 A) for pickling and 1199 ha (2962 A) for fresh-market (Table 1). A total of 12,672 ha (31,300 A) were estimated to be planted statewide in 1990.

The primary reason for the survey was to determine pesticide usage patterns of cucumbers. However, additional information was obtained about cultural practices such as scouting, types of sprayers, plant and tissue sampling, cultivation practices, and cultivar. This report focuses on the cultivars grown in North Carolina.

The predominant pickling cultivar grown in North Carolina was Calypso, while Dasher II was the dominant fresh-market cultivar (Table 1). Primepak and Regal pickling cucumber cultivars were grown on nearly one-third of the state's area. Small but substantial fresh-market cucumber production area of Poinsett 76, Centurion, Marketmore, Supersett and Revenue and comprised approximately 25% of the North Carolina production area. New cultivars such as H-19 Little leaf and Striker were still being evaluated by growers, so usage was low.

Yields varied considerably depending on the cultural practices employed. The yield for pickling cucumbers ranged from 12 to 528 q·ha^{-1} (23 to 981 bu·A^{-1}) with an average of 119 and 108 q·ha^{-1} (221 and 200 bu·A^{-1}) in the spring and fall, respectively. For fresh-market cucumber, yield ranged from 8 to 175 q·ha^{-1} (14 to 325 bu·A^{-1}) with an average of 105 and 135 q·ha^{-1} (196 and 251 bu·A^{-1}) in the spring and fall seasons, respectively. The low yields, in many cases, were attributed to lack of irrigation. About one-third of the cucumber production area is irrigated in North Carolina. In addition, cucumber is often grown as a secondary crop in order to utilize and keep labor prior to the harvest season of the primary crop. The crops most often planted with cucumber were (in order of importance): 1) tobacco, 2) corn, 3) soybeans, 4) wheat, 5) beans, 6) pepper and 7) sweetpotato.

Table 1. Cucumber cultivars planted in 1990 in North Carolina.

Pickling type				Fresh-market type			
Rank	Cultivar	Area(ha)	%	Rank	Cultivar	Area(ha)	%
1	Calypso	2384.1	54.1	1	Dasher II	663.8	60.6
2	Primepak	760.7	17.3	2	Poinsett 76	82.1	7.5
3	Regal	655.5	14.7	3	Centurion	63.6	5.8
4	Cross Country	189.9	4.3	4	Marketmore	57.7	5.3
5	Carolina	124.0	2.8	5	Supersett	48.2	4.4
6	Discover	72.5	1.6	6	Revenue	42.5	3.9
7	Royal	42.1	1.0	7	Striker	30.2	2.7
8	Napoleon	41.7	1.0	8	Guardian	25.3	2.3
9	Vlaspik	36.4	0.8	9	Comet A .	24.3	2.2
10	Flurry	25.3	0.6	10	Sprint 440S	24.1	2.2
11	Little leaf H-19	23.1	0.5	11	Slicemaster	20.2	1.8
12	Little leaf H-16	20.2	0.5	12	Maximore 100	6.1	0.6
13	Little leaf unknown	21.1	0.5	13	Raider	4.5	0.4
14	Sumter	11.3	0.3	14	Everslice	3.2	0.3
15	Raleigh	0.2	0.0	15	Medalist	0.1	0.0
16	Fancipak	0.2	0.0				
Total		4408.3	100.0			1095.9	100.0

Some Relationships of Seed Production with Parthenocarpy and Relative Humidity in the Cucumber

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An important advantage of the cucumber plant that makes it suitable for breeding and genetic studies is its ability to produce many seeds in each pollinated fruit. A mature, hand-pollinated fruit containing more than 150 seeds is not rare. However, with parthenocarpic plants the situation is different. We have observed that the strong parthenocarpic Dutch type slicers produce generally fewer seeds per pollinated fruit than the non-parthenocarpic. Since commercial seeds of parthenocarpic cultivars are produced by hand-pollination, seed cost is dependent on the number of seeds produced per pollination. The present investigation was initiated to investigate the factors affecting seed yield of parthenocarpic cucumbers in hopes of reducing seed cost.

Methods. Self- and cross-pollinations in various combinations were made among 6 inbred lines from our cucumber breeding program. Five of them were the strongly parthenocarpic, gynoecious lines 986, 987, 988, 989 and 990. The sixth was the non-parthenocarpic, monoecious line 991 (Table 1). We counted seeds per fruit after seed extraction.

The number of seeds per fruit under two different levels of relative humidity (RH) was also investigated with several cultivars (Table 2). Two greenhouse chambers were used, one with normal (uncontrolled) RH and the other with controlled RH. Relative humidity in the uncontrolled chamber ranged from 60 to 100%. Our objective was to increase the minimum RH level to 75% in the controlled room during pollination.

Results. A wide range in the number of seeds per fruit was observed among and within crosses of the first experiment. The number of seeds varied, depending on the female parent (Table 1). Also cross-pollinations of the lines (when used as female parents) produced generally more seeds than self-pollinations. The number of seeds was dramatically decreased in the parthenocarpic fruits (mean of 51) compared to that of the non-parthenocarpic (mean of 243).

In the second experiment, the number of seeds of the parthenocarpic fruits was again much smaller compared with the non-parthenocarpic fruits in both levels of RH (Table 2). In most cases, the number of seeds per fruit was higher at high RH. However, 4 entries produced fewer seeds per fruit at high RH. Similarly, cross-pollinations of the lines did not always produce more seeds per fruit than self-pollinations.

Strongly parthenocarpic cucumber plants produced much fewer seeds than the non-parthenocarpic. However, wide variation was observed within lines. Cross-pollination of the parthenocarpic flowers did not always provide more seeds than self-pollination. The increase of RH during pollination could affect positively the number of seeds per fruit. However, other factors that influence seed production should be investigated.

Table 1. Seed number per fruit for cross- and self-pollinations of 6 cucumber lines (986 through 991).

Statistic	986		987		988		989		990		991	
	Cross	Self	Cross	Self	Cross	Self	Cross	Self	Cross	Self	Cross	Self
Mean	46	21	47	47	25	21	95	23	80	35	259	226
Low	0	0	0	14	1	0	18	0	0	0	133	81
High	122	44	117	93	56	51	161	45	161	98	380	306

Table 2. Seed number of lines cross- and self-pollinated under normal (uncontrolled) and high (controlled) relative humidity.

Lines	Type	Controlled RH		Uncontrolled RH	
		Mean	Range	Mean	Range
1092 parth	Selfed	20	0-74	55	13-119
"	Crossed	30	0-87	31	0-70
1098 "	Selfed	17	0-92	5	0-24
"	Crossed	17	0-40	40	3-125
1099 "	Selfed	55	0-201	48	0-192
"	Crossed	61	0-195	46	0-197
1100 non-parth.	Selfed	150	150	147	57-150
"	Crossed	139	25-150	127	2-150
1094 parth	Selfed	14	0-50	0	-
1096 "	"	68	0-150	45	0-150
1113 "	"	31	0-64	14	0-72
1114 "	"	67	0-150	37	0-150
1115 "	"	75	0-150	29	0-100
1116 "	"	83	1-150	117	40-150
1117 "	"	80	1-150	65	1-150
1118 "	"	38	1-150	133	15-150
1119 "	"	129	1-150	105	6-150

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Correlation Between Parents and F_1 Progeny in Earliness Heterosis and the Estimation of Traits Limits of Parents

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Cucumber (*Cucumis sativus* L.) is one of the important summer vegetables in China. There is an urgent requirement for early cultivars of cucumber for use in plastic tunnel culture in the spring.

Early maturity is an important trait and is composed of many components. It has been suggested that early maturity of cucumber is related to position of first pistillate flower, growth rate of the fruits, percentage of pistillate flowers, and number of pistillate flowers per plant. The objective of this research was to study the relationship of those traits and early yield of F_1 hybrids, and to determine an optimum range for each trait to help plant breeders in the development of early maturing cucumbers.

Methods. Field work was conducted at the Vegetable Station, Northwestern Agricultural University, China in 1988 and 1989. Four inbred lines were chosen as female parents: 'Yue 82', 'Jing 4-3-1', 'Xinong 58-5', '7742'. Four inbreds were chosen as male parents: 'Changchun-1', 'Zhangqui M' and 'Black 235'. An incomplete diallel cross was used to generate 16 F_1. 'Changchun Mici' was used as the check. Soil for the experiment was covered by plastic film in the spring. A randomized complete block design was used in the experiment with 3 replications. There were 30 plants in each plot, with 0.67 m between rows and 0.23 m between plants in rows.

Ten plants in each plot were sampled for observation and measurement. The 14 traits observed were: yield per plant in early stage, harvested fruit numbers per plant in early stage, fruit set percentage in early stage, frequency of pistillate flowers/node on main stem in early stage, number of pistillate flowers on main stem in early stage, daily weight increment in grams of commercial fruits, total number of branches, number of fruiting branches, number of non-fruiting branch stem in early stage, leaf area of per plant in early stage, yield of a plant in early stage, number of leaves of a plant at flowering time, days from sowing to first staminate flowering three heterosis were described as follows:

Midparent heterosis = MP = (F_1-Midparent)/Midparent;
High parent heterosis = HP = (F_1-High parent)/High parent;
Performance relative to check = CP = (F_1-Check)/Check.

Correlations and regressions were performed with the ANALYST program.

Results. Correlation between heterosis of F_1 progenies and midparent, and the diversity of two parents are the most direct features of parent combination. Correlations between such features and heterosis of F_1 progeny were variable both in the size and direction (Table 1). MP and HP were comparable, but the correlation of parents and F_1 on CP showed large differences in size and direction.

Early maturity contributes to early yield. MP for early yield of F_1 progeny was significantly negatively correlated with midparent ($r = -0.831^{**}$) and positively correlated with diversity values of the two parents ($r = 0.347$). In that case, MP depended on the maturity of the parents (the later they mature, the easier it is to have large MP), and the diversity of the two parents for earliness.

HP for early yield was negatively correlated with diversity values of two parents ($r = -0.473^{*}$), but not correlated with the mean values of the two parents ($r = -0.174$). In order to obtain F_1 progenies with HP, parents with small diversity should be used.

We consider CP more important for cucumber breeding than MP. Correlation between CP for early yield of F_1 progeny and the mean values of the two parents was significantly positive ($r = 0.693^{**}$), so parents with early maturity should be chosen. Also, the correlation between CP for early yield of F_1 progeny and diversity of the two parents was not significant ($r = -0.286$), so parent diversity should be small.

Early yield was affected by many secondary traits or components. Analysis of the relations of those components and the F_1 seemed very important to the breeding of earliness in cucumber. Results indicated that CP for number of fruits harvested per plant in early stage was similar to that of early yield. Number of pistillate flowers on the main stem, and pistillate flower density or frequency of pistillate flowers/node were also the same except that MP and CP seemed correlated with diversity of the two parents in those two traits. The more total branches the parents had, the more difficult it was for the F_1 to exceed the midparent value for branch number. Correlation between MP or HP for number of branches in the F_1 and the mean value of the two parents were significantly negative ($r = -0.628^{**}$ and $r = -0.676^{**}$, respectively). Therefore, more diverse parents had F_1 progeny with less MP. CP was different in that the forming of fruiting branches at early stage was almost uncorrelated with the features of the two parents. On the other hand, CP for total number of branches and number of non-fruiting branches at early stage in F_1 showed significantly positive correlation with mean values of parents ($r = 0.670^{**}$ and $r = 0.529^{**}$, respectively).

In order to obtain F_1 progenies with few non-fruiting branches, parents with fewer branches should be chosen. The more diverse the parents were in flowering time and pistillate flowering time, the more likely it was that F_1 progenies would be early flowering with a low node of pistillate flower than the midparents. CP for time of pistillate and staminate flowering, the node of first pistillate flower and the leaf number at flowering time showed significantly positive correlation with midparents ($r = 0.828^{**}$, $r = 0.856^{**}$, $r = 0.724^{**}$, respectively). Therefore, in order to get negative CP for those four traits and improve earliness of F_1 progenies, the parents with the features of early flowering, lower node of first pistillate flower and fewer leaves at flowering time, and the parents with less diversity in these traits should be used.

The previous discussion relates to the limits for early yield. If mean value of the two parents exceeded the limit value, it would be impossible for F_1 progenies to produce positive (or negative, if desired) CP. Regression analysis was used to estimate that limit value (Table 2). F_1 progenies would not produce positive MP in each trait if early yield, fruit set percentage on main stem, percentage of pistillate nodes, number of pistillate flowers, daily increment of fruit, and number of fruiting branches at early stage were higher on average than 0.395 kg/plant, 73.38%, 30.021%, 2.954, 16.790 g/day, and 0.135 branch/stem, respectively.

Similarly, F_1 would not produce negative MP if the total number of branches per plant, number of leaves per plant at flowering time, days from seed sowing to first staminate flower, days from seed sowing to first pistillate flower and node of first pistillate flower for two parents were on average more or higher than 2.9, 7.0, 38.8, 43.8 and 2.5 respectively. Although our conclusions were influenced by materials, samples, experimental regions and seasons, those trends have some general implications for cucumber breeders.

Discussion. Plastic tunnels for cucumber production in early spring is important in China. Thus, breeding of early maturing F_1 hybrids is a high priority. Available information on earliness dealt mainly with first pistillate flower position (1, 2). Cui (3) and Lu (4) studied earliness in cucumber, and suggested that earliness was related to the first pistillate flower position, pistillate flower ratio (pistillate flower density), fruit growth rate, and other traits. Sterlenikova (5) suggested that the early maturity of cucumber was related to leaf area index at early stage. Other components of earliness were rarely reported.

In summary, F_1 progeny were earlier when there was positive or negative correlation with parent traits, especially when MP and HP depended on the parent mean. Estimation of parent feature limits was important in choosing parents for maximizing the use of heterosis. The suggestion that there should be fewer leaves on parents and F_1 hybrids during flowering, was different from the results of Sterlenikova. That may be due to use of different genotypes and environments. Additional research is needed in that area.

Table 1. Correlation between F_1 heterosis and parent trait in cucumber.

	Midparent heterosis		High-parent heterosis		Performance relative to check	
Trait[z]	Parent mean	Parent diversity	Parent mean	Parent diversity	Parent mean	Parent diversity
EYP	-0.831**	0.347	0.174	-0.473*	0.693**	-0.286
EFP	-0.461	0.028	-0.308	-0.628**	0.776**	-0.183
EFS	-0.967**	0.810**	-0.524*	-0.003	-0.326	0.150
FD	-0.529*	0.056	0.091	-0.610**	0.751**	-0.027
FFS	-0.543*	0.045	-0.0102	-0.424	0.737	0.053
DWI	-0.886**	0.821**	-0.395	0.184	0.193	-0.134
TBS	-0.38*	-0.685**	-0.380	-0.713**	0.670**	-0.047
EBS	-0.595**	-0.604**	-0.582*	-0.615**	-0.083	-0.001
NFBS	-0.470	-0.628**	-0.478*	-0.676**	0.529**	-0.039
LAP	-0.285	-0.376	-0.168	-0.172	0.613**	-0.080
LSP	-0.789**	-0.801**	-0.824**	-0.886**	0.699**	0.474*
DMF	-0.477*	-0.429	-0.551*	-0.749**	0.856**	0.368
DFF	-0.547**	-0.524*	-0.653**	-0.815**	0.828**	0.369
NFF	-0.515**	-0.677**	-0.700**	-0.781**	0.724**	0.424

[z]EYP-early yield per plant, EFP-early fruits harvested per plant, EFS-early fruit set percentage of main vine, FD-pistillate flower density of main vine, FFS-pistillate flowers of main vine, DWI-daily weight increment in gram per commercial fruit, TBS-total branches per plant, FBS-fruiting branches per plant, NFBS-non-fruiting branches per plant, LAP-leaf area per plant in early stage, LSP-leaves per plant in flowering time, DMF-days from sowing to staminate flowering, DFF-days from sowing to pistillate flowering, NFF-node of first pistillate flower.

Table 2. Approximate estimation of limits from F1 MP and parental mean values in a regression model.

Trait	Regression constant	Regression coefficient	Limit
Early yield per plant	183.25	-463.51**	0.395
Early fruit-set (% on main stem)	294.65	-4.015**	73.385
Pistillate flower density of main stem	81.33	-2.790*	30.021
No. pistillate flowers/main stem	68.12	-26.264*	2.594
Marketable fruit wt. gain (g/day)	257.30	-15.325**	16.790
Total branches/plant	46.29	-15.878*	2.915
Fruiting branches/plant	4714.29	-30774.67**	0.153
leaves/plant at flowering	22.67	-3.248**	6.989
Days from sowing to staminate flowering	19.07	-0.492*	38.779
Days from sowing to pistillate flowering	27.08	-0.618**	43.805
Node of first pistillate flower	9.88	-3.994**	2.474

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The Significance of Some Traits and Their Combinations in the Usage of U. S. Cucumber Varieties

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The excellent history of U. S. cucumber improvement by Wehner and Robinson (1) prompts me to make some corrections and add some thoughts about the importance of the right combination of traits as contrasted with improvement in a single trait. The slight corrections involve the Cornell releases 'Tablegreen' and 'Marketmore' which contrary to the history (1), did not become important as open-pollinated varieties and perhaps not as parents of F_1 hybrids either. Soon after 'Tablegreen' was released, it became clear that its susceptibility to scab (*Cladosporium cucumerinum* Ell. & Arth.) negated much of the value of its high cucumber mosaic resistance (CMR). Because of this, the release of 'Marketmore' was delayed until the addition of scab resistance was completed, and this resistance should be added to its traits in Table 1 of the history (1). The scab resistant version of 'Tablegreen' was named 'Tablegreen 65' and became much more important than the original 'Tablegreen'.

Along with high CMR, another novel trait of 'Tablegreen' was its uniform fruit color. It apparently was the first U. S. variety to carry the *u* gene, which came from 'Chinese Long', the source of CMR. lack of the *u* gene limited the use of 'Marketmore' because the fruit color became too light at high temperatures. The *u* gene was transferred from 'Tablegreen' by backcrossing and the resulting 'Marketmore 70' achieved far more usage than 'Marketmore'. It still, however, lacked the powdery mildew resistance of 'Tablegreen'. This was corrected by adding a higher level of PMR from 'Spartan Salad" and releasing 'Marketmore 76'.

Dating back to the first commercial trials of the scab susceptible 56-388, the recurrent parent of 'Marketmore', growers commented on its high yield and high percentage of marketable fruit. This trait was reflected in the name, and because of the difficulty of identifying it in segregating material, the backcross method has been particularly appropriate as a means of retaining it while improving other features. This is probably the principal trait that has made 'Marketmore 76' far more useful than 'Tablegreen 65' which is so similar in other respects. "High marketable yield" might well be added to the list of 'Marketmore' traits in Table 1 of Wehner and Robinson (1). Of the 5 principal traits of 'Marketmore 76' (CMR, SR, PMR, uniform color, and high marketable yield), not one could be omitted without seriously impairing the usefulness of the variety.

The listing of "fruit type" as the trait of interest for 'Marketer' (1) does not make it clear why that was the dominant slicing cucumber variety for many years. Consequently an anecdote about the development of 'Marketer' may be worth recording. Dr. Floyd Winter, Asgrow's director of research at that time told of a meeting in which company salesmen requested better slicing cucumbers. Invited by Winter to give their specifications, they agreed on a cylindrical fruit with blunt ends. One of Asgrow's breeding stations proceeded to breed such a cucumber. A trial sample resulting from this effort planted at Cornell in 1943 was impressively cylindrical and promptly used in crosses in the Cornell CMR program. In the same trial, a second unnamed slicer bred independently at another Asgrow station had tapered ends and got little attention. The variety bred to specifications was subsequently named 'Cubit' and soon disappeared. The one with tapered ends was named 'Marketer' and became the leading slicer. The reasons for this unexpected outcome are not entirely clear, but we observed in later trials that 'Cubit' produced many more cull fruits if conditions were less than ideal: 'Cubit' fruits were said to have a tendency to "jumbo" (become oversized) whereas 'Marketer' remained of marketable size for a longer time. In short, shape itself had less influence on success of the variety than did marketable yield.

'Poinsett 76' may deserve listing in a history of U. S varieties because it so rapidly and completely replaced the extremely important 'Poinsett'. Bred primarily for scab resistance, the *u* gene for color was incorporated at the same time, and a slight increase in fruit length demonstrated that the earlier linkage between scab resistance and short fruit had been broken. Since scab is not important in most areas where 'Poinsett' is adapted and the difference in length is not great, it is likely that improved color was the main reason for the success of 'Poinsett 76'. Only a few years after its introduction, slicers lacking the *u* gene disappeared from U. S. supermarkets.

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Screening Cucumber for Resistance to Belly Rot Caused by *Rhizoctonia solani*

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Belly rot caused by *Rhizoctonia solani* Kuhn (AG-4) is one of the more important cucumber (*Cucumis sativus* L.) diseases in the southeastern United States. Crop loss in the southern United States averaged 7 to 9 %, with potential yield loss of up to 80 % (3). In North Carolina, the average annual loss was approximately 3.5 % (4).

Efforts to control belly rot, which have been primarily by chemical means in the past, were generally unsuccessful or inefficient (2, 3). Resistant cultivars would provide adequate and economical protection. Therefore, in these experiments we were studying resistance available in the gene pool. In a series of experiments over several years, the majority of the cucumber germplasm collection has been screened using field and detached fruit tests (5, 7). That has provided a good base of information for future breeding work.

The narrow-sense heritability for resistance to belly rot was estimated at 0.24, which is low to moderate heritability (6). Thus, resistance could be transferred to horticulturally acceptable material, provided that resistant germplasm can be identified.

Damping off, which can also be caused by *R. solani*, has also been studied as a method of screening for resistance to belly rot. Booy et al. (1) found that resistance to damping off was not correlated with either field or detached fruit tests. This indicates that this method could not be used to screen for belly rot resistance.

The objective of this study was to screen the cucumber germplasm collection to detect large differences in resistance, and to identify the best sources of resistance to belly rot to use in further studies.

Methods. Two methods were used to screen for belly rot resistance, field and detached-fruit tests. Field tests were conducted at the Horticultural Crops Research Station, Clinton, NC. Detached-fruit tests were conducted in a greenhouse in Raleigh, NC. Tests were run in 1981 through 1985. Work done in 1984 dealt with inoculum concentration and further improvement of the test method, and will not be presented here. Experiment design, inoculum concentration, and dates of planting and harvest are given in Table 1.

The field tests involved replicated plots of the cultigens of interest, with *R. solani* infested oat grains added to the soil surface at the vine tip-over stage (6 to 9 nodes). When fruits were of the appropriate size (50 mm diameter) they were rated for percentage infected with *R. solani*.

Inoculum was prepared by first autoclaving 300 cm^3 of oats and 250 ml of water for 1 hour for two consecutive days in autoclavable bags. *R. solani* colonized potato dextrose agar disks approximately 1 cm^2 were transferred to the sterile oats and allowed to incubate for 7 days. Once colonized, the oats were dried and stored at 4°C until needed (7).

Detached-fruit tests consisted of growing plants in the field, and harvesting fruits for testing in a closed chamber. Flats were filled with steam-sterilized field soil and infested with *R. solani* by adding colonized oat grains. Fruits were then dipped in 10 % Clorox (0.52 % NaOCl), placed on the flats kept in a growth chamber maintained at greenhouse temperature and high humidity. We did not follow the Clorox dip with a water rinse (to avoid spreading disease). Temperatures averaged 35°C day and 27°C night. Humidity was not controlled except through the use of a closed chamber.

The soil was moistened at the start of the experiments and allowed to dry out for 3 days before re-wetting. Soil moisture was not critical for good disease development as long as the soil did not become completely dry (unpublished data). Fruits were rated as close to 7 days after inoculation as possible, although it was sometimes necessary to use shorter (4 days) or longer (12 days) periods to allow for proper development of disease symptoms.

Fruit were rated by estimating the percentage of the fruit surface that was colonized by the fungus. A rating of 30 % indicated that the entire belly of the fruit was colonized. Ratings greater than 30 % occurred when the fungus spread throughout the fruit. Primary symptoms consisted of brown, sunken, necrotic lesions. *Rhizoctonia* or secondary pathogens often colonized susceptible fruit so that they acquired a soft and mushy appearance. Data were standardized to the same mean and variance for each year to determine whether that improved the rating system over environments. However, since the most resistant and susceptible cultigens were not included in all tests, the original data provided a better estimate of resistance. Therefore, we used unstandardized data for analysis and presentation.

Results. Cultigens with no fruit damage in any of the years tested were PI 197088, PI 357852, PI 280096 and 'P 51' (Table 2). Cultigens showing the greatest susceptibility were 'Supergreen', PI 419108, PI 163218 and PI 177360 (Table 2). Most cultigens were not tested in all years, so care must be used in interpreting relative resistance. Fruit damage was variable as indicated by the high coefficients of variation (CV) in all of the experiments. We believe environmental conditions played a large role in that variability.

The check cultivar Marketmore 76 was shown to have good resistance. Slicing cucumbers like 'Marketmore 76' have tough skin for shipping. There appears to be a relationship between skin toughness and belly rot resistance. Also, PI 197088 had good resistance and a brown, netted skin. PI 163216 and PI 177360 differed in resistance in field and detached tests (Table 2). The correlation between field and detached-fruit tests was not significant in 1982 ($r = 0.07$), and significant in 1983 ($r = 0.50$).

Other potential problems with these experiments is the fact that all fruit do not mature at the same time. This leads to problems at the time of rating when fruits are not all of equivalent size and age. Later-maturing cultigens will often be rated for resistance using small, immature fruits. To correct for that problem, it would be useful to have a technique for screening at the seedling stage.

This study demonstrates the large differences in belly rot resistance in the cucumber germplasm collection. From this data it is possible to select resistant parents and susceptible checks for further study. Future work needs to be done to verify the sources of resistance and to improve the test methods.

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Table 1. Experiment design and methods used in evaluating cucumber cultigens for resistance to belly rot (1981 through 1985).

Year	Test type[z]	No. cult.	No. reps	No. fruit[y]	Inoc. conc.[x]	Date Planted	Date Rated[w]
1981	Det	1063	1	1	1600	27 May	25, 28 July, 1 Aug.
1982	Fld	174	2	3	3200	13 June	1, 6, 12 Aug.
	Det	174	3	3	3200	7 June	13, 16, 18, 21 June
1983	Fld	149	2	2	4800	24 May	11, 18, 25 July, 4 Aug.
	Det	149	3	3	4800	13 May	15, 25 July
1985	Fld	85	3	8	4800	29 April	5, 11 July
	Det	85	3	4	4800	29 April	24 July

[z]Det=detached fruit; Fld=field test.
[y]Number of fruits per replication.
[x]Inoculum concentration in grains per m^2.
[w]Fruits for detached test were harvested 4 to 12 days before rating.

Table 2. Belly rot resistance of resistant, susceptible, and check cultigens tested 1981 through 1985[z].

Cultigen	Seed source	Mean[y]	Field test			Detached test			
			1982	1983	1985	1981	1982	1983	1985
Resistant									
PI 197088	India	0	0	0	-	0	0	0	-
PI 357852	Yugoslavia	0	0	0	-	0	0	-	-
PI 280096	USSR	0	0	-	-	0	0	-	-
PI 285606	Poland	0	0	-	-	-	0	-	-
PI 271328	India	1	0	2	-	0	1	1	-
PI 379282	Yugoslavia	1	0	-	-	0	3	-	-
PI 163216	India	1	0	0	2	0	0	0	5
Check cultigens									
P 51	Ferry-Morse	0	0	-	-	0	-	-	-
Marketmore 76	Cornell Univ.	1	-	-	1	-	-	-	1
Pioneer	Clemson Univ.	1	0	2	-	0	1	1	-
Guardian	Rogers NK	2	-	-	1	-	-	-	2
M 21	NC State Univ.	2	-	-	2	-	-	-	1
M 16	NC State Univ.	2	-	-	1	-	-	-	3
Castlemaster	SunSeeds	2	-	-	2	-	-	-	2
Poinsett 76	Cornell Univ.	2	-	-	2	-	-	-	2
Wautoma	Wis-USDA	2	-	-	2	-	-	-	2
Calypso	NC State Univ.	3	-	-	2	-	-	-	4
Little John	Univ. Ark.	4	-	-	2	-	-	-	5
Sumter	Clemson Univ.	4	-	-	3	-	-	-	4
Score	Asgrow Seed	4	-	-	4	-	-	-	3
Carolina	Clemson Univ.	4	-	-	3	-	-	-	5
Wis. SMR 18	Univ. Wis.	5	-	-	4	-	-	-	5
WI 1701	Wis-USDA	5	-	-	4	-	-	-	6
WI 2757	Wis-USDA	15	-	-	6	-	-	-	23
Supergreen	Harris-Moran	22	-	-	10	-	-	-	34
Susceptible									
PI 344433	Iran	5	5	-	-	-	-	5	-
PI 418962	China	5	6	-	-	3	9	0	-
PI 267741	Japan	5	8	-	-	2	-	5	-
PI 177360	Turkey	6	1	-	-	5	9	10	-
PI 169382	Turkey	10	12	-	-	8	12	6	-
PI 181752	Syria	12	10	-	-	23	4	-	-
PI 419108	China	14	-	-	11	8	15	6	30
Mean			3	4	3	-	3	3	7
LSD (5%)			9	7	3	-	7	7	11
CV (%)			115	76	54	-	126	84	77

[z]Data are mean percentage of fruit surface damaged by belly rot.
[y]Mean across experiments without standardizing data.

Development of a Seedling Test for Resistance to Gummy Stem Blight in Cucumber

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Gummy stem blight, caused by *Didymella bryoniae* and possibly by *Phoma exigua*, is the second most important pathogen of field grown cucumber (*Cucumis sativus* L.) in North Carolina (2). The disease is caused by *Didymella bryoniae* (teleomorph stage of *Phoma cucurbitacearum*, usually reported as *Ascochyta cucumis*). Gummy stem blight causes conspicuous 'V' shaped yellow-brown lesions on leaves, and pale-fawn lesions on stems which are present mainly at the nodes. On fruits, the disease causes a soft rot with black discoloration of the skin (exocarp). In the lesions of different organs, pycnidia of the anamorph stage and perithecia of the teleomorph can be observed easily as abundant black dots. The disease causes much defoliation of the cucumber crop in the late stages of production. Gummy stem blight is a serious disease of greenhouse cucumbers in The Netherlands, where it causes fruit rot (5).

The disease organism is apparently stimulated by volatile compounds contained in plants of species belonging to *Cucumis* and *Cucurbita* (1). Infection caused by *D. bryoniae* appears to be dependent on relative humidity, with more infection occurring at 95 % than at 50 % relative humidity. Greatest infection is produced by free-standing water on leaves. In the case of older leaves, wounding of the leaves is essential for infection (6). A high incidence of main stem lesions and fruit rot is also apparent in plants that are grown with a long period of high humidity and free water on the fruits, for example when changing greenhouse conditions to normal day conditions in late morning (5).

There was no correlation between gummy stem blight on seedlings, and fruit rot caused by gummy stem blight in 2- to 3-month-old plants (4). A seedling test can be used to show differences between susceptible and resistant cultigens (cultivars, breeding lines, plant introduction accessions, populations and families) of cucumbers, using a virulent isolate of *Didymella bryoniae* inoculated at the first true leaf stage of plants kept at 100 % relative humidity for 2 days (3).

There is little evidence that field tests for gummy stem blight are correlated with seedling tests. Two tests involving 954 and 1208 cultigens, inoculating at the 2nd and 3rd true leaf stage and repeated 9 days later, rating hypocotyl and stem cankers along with foliar lesions, showed no significant correlations with field ratings for the same cultigens grown in Florida and Wisconsin (7). However, several resistant cultigens were identified from the tests. The ability to distinguish large differences in resistance to gummy stem blight was demonstrated in another seedling test using seedlings at the 2nd and 3rd true leaf stage incubated for 2 days at 100 % relative humidity, and rated for leaf lesions 4 days after inoculation (7).

A reliable seedling test should reveal significant differences among cultigens and a significant correlation with field test results. The objective of this study was to continue the development of a reliable seedling test for resistance to gummy stem blight in cucumber.

Methods. A field test, run in 1983 to determine resistance in cucumber to gummy stem blight, was used for comparison with the seedling tests. Three seedling tests were run in the greenhouse in 1988 and 1989 to evaluate several methods of inoculation, incubation and plant injury, and to choose the most pathogenic isolate. PI 200818, 'Slice', 'Poinsett 76', M 12 and M 17 were used as resistant checks, while 'Marketmore 76', Wis.SMR 18, 'Addis' and 'Colet' were susceptible checks. All but 'Colet' and PI 200818 are monoecious inbreds.

The field test was planted at the Horticultural Crops Research Station in Clinton, N. C. Plots were 1.5 m long with 1.5 m alleys at each end, and were arranged in rows 1.5 m apart. Plots were planted on 25 May with 15 seeds each, and thinned on 13 June to 10 plants. The field was surrounded by susceptible Wis.SMR 18 border rows. Plots were inoculated on 26 June with one selected isolate of *Didymella bryoniae*. Also, there was natural disease in the field for gummy stem blight, and to a lesser extent for anthracnose (*Colletotrichum orbiculare* (Pass.) Ellis & Halst). The weather conditions of the summer season with high humidity helped to spread the inoculum.

Plots were rated on 12 and 19 July for foliar lesions using a 0 to 9 scale (0 = no disease, 1 to 2 = a trace of infection, 3 to 4 = few small lesions, 5 to 6 = 20 to 50 % of leaves covered with small lesions, 7 to 8 = plant wilted and covered with 50 % or more with lesions, 9 = plant dead). Ratings were averaged over the 10 plants in a plot.

Inoculum. In a preliminary test of 12 *Didymella bryoniae* isolates, we chose those that were virulent and that represented diverse sources of the pathogen. Three isolates of *Phoma cucurbitacearum* collected in 1988 (two from cucumber fields in North Carolina and one from California) were used in greenhouse tests. The isolates were increased on malt extract agar medium at 20°C grown near a window for a 16 hr day (3). The fungus was incubated for 7 days until abundant pycnidia formed (an important characteristic for virulent isolates). Inoculum was prepared in 100 ml of distilled water by flooding plates with 15 ml of sterile distilled water, scraping the surface of the agar with a rubber spatula, and collecting the spores from one plate per isolate. Inoculum concentration was standardized using a hemacytometer to 10^6 spore/ml in 100 cc/ml. One drop of Tween 80 was added to the suspension before inoculation. The spore suspension was applied with an artist's airbrush until a fine film covered the inoculated leaves.

Of the many tests run, three will be described here. The first involved seedlings of 6 cultigens using 4 isolates (2 from North Carolina, 1 from Wisconsin and 1 from Arizona), 3 humidity chamber durations (1, 2 or 3 days), and 3 preinoculation treatments (Carborundum, florist's frog, or none). Preinoculation damage of the leaf produced mechanical damage in addition to the disease symptoms. The experiment was a split-plot with 2 replications of 4 plants each. Whole plots were chamber durations, subplots were leaf damage treatments, and sub-subplots were cultigens. Plants were inoculated with a hand-pumped mist sprayer until droplets formed on the leaf. The Carborundum treatment involved dusting the 1st true leaf with the finely-powdered compound before spraying, then rubbing lightly to damage the leaf epidermis. Damage by the florist's frog (9 needles mounted in a 20 mm diameter base) was produced by pressing lightly on the leaf surface before spraying, leaving a grid of small holes.

For all tests, seedlings were thinned to 1 plant per pot 7 days after seeding, inoculated at first true leaf stage 7 days after thinning, and placed in a dark humidity chamber at 100 % relative humidity and 18 to 21°C air temperature. Plants were removed after 1 day of dry-out (chamber doors open, humidifiers off), and rated for leaf damage 7 to 10 days after inoculation (21 to 24 days after seeding).

The second test involved seedlings of 5 cultigens, 3 isolates, 3 humidity chamber durations (0, 1 or 2 days), and 2 inoculum concentrations (10^6 or 10^7 spores·ml^{-1}). The experiment was a split-plot with 2 replications of 1 plant each. Whole plots were chamber durations, subplots were isolate-concentration combinations, and sub-subplots were cultigens.

The third test involved seedlings of 5 cultigens, 3 isolates, and 2 inoculum concentrations (10^5 or 10^6 spores·ml^{-1}). The experiment was a split-plot with 2 replications of 1 plant each. Whole plots were isolate-concentration combinations, and subplots were cultigens. Seedlings were incubated 1 day in the humidity chamber.

Results. The coefficient of variability was 33 % for the gummy stem blight rating in the 1983 field test, which is not unusually large for disease ratings. Occasional anthracnose lesions on the plants in that study made it more difficult to rate some plants for gummy stem blight. There may also have been an interaction between the two diseases. However, it is difficult to keep anthracnose out of field tests, since gummy stem blight and anthracnose occur naturally at about the same time in cucumber production areas of North Carolina. That is one of the reasons for our interest in developing a seedling test.

The first test showed no significant differences among treatments (Table 1). The only obvious trend was that longer durations in the humidity chamber produced more leaf lesions. The test was included to show that many of the seedling tests we conducted did not provide useful results. It was not possible to get correlation analysis between field and seedling tests since the seedling test did not differentiate the cultigens.

The second test showed that 1 day in the chamber provided greater differences among cultigens than 2 days, isolate DB-H-23 was more virulent than isolates DB-H-21 and DB-H-22, and cultigens PI 200818 and M 17 were more resistant than 'Colet' (Table 2). 'Slice' and M 12 were variable in their reaction. A high concentration was used in the test (10^7 spores·ml^{-1}), but proved too much for the seedlings, since all plants had ratings of 7 to 9 (data not shown).

The third test had much variability (CV = 66 %) so there were few significant differences among treatments (Table 3). The low inoculum concentration (10^5 spores·ml^{-1}) provided few differences among cultigens, whereas the high concentration caused reactions similar to the ranking in the second test. Also, isolate DB-H-23 was not as virulent as in the second test.

Seedling tests for gummy stem blight resistance work well for us only half the time, so they are not as reliable as we would like. However, they are useful for plant breeders wishing to test large numbers of plants off-season, or where limited greenhouse space is available. Based on the tests conducted, we recommend that seedlings be inoculated by spraying undamaged plants in the 1st or 2nd true leaf stage with 10^6 spores·ml^{-1} of an isolate that sporulates well, followed by 1 day of incubation in 100 % relative humidity. Seedlings can be rated 7 to 10 days after inoculation if held at 25 to 30°C after incubation. Progeny of selections that are resistant in seedling tests should be tested in the field to verify resistance.

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Table 1. Disease rating of seedlings of 6 cucumber cultigens inoculated using 1 isolate of *Didymella bryoniae* 3 incubation times (1, 2 or 3 days in the humidity chamber) and 3 damage treatments (Carborundum powder prior to inoculum spray, florists frog to puncture the leaf prior to inoculum spray, or none) compared with field rating[z].

	1 day in chamber			2 days in chamber			3 days in chamber			1983
Cultigen	Carb.	Frog	None	Carb.	Frog	None	Carb.	Frog	None	Field
Slice	1	1	2	3	5	5	5	4	5	2.9
Poinsett 76	1	1	0	4	4	6	5	3	5	4.0
M 17	0	1	1	4	4	5	6	4	5	4.1
Marketmore 76	1	2	2	4	4	7	5	4	5	5.3
Wis. SMR 18	1	1	0	6	1	7	8	4	6	5.6
Addis	1	1	1	4	6	5	5	4	6	6.2
LSD (5%) for row and column comparisons						NS				0.4
CV (%)						43				16

[z]Seedling data are means of 2 replications of 4 plants each (field ratings are means of 6 replications).

[y]Foliage damage rated 0 to 9 (0 = no disease, 1 to 2 = a trace of infection, 3 to 4 = few small lesions, 5 to 6 = 20 to 50 % of leaves covered with small lesions, 7 to 8 = plant wilted and covered with 50 % or more with lesions, 9 = plant dead) 7 days after inoculation at the first true leaf stage.

Table 2. Disease resistance of seedlings of 5 cucumber cultigens sprayed with 10^6 spores·ml^{-1} using 3 isolates of *Didymella bryoniae* and 3 incubation times (0, 1 or 2 days in the humidity chamber)[z].

	1 day in chamber			2 days in chamber			0 days in	
Cultigen	DB-H-21	DB-H-22	DB-H-23	DB-H-21	DB-H-22	DB-H-23	chamber	Water
PI 200818	2	2	6	5	6	9	0	0
M 17	4	4	8	7	4	9	0	0
Slice	4	9	9	6	7	9	0	0
M 12	4	6	6	6	8	9	0	0
Colet	6	7	9	6	9	9	0	0
LSD (5%) for row and column comparisons					3			
CV (%)					40			

[z]Data are means of 2 replications of 1 plant each. A high concentration (10^7 spores·ml^{-1}) was used which provided less consistent results (data not shown).

[y]Foliage damage rated 0 to 9 (0 = no disease, 1 to 2 = a trace of infection, 3 to 4 = few small lesions, 5 to 6 = 20 to 50 % of leaves covered with small lesions, 7 to 8 = plant wilted and covered with 50 % or more with lesions, 9 = plant killed) 7 days after inoculation at the first true leaf stage.

Table 3. Disease resistance of seedlings of 5 cucumber cultigens sprayed with 10^6 spores·ml^{-1} using 3 isolates of *Didymella bryoniae* and 3 incubation times (0, 1 or 2 days in the humidity chamber)[z].

	DB-H-21		DB-H-22		DB-H-23		Water
Cultigen	10^5	10^6	10^5	10^6	10^5	10^6	control
M 17	6	4	5	2	4	0	0
PI 200818	3	6	1	7	0	4	0
Slice	3	6	6	9	2	4	0
M 12	6	8	3	2	3	6	1
Colet	6	9	4	9	2	8	0
LSD (5%) for row and column comparisons				5			
CV (%)				66			

[z]Data are means of 2 replications of ? plants each.

[y]Foliage damage rated 0 to 9 (0 = no disease, 1 to 2 = a trace of infection, 3 to 4 = few small lesions, 5 to 6 = 20 to 50 % of leaves covered with small lesions, 7 to 8 = plant wilted and covered with 50 % or more with lesions, 9 = plant killed) 10 days after inoculation at the first true leaf stage.

Use of Reproduction Factor and Gall Index in Determining Resistance in *Cucumis* spp.

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According to Taylor and Sasser (4), nematode reproduction can be used to measure root-knot nematode resistance, since reproductive ability on a given host is directly related to resistance. Oostenbrink's (2) reproduction factor (R = final nematode population/initial nematode population) is used to measure the reproductive capacity of nematodes. An R-factor < 1 indicates an inefficient host (poor host); R-factor > 1 indicates an efficient host (good host). Gall index measures plant damage (host response), whereas reproduction factor measures nematode reproduction (nematode response). Sasser et al. (3) proposed using a resistance rating system based on gall index (GI) and reproduction factor (R). We used a modification of this system for designating resistance: susceptible (R > 1 and GI > 40), moderately resistant (R ≤ 1 and GI > 40), slightly resistant (R > 1 and GI < 40), and resistant (R ≤ 1 and GI < 40).

Methods. A greenhouse study was conducted to see how well reproduction factor ($R = P_f / P_i$) along with gall index could determine resistance in species of *Cucumis*. One cultivar of *C. sativus* ('Sumter') and five accessions of *C. metuliferus* (PI 482448, PI 482450, PI 482452, PI 482454, and PI 482461) were inoculated with four different species of root-knot nematodes, *M. hapla*, *M. arenaria* race 1, *M. incognita* race 1, and *M. javanica*. In previous studies, African horned cucumber (*Cucumis metuliferus* Naud.) was resistant to root-knot nematodes, whereas cucumber (*Cucumis sativus* L.) was susceptible (6).

The experiment was a factorial treatment arrangement in a randomized complete block design with four replications. A treatment combination consisted of one *Cucumis* cultigen and one root-knot nematode species in a 100-mm diameter (1450 cm^3 volume) pot. Pots contained sterilized sand and soil in a 1:1 ratio. Plants were grown from seed, each pot having one plant. Each plant was inoculated two weeks after planting with 5000 root-knot nematode eggs using the technique developed by Hussey and Barker (1). Plants were rated 8 weeks after inoculation (10 weeks after planting) first evaluating root-knot nematode damage using a gall index (0 to 100 % of roots galled) and then measuring the numbers of eggs on roots. The number of eggs on the roots of each plant was determined using the technique for obtaining root-knot nematode eggs as described by Hussey and Barker (1).

Results. The number of juvenile nematodes in stage two (J2s) was not determined but R-factor and gall index should be sufficient to determine resistance to these nematodes. *Meloidogyne incognita* race 1 was the most pathogenic species used, with an R-factor and gall index mean of 5.3 and 73, respectively (Table 1). Furthermore, all but one cultigen had a susceptible rating when infected with *M. incognita* race 1 (Table 2). Five of six cultigens tested had R-factors greater than four (an efficient host has a R-factor > 1). *Meloidogyne arenaria* race 1 and *M. javanica* produced R-factors over all cultigens of 2.6 and 3.3, respectively (Table 1), indicating that most cultigens evaluated were efficient hosts for those nematodes.

All cultigens evaluated were resistant to *M. hapla* based on R-factor and Gall Index (Table 2). Resistance of *C. sativus* and *C. metuliferus* to *M. hapla* has previously been reported (5). *Cucumis sativus* 'Sumter' was the most susceptible cultigen tested with a mean R-factor of 10.4, indicating that 'Sumter' was a good host for root-knot nematode reproduction. Of the *C.*

metuliferus accessions, only PI 482452 had an R-factor below one, indicating it was a poor host for root-knot nematode reproduction.

Of all root-knot nematodes evaluated, all plants were susceptible except PI 482452 (Table 2). Also, that accession was the only cultigen tested that was an inefficient host for *M. incognita* race 1. However, with respect to the root-knot nematodes evaluated, all *C. metuliferus* accessions tested were significantly different from *C. sativus* 'Sumter' except for *M. hapla* (Table 1). In this study, we found that R-factor can be used to determine degree of resistance in species of *Cucumis*, since gall indices and R-factors were somewhat comparable for each cultigen-nematode combination, which can be seen by the positive correlation (R = 0.68) between gall index and R-factor.

Table 1. Reproduction factors (Rf) and gall indices (Gall) for 6 cultigens of *Cucumis* spp. infected with 4 species of root-knot nematode[z].

	Mean		M. hapla		M.arenaria1		M.incognita1		M.javanica	
Cultigen	Rf	Gall	Rf	Gall	Rf	Gall	Rf	Gall	Rf	Gall
Sumter*	10.4	80	0.1	30	13.8	96	12.6	96	15.0	96
PI 482448†	1.7	56	0.0	20	0.7	50	4.5	86	1.4	66
PI 482461†	1.7	50	0.1	16	0.2	56	5.3	80	1.0	46
PI 482454†	1.6	42	0.0	6	0.4	40	4.5	60	1.3	60
PI 482450†	1.3	43	0.0	6	0.5	60	4.3	66	0.4	40
PI 482452†	0.3	35	0.0	6	0.1	36	0.5	50	0.7	46
Mean	2.8	51	0.0	14	2.6	56	5.3	73	3.3	59

*Cultivar of *Cucumis sativus*.
†Accession of *Cucumis metuliferus*.
[z]Data are means of four replication of one plant each. R-factor ($R = P_f/P_i$) is calculated as the average final count divided by 5000 eggs (initial number of eggs that pots were inoculated with). Gall index is the percentage of roots with galls (0 to 100 %). LSD (5 %) for R-factor = 3.8 and gall index = 22.

Table 2. Ratings of cultigens evaluated with root-knot nematodes.[z]

Cultigen	Mean	M. hapla	M.arenaria1	M.incognita1	M.javanica
Sumter*	S	R	S	S	S
PI 482448†	S	R	MR	S	S
PI 482461†	S	R	MR	S	MR
PI 482454†	S	R	R	S	S
PI 482450†	S	R	MR	S	R
PI 482452†	R	R	R	MR	MR

*Cultivar of *Cucumis sativus*.
†Accession of *Cucumis metuliferus*.
[z]Resistance ratings: susceptible (R > 1 and GI > 40), moderately resistant (R ≤ 1 and GI > 40), slightly resistant (R > 1 and GI < 40), and resistant (R ≤ 1 and GI < 40). S=susceptible, MR=moderately resistant and R=resistant.

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Non-linkage of Bitterness and Resistance to Red Spider Mite in Cucumber

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The role of bitterness (caused by cucurbitacins) in cucumber resistance to twospotted spider mite *(Tetranychus urticae)* is not conclusive. North American researchers reported that bitterness caused resistance to spider mite (2, 3). However, in Europe, De Ponti and Garretsen (6) hypothesized only a genetic relation in terms of linkage of genes for resistance and bitterness. Interestingly, the resistant non-bitter lines of cucumber selected in The Netherlands were susceptible to twospotted spider mite in the United States, and the resistance of bitter lines was inconsistent (7). Those data support the earlier results of causal relation between resistance and bitterness.

Red spider mite *(Tetranychus cinnabarinus)* is a major pest of cucumber in India and other tropical countries like Pakistan, Bangladesh, Sri Lanka and Burma. It is closely related to *T. urticae* (1). It is an economically important pest of cucumber in Europe and North America.

The objective of this research was to investigate further the relationship between bitterness and resistance to red spider mite, and to evaluate the germplasm of cucumber reported to be resistant to twospotted spider mite for resistance to red spider mite.

Methods. During March, 10 plants of each of 16 lines were sown in the field in a completely randomized design. The 16 lines consisted of 4 pairs of near-isogenic bitter and non-bitter lines ('Hokus', 'Nimbus', 'Marketmore 70', 'Marketmore 72', 'Marketmore 76', 'Marketmore 80', 'Poinsett 83-10 Bi', and 'Poinsett 83-10 bi'), and 8 bitter lines (PI 163222, PI 178885, PI 218036, PI 220860, 'Ohio MR 200', 'Robin 30', 'Taipei No. 1', and 'Aodai'). Bed width was 0.75 m and plants were spaced 25 cm apart. Observations on damage index were recorded during the peak natural infestation period of the middle of May. Damage was assessed on a scale of 1 to 5 (1 = slight damage; 5 = heavy damage) described by De Ponti (4). At each observation, five leaves per plant were evaluated and their mean value represented the damage index of the plant. The experiment was run twice in two years.

Results. In both years, the cucumber lines tested were susceptible to *T. cinnabarinus* (damage index > 3). Bitter cucumber lines 'Hokus', 'Marketmore 70', 'Marketmore 76' and 'Poinsett 83-10 Bi' did not differ in damage level from their near-isogenic non-bitter counterpart (Table 1). An unreplicated trial involving single plants of each of those lines behaved similarly at two different sites in a farmer's field (data not presented). That suggests no causal relation between bitterness and resistance to *T. cinnabarinus.*

Using the same set of bitter and non-bitter isogenic lines of cucumber ('Hokus', 'Nimbus', 'Marketmore 70' and 'Marketmore 72'), De Ponti (5) also observed that resistance of cucumber to *T. urticae* was not related to bitterness. Eight other bitter lines of cucumber were also found susceptible to *T. cinnabarinus* (Table 2). In The Netherlands, those lines proved resistant to *T. urticae* (4).

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Table 1. Mean damage index of 4 pairs of near isogenic (Bi vs. bi) cucumber lines tested for resistance to *T. cinnabarinus* under field conditions.

Cultigen	Bitterness	Origin	Damage rating (0 - 5) Year 1	Year 2
Hokus	+	Netherlands	3.6	3.7
Nimbus	-	Netherlands	3.2	3.4
Marketmore 70	+	USA	3.5	3.6
Marketmore 72	-	USA	3.5	3.6
Marketmore 76	+	USA	3.5	3.1
Marketmore 80	-	USA	3.3	2.8
Poinsett 83-10 Bi	+	USA	3.7	2.9
Poinsett 83-10 bi	-	USA	3.8	3.0

Table 2. Mean damage index of 8 bitter cucumber lines evaluated for resistance to *T. cinnabarinus* under field conditions.

Cultigen	Origin	Damage rating (0 - 5) Year 1	Year 2
PI 163222	India	3.9	3.0
PI 178885	Turkey	3.8	3.0
PI 218036	Iran	3.7	3.1
PI 220860	Korea	4.0	3.3
Ohio MR 200	USA	4.0	3.3
Robin 50	USA	3.9	3.4
Taipei No. 1	Taiwan	3.8	3.5
Aodai	Japan	3.8	3.5

Cucumber (*Cucumis sativus* L.) Induced Mutations. II. A Second Short Petiole Mutant

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The *short petiole-2* mutation of cucumber is part of our collection of chemically-induced mutants obtained by Kubicki (1). The first mutant line from the collection was reported last year (3), and we are going to continue this series of descriptions of items not published before.

Methods. The morphological description of the mutant was made for 20 plants compared with the standard inbred, 'Borszczagowski', while its genetic analysis concerned as many as several hundred individuals. The results were verified using statistical methods.

Results. The *short petiole-2* mutant can be distinguished at the 2- to 3-leaf stage. Compared with 'Borszczagowski', plants of *short petiole-2* were smaller, with shorter hypocotyl, shorter, darker green petioles, and crinkled leaves showing some developmental delay. Mature individuals (Fig. 1) were characterized by a significantly shorter hypocotyl (45.0 ± 8.4 mm for *short petiole-2*, 71.6 ± 8.2 mm for 'Borszczagowski'), smaller size of plant (1203.7 ± 74.8 mm for *short petiole-2*, 1856.6 ± 121.7 mm for 'Borszczagowski'). There was almost no branching and if any, branches were usually very short, while the standard had 20 branches on average. Leaf petioles were significantly shorter (5 to 70 mm for *short petiole-2*, 120 to 210 mm for 'Borszczagowski') (Fig. 2). In addition, flowers of the tested plants differed from those of the standard by the shape of ovary (Fig. 3), thicker petals and lack of netting on mature fruits. The mutant appeared to be fertile, which allowed for obtaining seeds after self-pollination. The seed germination was relatively high (90 %) although slightly delayed.

Genetic analysis of the mutant indicated that short petioles was governed by a single recessive gene for which we suggest the symbol, *sp-2*. The F_2 phenotype ratio varied from the expected results (Table 1). However, from the first backcross to *sp-2*, it was possible to confirm control by a single recessive gene.

Table 1. Genetic analysis of *short petiole-2* (*sp-2*) cucumber mutant in a cross with 'Borszczagowski' (B).

	No. obtained		No. expected		Tested		
Generation	Normal	Mutant	Normal	Mutant	ratio	X^2	P
P_1 (B)	39	--	39	--	1:0	--	--
P_2 (*sp-2*)	--	84	--	84	0:1	--	--
F_1	99	--	99	--	1:0	--	--
F_2	333	93	319.5	106.5	3:1	0.804	0.05
BC_1P_1	160	--	160	--	1:0	--	--
BC_1P_2	90	88	89	89	1:1	0.022	0.05

A similar mutant *sp* was obtained in the Soviet Union by Dr. Efimov and described in 1985 by Nijs and Boukema (2). The two mutants are similar except that seeds of the *sp* mutant had only 30 % germination. Also, *sp-2* had bushier plants, slower growth at later stages, and rougher leaves compared with *sp*. We were not able to perform diallel analysis of *sp* and *sp-2* mutants since only one plant was obtained from the seed samples supplied in 1989.

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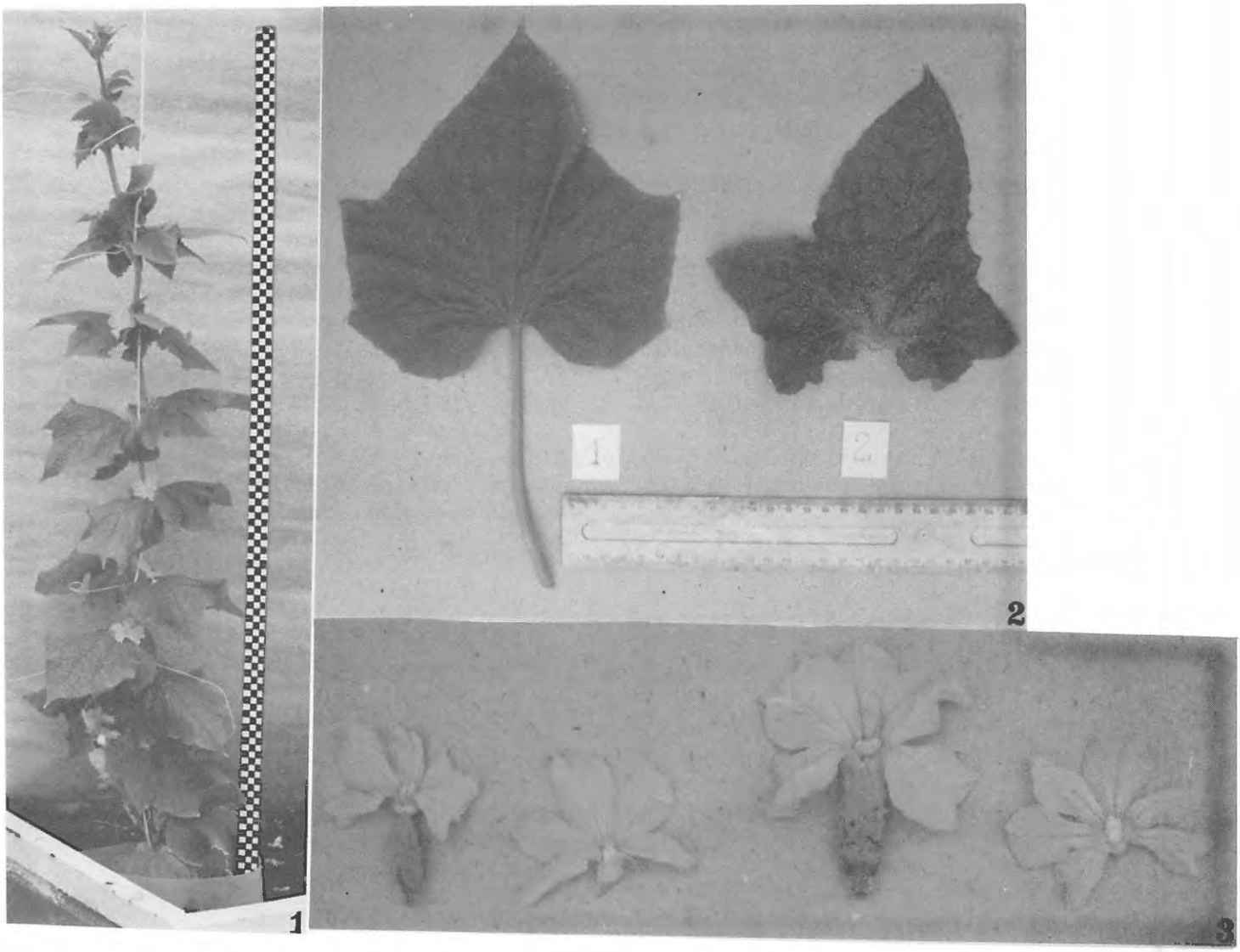

Fig. 1. *Short petiole-2* mutant at the flowering stage.
Fig. 2. Comparison of leaves: 1='Borszczagowski', 2=*short petiole-2*.
Fig. 3. Comparison of flowers: 'Borszczagowski' (on the left), *short petiole-2* (on the right).

Initiation, Maintenance and Plantlet Regeneration from Long-term Suspension Cultures of Pickling Cucumber

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Methods for the culture and sustained cell divisions and growth of plant cells or cell aggregates in liquid medium containing appropriate growth regulators have been reported for a number of important crop species, including carrot (1), bell pepper (12), white poplar (9), tepary bean (6) and cotton (4). In cucumber (*Cucumis sativus* L.), a several methods for initiating suspension cultures and regenerating plantlets from several fresh market cultivars have been described (2, 3, 7). The frequency of regeneration from those suspension cultures was affected by genotype and the growth regulators used (2, 7), necessitating the modification of procedures for different cultivars.

In general, suspension cultures offer several advantages over plant tissue growth on solid media. Since the cells and developing embryos are evenly exposed to nutrients and growth regulators in the liquid medium, the effects of any gradients can be eliminated, allowing more synchronous control over plantlet development. Second, the cell clusters and embryos usually separate from each other in the medium, allowing easy handling and recovery. Third, the number of potential plantlets which can be obtained from a single culture can be large if the conditions are optimized, thereby achieving exponential increases in plant numbers. Fourth, by exposure to specific selection pressure (for example toxins and other chemicals), cell lines could be recovered which display an enhanced level of tolerance.

The objective of this study was to develop a procedure for the initiation, maintenance and regeneration from suspension cultures of the pickling cucumber cultivar Endeavor. We describe conditions for the maintenance of a regenerable culture for a period of up to 15 months and evaluation of techniques for regeneration of normal appearing plantlets.

Plant material. Seeds (provided by Campbell Inst. Res. & Tech.) of the pickling cucumber cultivar Endeavor, a gynoecious hybrid (WI 2870G × Clinton) were soaked in water for about 30 min. and the seed coat was removed carefully using a sharp scalpel. This was followed by surface-sterilization in 70 % ethanol for 30 sec., a 4 min soak in 10 % solution of commercial bleach (Javex, 6.25% sodium hypochlorite) and three rinses in sterile distilled water. Seedlings were grown in Magenta boxes (Magenta Corp., Chicago, IL) containing 50 ml of half-strength Murashige and Skoog (MS) basal medium (8), with 100 $mg \cdot l^{-1}$ ampicillin to minimize bacterial contamination. To ensure uniform germination, the boxes were incubated in the dark at a temperature of 29°C prior to placing them in the light (provided by cool-white fluorescent lamps) at ambient temperatures of 25 to 28°C with a 16 $h \cdot day^{-1}$ photoperiod.

Initiation of callus. Petiole segments 3 to 5 mm long from seedlings grown in vitro were used as the explant source. The medium used for callus initiation was full-strength MS (8) (with full complement of major and minor salts, vitamins, 30 $g \cdot l^{-1}$ sucrose), with 100 $mg \cdot l^{-1}$ ampicillin. The medium was supplemented with 2,4-D/BA at 5/5 µM and solidified by addition of 10 $mg \cdot l^{-1}$ Sigma tissue culture agar. The pH was adjusted to 5.8 prior to autoclaving at 15 psi for 20 min. Approximately 25 ml of medium was dispensed into disposable Petri dishes (20 × 100 mm). Eight explants were placed in each Petri dish, and the dishes were sealed with Parafilm®. All dishes were incubated in the dark for 3 weeks prior to placing in the light at an intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ (provided by cool-white fluorescent lamps), 16 $h \cdot day^{-1}$ photoperiod, and at ambient temperatures of 25 to 28°C.

Initiation. Eight weeks after explants were plated, creamy-yellow embryogenic calli which developed were dissected and used as the donor of cells or cell aggregates for suspension culture utilizing liquid MS medium containing full strength major and minor salts, vitamins, and 30 $g \cdot l^{-1}$ sucrose. Several combinations of growth regulators were evaluated: NAA/BA (1.0/1.0 μM), NAA/Z (1.0/1.0 μM), NAA/adenine-SO_4 (1.0/200 μM), 2,4-D/BA (1.0/1.0 μM), and 2,4-D (1.0 μM). Embryogenic callus from one explant was added to each 250 ml Erlenmeyer flask containing 75 ml of medium. There were three flasks for each combination of growth regulator tested. The flasks were kept in dim light at ambient temperatures of 24 to 28°C and shaken continuously at 120 rpm (on a gyratory shaker). After 4 weeks, the condition of the suspension cultures was noted and healthy-appearing cultures were subsequently subcultured into fresh medium containing the same combinations of growth regulators. This first subculture was carried out by sieving the suspension culture through Sigma cell sieve (with 0.38 mm openings), followed by two rinses of the cells/aggregates using liquid MS medium without growth regulators and transferring the cells/aggregates into the fresh medium.

Maintenance. Two weeks after the first subculture, suspension cultures initiated in medium with 2,4-D/BA (1.0/1.0 μM) showed the best appearance. The best cultures produced a large number of yellow cells/aggregates, did not result in formation of roots or embryo elongation, and did not show evidence of browning (Fig.1a). Only those suspension cultures were maintained further. The suspension cultures were subcultured every 2 to 3 weeks as follows. The flasks were removed from the shaker, the cells/aggregates were allowed to settle down to the bottom of the flask; then about 10 ml of the suspension (with dense cells/aggregates) was pipetted (using a volumetric pipet of 10 ml capacity with a large-holed tip) into 75 ml of fresh medium with 2,4-D/BA (1.0/1.0 μM) and the flask was shaken manually. After all of the cells/aggregates had settled, 10 ml was once again pipetted and dispensed into each of 4 to 5 flasks with 75 ml of fresh maintenance medium containing 2,4-D/BA (1.0/1.0 μM). The suspension cultures were maintained on a gyratory shaker at 120 rpm, at the same conditions as those for their initiation.

Regeneration. The first attempt to induce regeneration was carried out using 5-month old cultures by plating 1 ml of the suspension culture, two weeks after last subculture, onto MS medium without any growth regulators (MS0), or MS0 with activated charcoal (0.5%), or five 100 μl drops were plated on MS with NAA (1 μM) (Fig.1b). However, this procedure did not induce formation of normal plantlets. A second procedure similar to that described by Bergevoet et al. (2) was tested with 11-month-old cultures. All the cells/aggregates from each flask 10 days after the last subculture, were pipetted and transferred into another flask containing 100 ml liquid MS medium without growth regulator and shaken manually. After all of the cells/aggregates had settled, approximately 10 ml of the medium with dense aggregates was pipetted out. To plate the aggregates, five 100 μl drops of the suspension were plated onto filter paper (Whatman) overlaid onto solid regeneration medium containing either IAA/kinetin (0.1/1.0 μM) or NAA/BA (2.0/1.0 μM) (Fig.1c).

Results. Petiole segments initiated callus after about one week on medium containing 2,4-D/BA (5/5 μM) and the callus developed embryogenic (yellow and friable) sectors and embryos 5 to 7 weeks later. This embryogenic callus was dissected and transferred into liquid medium to initiate the suspension culture. After 2 to 3 weeks of shaking on a gyratory shaker, callus in the medium with 2,4-D/BA (1.0/1.0 μM) started to break apart, forming suspension of cells/aggregates (Fig.1a). In the medium containing either of NAA/BA (1.0/1.0 μM), NAA/Z (1.0/1.0 μM), NAA/adenine-SO_4 (1.0/200 μM), or 2,4-D (1.0 μM), the callus did not break apart or formed elongated and rooted embryos, resulting in browning of the tissue or the liquid after 4 weeks. The medium containing 2,4D/BA (1.0/1.0 μM) also appeared to be most appropriate for long-term maintenance of the suspension culture. Using this medium, healthy and regenerable suspension cultures have been maintained for more than 15 months. A subculture every 2 to 3 weeks appeared to be optimal for long-term maintenance of the suspension culture; after more than 3 weeks, the aggregates increased in size and developed elongated and rooted embryos, resulted in the browning of the liquid and rapid decline of the culture. Aggregates from this

suspension culture were able to form normal shoots when plated onto filter paper overlaid on medium containing NAA/BA (2.0/1.0 μM) (Fig.1c). Shoots developing on regeneration medium were excised after 3 weeks and transferred onto MS medium without growth regulators where the shoots elongated and developed roots. Although the first leaves appeared distorted, subsequent leaves usually looked normal. The frequency of plantlet formation is given in Table 1. About 20 plantlets were obtained from each Petri dish containing 500 μl of the initial suspension.

Discussion. Procedures to regenerate plantlets from suspension cultures of different genotypes of cucumber have included various techniques, such as direct plating of embryoid aggregates onto MS medium (solid, semi-solid, liquid) without growth regulators (7), plating the aggregates on MS medium containing activated charcoal or germinating the embryos from suspension culture in MS basal medium (after a pre-wash in MS medium containing activated charcoal (3), and plating the aggregates on MS medium with IAA and kinetin (2). The addition of activated charcoal, which was useful for initiating regeneration from callus as well as suspension cultures in other studies (3,5), was not effective in this study. That might be due to the fact that our suspension culture had been maintained over a longer period or due to genotype differences.

In our study, MS medium with IAA/kinetin (0.1/1.0 μM) gave regeneration of adventitious shoots through organogenesis. However, the shoots dedifferentiated very quickly prior to the stage where they could be transferred to MS free of growth regulators (for plantlet development). The use of NAA/BA at 2.0/1.0 μM in the plating medium provided the highest recovery of plantlets in this study. That combination of growth regulators has also been used in the regeneration of a range of pickling cucumber cultivars from explants (11) and from protoplasts (10). The procedure described in this study may be applicable to regeneration from suspension cultures of other cultivars of pickling cucumber. In preliminary studies, the cultivar Calypso has also responded in a similar manner.

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Table 1. Regeneration and appearance of regenerants of *Cucumis sativus* cv. Endeavor 3 weeks after plating aggregates from suspension culture onto regeneration medium.

Plating medium	No.plantlets/dish Total	Normal	Appearance
MS0[z]	0	0	aggregates forming calli, embryo dedifferentiation, rooting, browning
MS+charcoal(0.5%)[z]	0	0	aggregates forming calli, embryo dedifferentiation, rooting, browning
MS+NAA(1μM)[y]	18	0	aggregates forming calli and shoots, shoot dedifferentiation
MS+NAA/BA(2.0/1.0μM)[x]	39	21	aggregates forming calli and normal shoots from embryos, less shoot dedifferentiation, rooting
MS+IAA/kinetin(0.1/1.0μM)[x]	35	0	aggregates forming calli and shoots, shoot dedifferentiation, rooting

[z]One ml of suspension culture was plated onto each dish of medium, 14 days after the last subculture.

[y]Five 100 μl drops of suspension culture were plated onto each dish of medium, 10 days after the last subculture.

[x]Five 100 μl drops of dense cell/aggregate suspension were plated onto filter paper overlaid onto medium, 10 days after the last subculture.

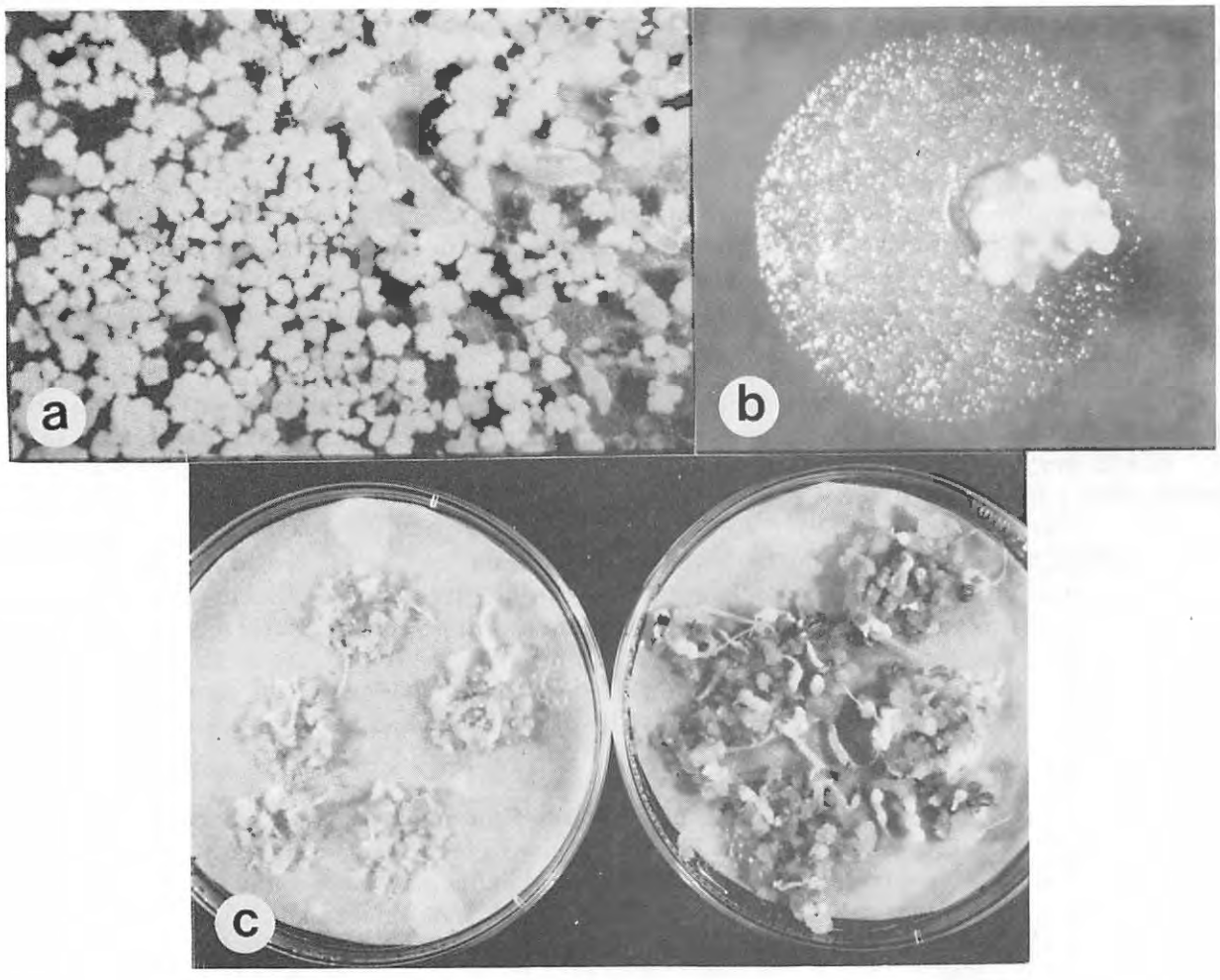

Figure 1. Cell aggregates and embryo development from suspension cultures of pickling cucumber cv. Endeavor. a) From medium with 2,4-D/BA at 1.0/1.0 μM. b) Callus development from a 100 μL droplet of suspension culture plate onto medium with NAA at 1 μM. c) Differentiation and regeneration from droplets of suspension culture plated onto filter paper overlaid on medium with NAA/BA at 2.0/1.0 μM; plate on left shows development after 10 days, plate on the right after 20 days.

Somatic Embryogenesis and Plant Regeneration from Cotyledon Protoplasts of *Cucumis sativus* Wisconsin 2843

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Wisconsin 2843 is a cucumber breeding population with multiple disease resistance (12). The population is homozygous for resistance to cucumber mosaic virus (CMV), *Pseudoperonospora cubensis* (Berk & Curt) Rostow (downy mildew), *Cladosporium cucumerinum* Ellis & Arthur (scab spot rot), *Pseudomonas lachrymans* (E. F. Smith & Bryan) Carsner (angular leaf spot), and *Colletotrichum lagenarium* (Ross.) Ellis & Halst (anthracnose). It is segregating for two levels of resistance to *Sphaerotheca fuliginea* (Schl. ex. Fr.) Poll. (powdery mildew, PM). One level is the intermediate type as seen in Gy 14; the other is the high resistance of WI 1983. The population is heterozygous for resistance to *Erwinia tracheiphila* (E. F. Smith) Holland (bacterial wilt) and to *Corynespora cassiicola* (Berk & Curt) Wei (target leaf spot). In addition, it is gynoecious with non-bitter fruits (lacking cucurbitacins) and has genes for parthenocarpy (12).

The transfer of other disease resistance to Wisconsin 2843 would be of great importance to the crop. However, the existence of sexual incompatibility barriers has hindered the exploitation of sources outside *Cucumis sativus*. Protoplast fusion could help to overcome those incompatibility barriers. However, before that biotechnological alternative can be applied it is necessary to develop good and reproducible methods of plant regeneration from protoplasts of the recipient cucumber line.

In this work, we present a method for obtaining cultures of cucumber protoplasts with high mitotic activity in liquid media, a culture system which has presented problems in other research (1, 11, 13). In addition, we show how to obtain embryogenic calli at an acceptable rate and to regenerate normal plants from somatic embryos.

Plant material. Seeds of Wisconsin 2843 were obtained from Dr. C. E. Peterson. Cotyledons from 5- to 6-day-old seedlings, axenically germinated in MG medium (8) were used as starting material. Before protoplast isolation, cotyledon explants were precultured for 3 days in darkness at 28°C in NB 2.5/1.0 solid medium consisting of 2.5 mg/l NAA, 1.0 mg/l BA, M&S (10) mineral salts, 100 mg/l myo-inositol, 1 mg/l thiamine-ClH, SH-vitamins (16), 30 mg/l gentamycin, 30 g/l sucrose and 8 g/l agar (Technical No.3, Oxoid). The pH was adjusted at 5.7 before autoclaving at 115°C.

Protoplast isolation. The method was previously described for melon (4, 7) with some modification. Briefly, cotyledons were cut into 1 to 2 mm wide strips and placed in enzymatic medium at the ratio of 1 g per 8 ml of medium, for 12 to 14 hours at 28°C in darkness in a reciprocal shaker at 100 strokes/min (amplitude 20 mm). Enzyme solution consisted of 1.5 % cellulase Onozuka-R-10 (Yakult-Honsha Co., Ltd.) in the washing medium LG-0.5 (M&S macronutrients, 0.4 M-mannitol, 0.1 M-glycine, 0.5 mM MES). The pH was adjusted to 5.7 and the solution was sterilized by filtration. Crude protoplast suspension was filtered through 85 µm nylon mesh and purified by centrifugation at 75*g* for 10 min, and resuspended in the purification medium F-0.65 (consisting of M&S macronutrients, 0.6M-sucrose, 0.05 M-glycine, 0.5 mM MES). An overlay of 1 ml LG-0.5 was added to the resuspended protoplast which was centrifuged at 50*g* for 5 min. The protoplast ring which formed at the interface between the sucrose solution and the overlaid mannitol solution was collected with a Pasteur pipette and resuspended in the washing medium. After two rinses in that medium by centrifugation at 75*g* for 10 min, the protoplasts were resuspended in the culture medium DNB 1.0/0.5/0.5 at the final density of 0.75 to 1 x 10^5 protoplasts/ml and distributed into 55 mm glass petri dishes at the rate of 2.5 ml/dish.

Protoplast culture. The culture medium DNB 1.0/0.5/0.5 consisted of 1.0 mg/l 2,4-D, 0.5 mg/l NAA, 0.5 mg/l BA, B5 mineral salts (3), 100 mg/l myo-inositol, SH-vitamins, 100 mg/l MES, 100 mg/l carbenicillin, 20 g/l glucose and 3.8 g/l xylitol. The medium was osmotically stabilized up to 0.5M with mannitol and the pH adjusted at 5.7 before autoclaving at 115°C. Medium osmolarity was gradually reduced throughout the culturing period by further additions of fresh mannitol-free culture medium: 0.5 ml after 5 days in culture and 1 ml each 5 days for two weeks until microcalli formation. Incubation was always at 28°C in darkness.

Embryogenic response. Microcalli (multicellular colonies) grown 20 to 25 days after protoplast isolation and culture were transferred to the embryogenic media and cultured by the double-layer method (7) in 90 mm plastic Petri dishes at 28°C in darkness for 25 days. Embryogenic response in diverse culture media was studied following a factorial design with three factors under control: 1) type of auxin (2,4-D and NAA); 2) concentration of auxin (1.0 and 2.5 mg/l); 3) concentration of BA (1.0 and 2.5mg/l). In this case the basal medium consisted of M&S mineral salts, 250 mg/l glutamine, 100 mg/l myo-inositol, SH-vitamins, 100 mg/l carbenicillin, 30 g/l sucrose and either 6 g/l (semisolid layer) or 8 g/l (solid layer) agar (Technical No.3, Oxoid). The pH was adjusted to 5.7 before autoclaving.

Minicalli grown in these semisolid media were transferred after 25 days to the same solid culture medium (with 8 g/l agar), distributed in 250 ml glass culture vessels filled with 40 ml solid medium, and incubated under the same conditions to obtain proembryogenic calli. Proembryogenic calli were transferred to new culture media with a reduced concentration of auxin and cytokinin to study their effect on formation and development. A factorial design was carried out with two factors: NAA (0.01; 0.1; 0.5 mg/l; and BA (0.01; 0.1; 0.5 mg/l). The basal medium was M&S mineral salts, 100 mg/l myo-inositol, SH-vitamins, 30 mg/l gentamycin, 30 g/l sucrose and 8 g/l agar (Technical No.3, Oxoid). Cultures were incubated under 16 h photoperiod (1000 lux = 45 $\mu E \cdot s^{-1} \cdot m^{-2}$) for 25 days.

Embryogenic lines and plant regeneration. The embryogenic calli obtained in any of the preceding media were kept as embryogenic lines by subculturing every 20 days in media NB 0.1/0.1 or NB 0.01/0.01. It was possible to regenerate normal plants by culturing on basal medium without regulators the cotyledonary somatic embryos developed in media with a low concentration of NAA and BA.

Results. The yield from culture was 0.5 to 1.0 x 10^6 protoplasts/g of precultured cotyledonary tissue. The culture in medium DNB 1.0/0.5/0.5 was as follows. There was a high rate of cell wall regeneration (80 to 90 % of cultured protoplasts) on the 2nd or 3rd day followed by intense mitotic activity leading to a plating efficiency (percentage of dividing cells) of nearly 80 % after 4 to 5 days in culture. That mitotic capacity was maintained during culture so that after 25 days, the dishes were covered by a mass of microcalli with the liquid medium almost consumed. Periodic addition of fresh medium facilitated the high mitotic rate of the cells and prevented browning problems.

Others have reported methods for cucumber protoplast culture (1, 6, 11, 13, 17). In order to attain a reasonable plating efficiency, most of them have made use of solid media or gelled microdroplets for culturing the protoplasts since they obtained low results when liquid culture media were used (1, 11, 13). Only Trulson and Shahin (17) reported high plating efficiencies (40 to 60 %) in liquid media. With our method we achieved higher efficiencies (80 %) with Wisconsin 2843. That increase in the mitotic activity did not seem to be a function of genotype used, since we obtained similar results when using other genotypes. That suggests the most important factor was the preculture of the cotyledon explants before isolation of protoplasts, always used as a routine protocol in our experiments.

The advantageous effect of preculture of plant material on the latter cell division rate of the cultured protoplasts had already been observed in previous experiments carried out in our

laboratory where the same medium (DNB 1.0/0.5/0.5) was used for culturing leaf and cotyledon protoplasts from melon (7,8,15) and from other *Cucumis* Species (2, 14).

Culture of p-microcalli in media with NAA/BA. Growth type and intensity were quite similar in the four assayed media (NB 1.0/1.0; NB 1.0/2.5; NB 2.5/1.0 and NB 2.5/2.5). In all cases, minicalli of 3 to 4 mm in diameter, white-cream in color, and most of them friable were formed. In any case visible proembryogenic structures developed. When those microcalli were subcultured in the corresponding solid media and incubated under darkness, calli of approximately 1 cm diameter, light cream color, friable (and which disintegrated in globular structures) were formed. Creamy calli with proembryogenic structures appeared only sporadically in media NB 1.0/2.5, NB 2.5/1.0 and NB 2.5/2.5 (Table 1). The remaining non-embryogenic calli were transferred to media with reduced levels of NAA and BA and no embryogenic response was obtained in any case.

Table 1. Influence of combinations of 2,4-D/BA and NAA/BA on cultural and embryogenic response from cucumber cotyledon p-minicalli.

Culture medium	Growth index[z]	% of embryogenic calli[y]
NB 1.0/1.0	1.69 ± 0.05	0.0 ± 0.0
NB 1.0/2.5	2.71 ± 0.05	3.3 ± 1.6
NB 2.5/1.0	1.86 ± 0.06	3.3 ± 1.6
NB 2.5/2.5	1.81 ± 0.06	0.8 ± 0.8
DB 1.0/1.0	2.00 ± 0.10	8.4 ± 3.6
DB 1.0/2.5	2.08 ± 0.08	6.7 ± 3.2
DB 2.5/1.0	1.67 ± 0.09	11.5 ± 4.4
DB 2.5/2.5	1.54 ± 0.07	15.0 ± 5.8

[z]Growth index was calculated by assigning an arbitrary value, ranging from 0 to 3, to each callus growth estimated qualitatively: 0=no growth, 1=little growth, 2=moderate growth, 3=much growth (mean ± SE).

[y]Frequency of embryogenic calli giving whole plants ± SE; $SE = \sqrt{p(1-p)/n}$, where p=% of calli with somatic embryos and n=total number of calli.

Culture of p-microcalli in media with 2.4D/BA. In the four semisolid media studied (DB 1.0/1.0; DB 1.0/2.5; DB 2.5/1.0 and DB 2.5/2.5) growth type and intensity were also quite similar. Most of the minicalli grown on those media were friable, light cream colored, without visible morphogenic structures, and of a reduced size (2 to 3 mm in diameter). Both the slow growing and the absence of light seemed to be advantageous in the prevention of browning. Besides this, although only sporadically, some proembryogenic minicalli had already appeared at that premature level. That response was, however, more generalized and at higher frequencies after subculturing in the same solid media (Table 1). In the four solid media, two kinds of calli were formed: non-embryogenic calli presenting friable, cream-white colored zones and glomerular structures (potentially embryoids); and soft, yellow colored embryogenic calli,, with proembryos and white embryos similar to, but bigger than those obtained in the semisolid media. The highest frequency of embryogenic calli able to regenerate plants (15 %) was achieved in medium DB 2.5/2.5.

Both the morphogenic potential and the plant regeneration capability of those calli are not lost by successive subcultures in media with reduced concentrations of NAA and BA. Moreover, the number of embryo-producing calli can be increased if the non-proembryogenic calli grown on the

2,4-D/BA media are transferred to those media. With that procedure, an additional 18 % of calli with somatic embryos could be obtained.

Plant regeneration. Whole plants were obtained without problem, since practically every embryogenic callus developed in any of the above-mentioned media gave rise to normal cotyledonary embryos which germinated and grew easily on the basal medium without plant regulators. The average of normal plants coming from each embryogenic callus ranged between 1 and 3.

Many have reported embryogenesis from cucumber protoplasts (1, 6, 11, 13, 17). Frequencies of enbryogenic calli, when reported, were comparable (1, 13, 17) to those described here. However, when plant regeneration was taken into account the results differed. Trulson and Shahin (17) reported frequencies of embryogenic calli giving whole plants less than 1 %; Punja et al., (13) reported 18 plants regenerated and Colijn-Hooymans et al. (1) reported problems with plant regeneration in their work on cotyledon-derived cucumber protoplasts. In our study, the frequency rose to 15 % in the first phase and was increased another 18 % by the second phase of subculturing. In previous works carried out with protoplasts from leaves and cotyledons of other cucumber genotypes, we observed that the frequency of calli with embryos was much higher than the frequency of embryogenic calli capable of giving whole plants. Fortunately, in this study we did not have those problems, perhaps due to the fortunate choice of the cucumber genotype, or to the fortuitous choice of cultural parameters.

The effectiveness of the regeneration procedure was, of course, too far from the ones previously achieved by us for melon protoplasts (4, 7). However, we are improving this protocol in successive experiments. The results of this study permit the setting up of a method for culturing cotyledon protoplasts of cucumber in liquid medium with a high mitotic activity. Moreover, the induction of somatic embryogenesis, the production of embryogenic lines and the regeneration of whole plants from somatic embryos in cotyledon protoplasts of Wisconsin 2843 over the relatively short period of 3 months had also been achieved.

Those results are valuable since they permit the use of this cucumber population as the recipient parent in a biotechnology program aimed at the introduction of new resistance to plagues and diseases by fusion of protoplasts. Genetic transformation by electroporation of protoplasts should also be a valuable alternative in the achievement of interesting improvements in that population.

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Database of Native Distributions for *Cucumis*

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A taxonomic monograph (7) of the genus *Cucumis* Linnaeus (Cucurbitaceae) was completed, and the morphological, cytological, and macrodistributional data were distributed in a format suitable for interactive specimen identification or for data interrogation (6). Thirty four botanical institutions around the world loaned 5,880 herbarium specimens of *Cucumis* and related genera for the study (acronyms according to 5): BH, BLAT, BM, BOL, BR, C, CAL, CANB, CM, E, GAT, HUJ, K, KUN, L, LE, LISC, LISU, LIV, LMU, LY, M, MEL, MO, MPU, NSW, PE, PRE, S, SRGH, TI, UPS, W, and the Vegetable Research Institute, Beijing. The morphological data were collected from the specimens themselves, and the collection data on their associated labels were entered into a fixed-length-field database on a microcomputer. Only information on labels accompanying *Cucumis* specimens with identifiable collectors were entered in the database; there were 3,719 collections, i.e., records, with 4,708 specimens in the database. Some collections had duplicate specimens deposited in more than one institution resulting in fewer collections than specimens. Collections examined at NY, P, and US were not loaned, and so are not included in the database.

A unique record number was assigned to each collection and lightly written in pencil in the lower right-hand corner of each duplicate specimen (Table 1). This number was used to sort out and eliminate duplicate records of collections with duplicate specimens. The species and infraspecific identifications were assigned by the author, and the taxon numbers are those which appeared in the monograph (7). The collectors' names and the location data were in numerous languages using various accents. To enter accents, the extended ASCII character set, 1 to 255, was used. The following letters with accents and symbols were input using extended ASCII characters 123 to 255: á, number 160, 13 times; à, number 133, once; â, number 131, twice; ã, number 132, 6 times; ä, number 204, once; ç, number 135, 27 times; é, number 130, 37 times; è, number 138, 16 times; ê, number 136, 4 times; í, number 161, 7 times; ñ, number 164, 3 times; ó, number 162, 3 times; ô, number 148, once; ö, number 206, 14 times; ü, number 129, 47 times; and, ±, number 241, once. If a well-known or prominent location, whether part of the detailed location data or separate, was cited on a label, then it was recorded in the Landmark field. This assisted in determining the position of obscure locations. Elevation above sea level in feet was converted to meters.

Few labels gave latitude and longitude of the location in degrees and minutes. These data were obtained from other sources, including geographical references (1, 2, 4, and 10), country gazetteers of the United States Board on Geographic Names, pencil notes on specimens, and the PRECIS database of the Botanical Research Institute, Pretoria, South Africa (3 and 8). In PRECIS a grid system is used for determining locations; each degree square is divided into four half-degree squares, 30' by 30', and 16 quarter-degree squares, 15' by 15', indicated by lower-case letters. Therefore, PRECIS data were used in conjunction with other sources, or when only PRECIS data were available, the latitude and longitude are given in degrees with zero minutes. In a few cases, place names had been used more than once in the same country, and so it was impossible to determine which of the different sets of coordinates applied to a location.

The MS-DOS diskette accompanying report number 15 of the *Cucurbit Genetics Cooperative* (CGC) contains the self-uncompressing file CUCUDIST.EXE. The *Cucumis* collections database is compressed within this file. It is an ASCII text file 1,966,435 bytes in length. To uncompress the database, create a suitably named directory on a hard disk, and change to it,

making it the default directory. Place the diskette accompanying CGC report number 15 in drive A:, and type A:\CUCUDIST at the DOS prompt, and press enter. The file CUCUMIS.DST with 1,966,435 bytes will be written to the default directory. Each line in CUCUMIS.DST contains the data for one collection of *Cucumis*. Using suitable database software, establish a database structure with the format given in Table 1; the data for each field occurs in the columns given in Table 1. Import the data into the database, and they are ready for use.

The *Cucumis* collections database can be used in numerous ways. Sorting it by collector and collector's number, the collections critically studied and identified for the monograph of *Cucumis* can be determined, and the identity of particular collections can be confirmed. Sorted by taxon, i.e., species and infraspecific epithet, the distribution of taxa can be ascertained in the degree of detail desired. Sorted by country and state, the occurrence of taxa in a geographic area can be outlined. Sorting by location is less useful because the location descriptions are so varied that they will not group together. These data can illuminate many aspects of *Cucumis* when different sorts and selections are used.

Table 1. Fields in the database of collections studied indepth by Kirkbride for his taxonomic monograph of the genus *Cucumis* Linnaeus (Cucurbitaceae).

Field name	Type[1]	Length	Columns	Description
record_numb	N	5	1-5	Unique number of each collection in database
genus	C	7	6-12	Always *Cucumis*
species	C	13	13-25	Species name
rank	C	6	26-31	Rank of the infrapsecific name
infraspecific	C	32	32-63	Infraspecific name
taxon_numb	C	8	64-71	Taxon number in Kirkbride's Monograph
collector	C	61	72-132	Collector's initials and full last name
collnum	C	17	133-149	Collector's number
month	N	2	150-151	Number from 1 to 12 corresponding to month
day	N	2	152-153	Number from 1 to 31
year	N	4	154-157	Number from 1752 to 1988
source	C	26	158-183	Botanical institution(s) where deposited[2]
country	C	29	184-212	Follows United States Government (9)
state	C	27	213-239	Name appearing on labels
landmark	C	48	240-287	Prominent location on label
location1	C	61	288-348	See footnote [3]
location2	C	61	349-409	See footnote [3]
location3	C	61	410-470	See footnote [3]
location4	C	37	471-507	See footnote [3]
elevat_me	C	9	508-516	Elevation above sea level in meters
latitdeg	N	2	517-518	Degrees of latitude
latitmin	N	2	519-520	Minutes of latitude
latitdir	C	1	521	Latitude direction, N = north; S = south
longideg	N	3	522-524	Degrees of longitude
longimin	N	2	525-526	Minutes of longitude
longidir	C	1	527	Longitude direction, E = east; W = west

[1]C = character, i.e., any character in the extended ASCII character set; N = numeric.
[2]Acronyms according to reference 2.
[3]Depending on its length, the location may be divided between location fields 1 to 4.

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Interactive Microcomputer Database for Identification of *Cucumis*

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Recently a taxonomic monograph (11) of the genus *Cucumis* Linnaeus (Cucurbitaceae), as it has been circumscribed since its inception (2, 3, 8, 9, 10, 12, 13, 14, and 15), was completed. Two subgenera, two sections, six series, 32 species, six subspecies, and two varieties were recognized in the genus. One section, five series, and four species were presented as new, and two new combinations and new statuses and one new name were proposed. The taxonomic history, morphology, cucurbitacins, flavonoids, isozymes, DNA, cytology, and crossability of the genus were summarized. Each taxon was presented with a morphological description, synonyms, distribution, and pertinent discussion. Single entry keys to the subgenera, sections, series, species, and infraspecific taxa were provided. Six hundred and fifty synonymous names are cited of which 522 are referred to *C. melo* and 70 to *C. sativus*. Nineteen doubtful names were cited, and 52 scientific names were excluded from the genus. Lists of the morphological characters scored and of the herbarium specimens critically examined were included.

Morphological, cytological, and macro-distributional data were stored on a microcomputer in DELTA format (4, 6, 7, and 16). The DELTA format was chosen because the International Working Group on Taxonomic Databases in Plant Sciences (TDWG) has designated it as the standard format for exchange of descriptive plant data (1) and because it is compact and allows comments and ranges of data to be entered. The DELTA system (4, 5, 7, and 16) for microcomputers was used to maintain the data and transform it into descriptions and various formats for single-entry key production, interactive querying, and phenetic and cladistic analyses. The descriptions and single-entry keys in the *Cucumis* monograph were produced using the DELTA system. Also, the descriptive data in DELTA format was transformed into a suitable format for interactive querying using Dallwitz's INTKEY program, version 3.0.

The MS-DOS diskette accompanying report number 15 of the *Cucurbit Genetics Cooperative* (CGC) contains two self-uncompressing files, CUCUMIS.EXE and INTKEY30.EXE. The *Cucumis* database is contained in CUCUMIS.EXE, and the program INTKEY, version 3.0, is contained in INTKEY30.EXE. The expanded database is 180 kilo bytes in size, and the expanded software is 350 kilo bytes. They can be installed either on a hard disk or dual floppy diskettes of an IBM compatible microcomputer. For hard disk installation, follow the instructions in the next paragraph and skip the following one, and for floppy diskette installation, skip the next paragraph and go to the following one.

To install the *Cucumis* database and INTKEY on a hard disk, create an appropriately named directory on the hard disk, and change to the newly created directory, making it the default directory. Place the diskette accompanying CGC report number 15 in drive A, type A:\INTKEY30 at the DOS prompt, and press enter. The file will decompress itself and install INTKEY in the default directory. Repeat the preceding process with A:\CUCUMIS. If the CGC diskette must be used in drive B: instead, substitute B: for A:. To start the program and access the *Cucumis* database, type INTKEY at the DOS prompt and press enter.

To install the *Cucumis* database and INTKEY on floppy diskettes, place a formatted, empty diskette in drive A:, and change to drive A:, making it the default drive. Place the diskette accompanying CGC report number 15 in drive B. Type B:\INTKEY30 at the DOS prompt, and press enter. The file will decompress itself and install INTKEY on the default drive. Repeat the preceding process with B:\CUCUMIS. If the CGC diskette must be used in drive A: instead, substitute A: for B:. To start the program, place the diskette containing the *Cucumis* database and INTKEY program in drive A:, change to drive A:, and type INTKEY at the DOS prompt and press enter.

As INTKEY starts, it will scroll through its name, version, author, and information on the *Cucumis* database. Highlighted bars at the top and bottom of the screen mark off the INTKEY window in which commands and material generated by the program are displayed. Commands may be typed on the command line directly below the bottom highlighted bar, or they can be selected from the pull-down menus. The bottom highlighted bar is the *prompt line* giving a brief summary of what can be typed at the current stage. The highlighted bar at the top of the screen is the *function line* and indicates some of the keys available at the current point in the program to carry out various functions. Single keys are indicated at the right-hand end of the function line, and keys used in combination with the ALT key at the left-hand end of the line. An arrow pointing down on the function line indicates that the down-arrow or PAGE-DOWN keys can be used to scroll through material in the window, and an arrow pointing up means that the up-arrow or PAGE-UP keys can be used. The ALT+M key combination is used to display the pull-down commands menu, and ALT+H displays helpful information about the current activity, function, or command. The ESC key can be pressed at any time to discontinue scrolling through material in the window, to close a pull-down menu, or to abort the current command. To exit from INTKEY, select QUIT from the commands menu, or type 'quit' on the command line and press enter.

When the INTKEY program is started, its defaults are set for the identification of specimens. Examine the specimen to be identified, and determine what its characteristics are. If you are unsure what characters are available in the database, call the commands menu (press the ALT+M key combination), use the cursor arrows to place the highlighted area on the command CHARACTERS, and press the enter key twice. A complete list of the characters will appear in the window to be scrolled through. If a particular structure is present, such as a fruit, then call the commands menu and use FIND. Select CHARACTERS, then type the letters "fruit" on the command line and press enter. All characters in which the letters "fruit" appear will be listed. A third method is to call the commands menu and use BEST. This will list the characters in the order of their separating powers; those with larger values are more useful in separating the remaining taxa. After selecting a character, type its number and press enter. For a numeric character, a window will appear describing it. The value or range of values should be typed on the command line and enter pressed. For multistate characters, a pull-down menu will appear with its states. If the specimen has a single state for the character, move the highlighted area to that state and press enter. If the specimen has more than one state for the character, move the highlighted area to each state of the specimen one-by-one and press space for each state, then press enter. Only those taxa possessing the selected character states will be considered as additional characters and their states are selected. Finally a single taxon will remain. Call the commands menu, and use DESCRIBE to generate a complete description of the remaining taxon to verify the identification of the specimen. To do another identification, call the commands menu and use RESTART to reinitialize INTKEY to do the next identification.

To query the database for information about the taxa, the defaults should be reset. Call the commands menu and use SET. A second pull-down menu of subcommands will appear; select MATCH, and set it to OVERLAP. To discover what characters are available, use the techniques described in the preceding paragraph. For example, to know what taxa occurring in Ethiopia have aculeate fruits, type '230', native distribution in Africa by country, on

the command line and press enter, and select Ethiopia from the pull-down menu. Next type '208', presence or absence of aculei on the surface of the fruit, on the command line, and select aculeate on the pull-down menu. The program reports that 11 taxa remain, i.e., there are 11 taxa in Ethiopia with aculeate fruit. To see which taxa remain, type 'taxa rem' on the command line and press enter, and the 11 taxa will be listed in the window. To do another query, call the commands menu and use RESTART to reinitialize INTKEY to do the next query.

INTKEY is a flexible program rich in useful commands; its capabilities are only limited by the imagination of the user. To learn more about INTKEY, put the CGC diskette in drive A:, type A:/LIST INTKEY.DOC at the DOS prompt and press enter. LIST is a shareware utility for browsing through ASCII files. The cursor keys and PAGE-UP and -DOWN keys move through the document, the '?' key is for help, and the ESC key exits from LIST. LIST.DOC on the CGC diskette has further information about the program. Both programs are shareware, i.e., they can be distributed freely with their documentation. If you seriously use them, please follow the instructions in their documentation for compensating their authors.

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'Pedigree' PI 414723 Melon

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PI 414723 from India has received much attention from breeders, pathologist and entomologists for resistances to: zucchini yellow mosaic and watermelon mosaic viruses including resistance to virus multiplication (5,7,8), powdery mildew resistance (4), melon aphid, *Aphid gossypii* Glover (3), and mosaic virus transmission by the melon aphid (6). It is, however, susceptible to western flower thrips, *Frankliniella occidentalis* Pergande (2). PI 414723 was derived from PI 371795 (10) which was obtained in 1948 by Walter N. Koelz in Mussoorie, Uttar Pradesh, India (altitude 1829 m) and which resembled the Indian variety 'Kjira' as described by Corley (1): the fruit have soft flesh and rind which split at maturity and are used in soups and stews, and the seeds are roasted and eaten.

In this report, we provide some details about the derivation of PI 414723 from PI 371795. Table 1 includes PI and progeny numbers that have appeared in reports by various authors about these PIs and related progenies. The present authors welcome any questions or comments about these plant materials.

In summer 1959, PI 371795 was planted in the field at Torrey Pines, California. Following a chance infestation of melon aphids, the single plant in PI 371795 was free of aphids; all of the others were covered with them. Seven or eight self-pollinated fruit were obtained from the plant to produce progeny 90234 (sometimes designated LJ 90234).

As might be expected, PI 371795 was not homogeneous for plant and fruit characters and aphid-resistance. Three self-pollinated progenies of PI 371795 produced by Fred Andrus at Charleston, South Carolina were susceptible to aphids and differed from 90234 for fruit and plant characters (unpublished data).

In 1969, resistance to the melon aphid in 90234 was confirmed in controlled, greenhouse tests at Riverside. Melon aphid-resistant inbreds of 90234 were bulked and designated PI 414723. Breeding for melon aphid resistance culminated in the release of three orange flesh melon aphid resistant breeding lines (3).

Table 1. 'Pedigree' of PI 414723, and related progenies.

PI/Progeny	Notes
PI 371795	The *C. melo* contaminant of PI 175111 (*C. sativus*) designated as to PI 371795 in 1972 (9,10).
90234	S_1 Self-pollinated seeds from a single plant of PI 371795. (1959)
91060	S_2 Melon aphid-resistant selection of 90234. (1969)
91213	S_3 Melon aphid-resistant selection of 91060. (1971)
PI 414723	S_1Sb_1 Bulk of seeds from 16 (91756-91772) sib-pollinated aphid-resistant 90234 plants. (1977)
92417	S_2 Bulk of nine (90303, 91202-91205, 91209, 91228, 91229A and 91381) self-pollinated melon aphid-resistant plants of 90234; not released. (1977)

[z]Some authors included LJ (La Jolla) for the former USDA station at La Jolla on Torrey Pines Mesa as a prefix to the five-digit pedigree numbers.

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Interaction and Linkage Tests of Flesh Color Genes in *Cucumis melo* L.

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The melon genes green flesh (*gf*) and white flesh (*wf*) have each been reported to be inherited as simple recessives to orange flesh (2,3). However, their interaction has not been described. I determined the interaction, using all three types in crosses. The orange-fleshed parent was a bush (*si-1*) crenshaw cultivar I recently developed, which had been inbred through F_7 and showed no segregation for flesh color. The green-fleshed parent was an advanced breeding line homozygous for flesh color and bush habit and derived from a cross of the bush crenshaw with the honeydew cultivar Earlidew. The white-fleshed parent was the casaba cultivar Golden Beauty (Burpee Seed Co.). Results for the three possible crosses are given in Table 1. F_1 flesh color in Tests 2 and 3 was orange and in Test 1 was white. Test 3 represents the pooled data for five different F_2 families, each from a different F_1 plant, but all five F_1's from a single P_1 x P_2 cross. The data were pooled following a heterogeneity chi square test ($X_T^2 = 7.79$, $p>0.50$). Family size differed among the three tests, because they were also being used to advance my breeding program.

As can be seen from Test 3, wf^+ is epistatic over gf^+-*gf*. My orange-fleshed parent was wf^+/wf^+ gf^+/gf^+, not wf^+/wf^+ *gf*/*gf*. This suggests that the crosses of Hughes (2) and Baines and Kang (1) of *gf* by gf^+, which gave no white-fleshed segregants, either involved wf^+/wf^+ *gf*/*gf* parents or the authors were misclassifying white-fleshed segregants, assuming that *wf* and *gf* in my material and theirs is the same. The variability I encountered in *gf* phenotypes could lead to such misclassification, and indeed I did observe a deficiency of *gf* and an excess of *wf* phenotypes in comparison to expected in Test 3 (Table 1).

No linkages were encountered among the three genes *gf*, *wf*, and *si-1*.

Table 1. Summary of linkage tests.

Test	Generation		Gene combination		Observed frequencies					
no.[z]	and phase[y]		a	b	++	+b	a+	ab	X^2[x]	p
1	F_2	R	*si-1*	*gf*	104	33	35	8	1.05	>0.70
2	F_2	R	*si-1*	*wf*	54	17	12	4	1.86	>0.50
3	F_2	C	*wf*	*gf*	(619)		166	41	2.97	>0.20

[z]Tests 1 and 2 are each of a single family; 3 is of 5 homogeneous, pooled families. Parental flesh colors were green x white in Test 1, orange x white in Test 2, and orange x green in Test 3.

[y]C = coupling; R = repulsion.

[x]Chi squares were calculated for fit to independent assortment, i.e., to a 9:3:3:1 in 1 and 2, and to a 12:3:1 in 3.

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Bitterness in Snake Cucumber *Cucumis melo* var. flexuosus Naud.

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The immature fruit of the Snake cucumber is a popular salad and pickling crop in the Sudan. Bitter fruits are occasionally discovered in the produce, and although in very small quantities, it is sufficient to discourage sales and reduce prices of a specific lot. This study was conducted to investigate the phenomenon so as to enable selection for non-bitterness prior to fruiting.

Seed of a local variety of snake cucumber was obtained from a local seed dealer at Wad Medani. Evaluation for bitterness in mature plants was done by testing a small part of a mature leaf blade, petiole, stem, and fruit. Dry seeds were evaluated after removal of the seed coat. Germinated seeds were evaluated by testing the unfolding cotyledons. Seedlings were evaluated at the cotyledon, first, and second leaf stages (5). Thin layer chromatography (TLC) was used to determine the bitter principle in bitter fruits.

When individually evaluating 2400 mature plants, it was observed that plants with bitter fruits also had bitter leaves and stems. This relationship can be exploited to select for non-bitter lines. Of the 2400 mature plants tested, 8 were highly bitter (0.3%), 14 were slightly bitter (0.6%), two relatively sweeter in taste (0.08%) and the rest had the normal, non-bitter taste. Non-bitterness has been reported to impart resistance to cucumber beetles (1,4,6) but it may result in susceptibility to other insects such as two spotted spider mites (2). A study of the inheritance of this trait is planned as it has been reported to be relatively simple (1,4,6).

In a nursery experiment in which approximately 1000 seedlings were evaluated individually by tasting the cotyledon, about 100 germinating plants (10%) were bitter. The same plants were evaluated a week later by tasting the first leaf and only five seedlings (0.5%) expressed bitterness. In another experiment in which plants were evaluated at the second leaf stage, 5 seedlings out of 450 (1.1%) were bitter. It has not been determined whether the seedlings will maintain the bitterness through fruiting. Thus it is suggested that evaluation be delayed at least until the first or second leaf stage.

One thousand of the dry seeds were evaluated individually and none gave the bitter taste. When another thousand seeds were germinated, 24 out of 230 (10%) germinating seeds had a bitter taste. It seems that the bitter principle develops after seed germination, soon reaches a maximum, followed by a substantial reduction at the first and second leaf stages and a gradual reduction up to maturity. The bitter principle was detected to be a triterpene (cucurbitacin).

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Search for Sources of Resistance to a Melon Dieback Disease in Spain

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In the Spanish Mediterranean coast a disease has appeared which brings about the sudden death of melon plants. In the Valencian area this disease has been associated with a ground fungus belonging to the *Acremonium* genus (2). This fungus implants itself in the plants in the early stages of development causing brownish discoloration and corky and necrotic areas in the roots and root collar (3). This deterioration of the root system brings about wilting and death of the plant a few days after hydric stress is reached. This usually occurs at first fruit set (3).

Inoculation with this fungus in hydroponic culture conditions produces root rot on melon seedlings (2). In a previous experiment (1) 45 melon accessions from the UPV germplasm bank were inoculated in this way, 35 of which were classified as susceptible, and the response of the other 10 was doubtful or variable.

Nine of these ten accessions together with another three were tested under outdoor field conditions at two locations in Valencia (Alcudia and Paiporta). In both places plants were grown in plots where the disease had previously been noted. In Paiporta, all accessions were also grown in a plot which had previously been disinfected with methyl bromide. The number of replicates per accession was three in Alcudia and two in Paiporta. The number of plants per replicate was five.

The objective of this experiment was to test up to what point the observed response when *Acremonium* sp. is inoculated in laboratory is a reflection of the incidence of this disease in field conditions. The accession Pat 82 acted as a control, given that it has already shown great susceptibility to *Acremonium* sp. in hydroponic culture. The subterranean part of the plants were carefully inspected for signs of root rot, brownish discoloration, and corky areas. The numbewr of wilted plants was also noted. On the basis of these characters we rated the severity of the attack using a point system (Table 1).

Wilting and sudden death of some plants were observed in Paiporta, but not in Alcudia, even though the roots were damaged in both places. Accessions Pat 46 and 81 showed little sign of disease (Table 1). In Pat 49 the symptoms were only apparent in the roots of the plants grown in Alcudia. Pat 39 and 22 showed distinct severity of the attack in both places. Plants of the accession Pat 80 grew well despit root damage in both Alcudia and Paiporta. The disease was substantial in the rest of the accessions at both places locations. However, in the plot which had been disinfected previous to planting (Paiporta), there were no signs of wilting or root damage. The presence of *Acremonium* sp. was confirmed in both the untreated plots.

At present, the inoculation of hydroponic cultivars does not seem to be an efficient way of selecting resistant sources. This could be due to the fact that other ground fungi, principally saprophytes, occupy the lesions produced by *Acremonium* sp. and cause more damage. So far we have been unable to find an accession which shows a clear resistance to *Acremonium* sp. in laboratory conditions. If we had one, we could confirm the usefulness of hydroponic inoculation and we would put forward more information about the part *Acremonium* sp. plays in the disease.

Table 1. Severity of melon dieback disease attack on accessions studied.

Accession	Root Appearance*					Number of withered plants	
	Alcudia replicates			Paiporta replicates		Paiporta replicates	
	1	2	3	1	2	1	2
Pat 46	0	1	1	1	1	0	0
Pat 81**	0	0	0	1	1	0	1
Pat 49**	2	3	2	1	1	0	0
Pat 39	1	2	1	4	4	5	4
Pat 22	3	3	3	1	2	0	0
Pat 80**	3	-	3	3	3	0	0
Pat 6	2	2	3	2	3	2	3
Pat 34	2	1	3	3	4	4	5
Pat 35	1	3	2	3	3	5	3
Pat 36	2	3	2	3	4	2	5
Pat 38	2	3	2	4	4	5	5
Pat 82***	3	3	3	4	-	2	-

* 0: Roots with no symptoms; 1: Slightly damaged roots; 2: Moderately damaged roots; 3: Strongly damaged roots; 4: Very strongly damaged roots.

** Accessions which were not tested by means of hydroponic culture.

*** The replicate 2 of Pat 200 in Paiporta was of 2 plants only.

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Acknowledgments

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Screening of *Cucumis melo* Parental, F_1, F_2, and Backcross Population for Determination of the Genetic Basis of Tolerance to *Myrothecium roridum*

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Detached leaf inoculation assays by Kuti and Ng (2) of fifty melon cultigens revealed no source of resistance to *Myrothecium roridum*. However, in assays performed using both spore and roridin E inoculations there was a differential response between cultigens. Inheritance studies using diallel analysis indicated that tolerance or susceptibility was not simply inherited and there were no maternal effects (3). Greenhouse experiments were critically designed to further examine the mode of inheritance of tolerance to *M. roridum* in melon.

Parental, F_1, F_2, and backcross populations: Four melon populations consisting of the parents, F_1, F_2, and backcrosses to both parents were derived from crosses of 'Perlita' x 'Iroquois', 'Perlita' x 'Hales Best', and from 'Hales Best' x 'Iroquois' and its reciprocal. The parents were crossed in the University of Maryland greenhouses in the winter of 1987-88 and the F_2, BC_1, and BC_2 crosses made in the winter of 1988-89. Parental, F_1, F_2 and backcross populations were seeded in square 9.2 cm pots in a commercial soilless mix (7.4 peat: 2.5 perlite: 1 vermiculite, v:v:v) in the late winter and spring of 1990. Each population consisted of 10 plants of each parent and F_1, 200 plants of each F_2, and 100 plants of each backcross.

Fungal culture: *Myrothecium roridum* (ATCC# 52485) was originally isolated from diseased melon fruit in Texas (1). Cultures were obtained from Dr. George Bean, Department of Botany University of Maryland, and maintained on PDA. Inoculum was obtained by placing mycelial plugs on fresh PDA and incubating under 16 hr photoperiods at 25° C. When sporulation occurred cultures were placed in the dark at 4°C until used. Spore suspensions were made by washing 2 or more plates with ddH_2O and standardized to 10^6 spores per ml of distilled water.

Detached leaf inoculation: Leaf inoculations were performed when the plants had approximately ten true leaves. Expanded leaves approximately 9 cm in diameter were excised from the fourth or fifth node, washed with sterile distilled water three times, and placed in petri dishes lined with sterile moistened filter paper (2 ml sterile distilled water). Each leaf was inoculated with 25 µl of spore suspension in an interveinal area and then covered with a moistened filter paper disc, 10 mm in diameter, for 24 hr. Inoculated leaves were incubated in a growth chamber at 25±1°C for seven days. Host reaction was determined by measuring necrotic lesion diameter and necrotic plus chlorotic lesion diameter.

Results and Discussion: No conclusions can be made about the genetics of inheritance of melon resistance to *M. roridum* from these studies. Typical results are shown in Fig. 1. The backcross populations in each cross had similar distributions within each inoculation for both necrotic and necrotic plus chlorotic lesion measurements. In general there was no clear difference between the parent populations. In one case 'Hales Best' and 'Iroquois' had opposite responses for the two inoculation dates. The F_1 and F_2 populations generally had the same distribution of individuals, although the small number of F_1 individuals made comparison difficult. The amount of time between inoculation dates may be important in obtaining repeatability of results. The inoculation two days apart (5/23 and 5/25) had very similar population responses while other inoculations with greater time intervals had less similar responses. However, there was insufficient data to test this hypothesis.

Studies with *Myrothecium roridum* have shown that host reaction can vary with changes in the host plant nutrition and growing conditions (4,5). Kuti (personal communication) indicated that the pathogenicity of the melon isolate of M. roridum is affected by host factors such as age, position of leaf on the plant, and general health of the plant. Host reaction to *Myrothecium roridum* may have been influenced by the presence of a virulent strain of powdery mildew in the greenhouses; a number of leaves were discarded due to obvious infestation of the leaves with powdery mildew shortly after inoculation with *M. roridum*. Powdery mildew appeared to suppress the progress of *M. roridum* on those leaves that were heavily infested. However, since these leaves were often discarded before the end of the incubation period no data was collected.

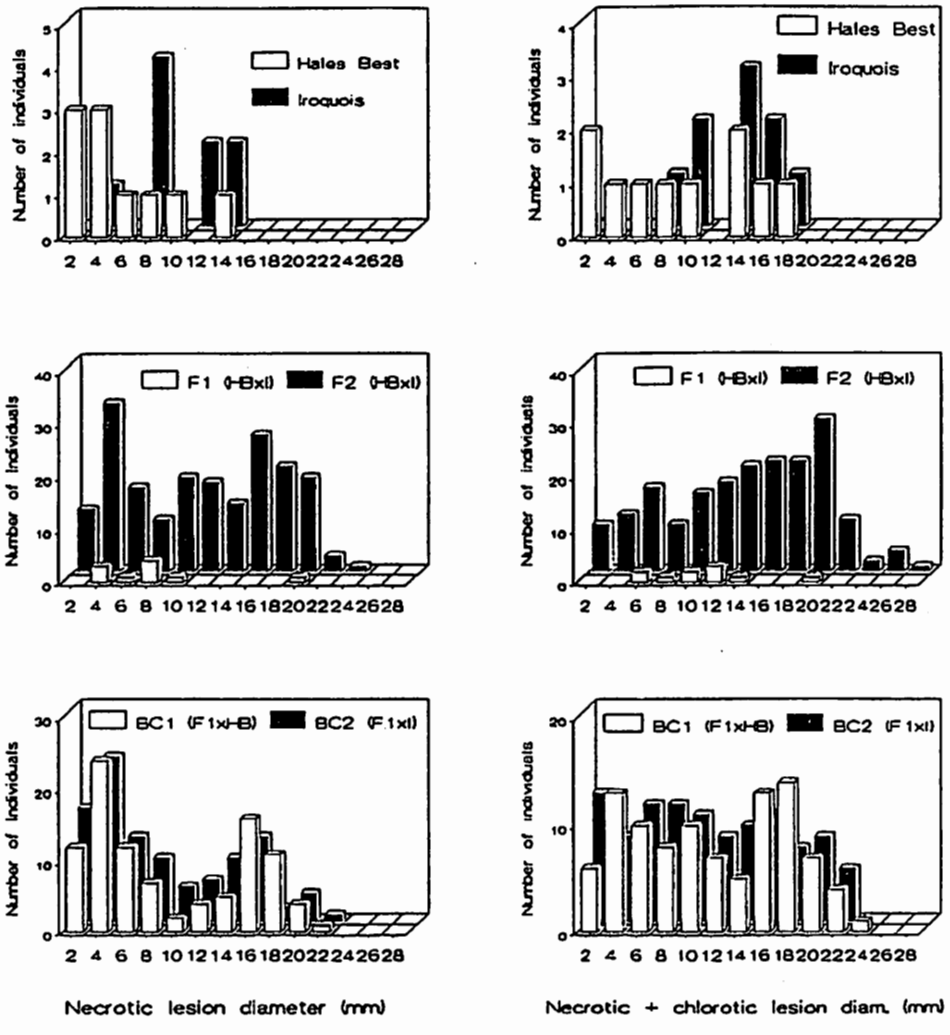

Figure 1. Frequency distribution of detached leaf lesion diameters for parental, F_1, F_2, and backcross populations of the cross 'Hales Best' x 'Iroquois' inoculated with Myrothecium roridum.

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Preliminary Screening of Melons for Sweetpotato Whitefly Resistance

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Lettuce infectious yellows (LIY), a sweetpotato whitefly, *Bemisia tabaci* Genn., (SPWF) transmitted virus disease of melons, has been a problem in fall melon production in the lower desert valleys of California, Arizona and Mexico from 1978 through 1990 (3). In 1990, a new biotype of SPWF was found in these areas. The new biotype, designated the poinsettia (SPWF-P) or IV-90 biotype, differed from the old biotype, designated the cotton (SPWF-C) or IV-81 biotype, by several significant characters including: broader host range, shorter life cycle, i.e. number of days from egg-to-egg, ability to induce silverleaf of squash and inability to transmit LIYV (1,4,6). By fall 1991, SPWF-P appeared to have completely replaced SPWF-C (1). In addition, the increased host range, shorter life cycle, and perhaps a favorable environment resulted in unprecedented numbers of SPWF-P that virtually destroyed the Arizona and California fall 1991 melon crop (2). Imperial Valley reportedly suffered $107 million in crop losses to SPWF-P (5).

The initial objective of the present study was to evaluate *agrestis* type melons for LIYV resistance. The changes in the SPWF population resulted, however, in a virtual absence of LIYV in the fall melons. The large numbers of SPWF and their devastating effect on melon plant growth presented an opportunity to evaluate these melons for resistance to SPWF feeding and reproduction.

A field screening test of 151 *agrestis* melon PIs was planted at the USDA-ARS, Irrigated Desert Research Station, Brawley on 22 August 1991. Plots were 8.4 m in length on 2 m centers and consisted of 10 hills spaced 76 cm apart; there were two replications. Two seeds were planted per hill; seedlings were thinned to one per hill at the first true leaf stage of growth. LIYV infection was determined by ELISA (3). Plant size and plant condition were evaluated on a plot basis on 22 October; 61 days post-planting.

The PIs were heavily infested with SPWF, plants were stunted, and ELISA samples were negative for LIYV. No data on LIYV resistance were obtained, but preliminary data on resistance to SPWF were obtained. There were significant differences among the PIs for plant size, and plant condition in response to SPWF (Table 1). Melon PIs are known to be variable for many plant characters because the original collection was a mixture, or because of subsequent contamination or cross-pollination during seed increase. For this reason and because these evaluations were done on a plot basis, the mean values presented do not show the true potential of these PIs for resistance to SPWF-P. For example, PI 164825 had a mean rating of 3.5 for size and for condition, but one plant of the 15 plants observed in the two replications was approximately 3-4 times larger than the other PI 164825 plants and was by far the largest plant in the field. Despite this difference in size within PI 164825, the plants were remarkably alike for other vegetative characters. This large PI 164825 plant was crossed with two lines that were growing in the greenhouse at Salinas: WMR 29 and I 5 a line from Bulgaria that carries the *ms*-4 gene. PI 124550 is another example. It had mean ratings of 4.5 for size and condition, but was rated 6 in one replication for both characters and rated 3 for both characters in the other replication. No crosses were obtained using this PI. Nineteen PIs had one or more plants in one or both replications that were more vigorous than most of the plants in the field; these PIs (marked with * in Table 1) should be investigated further for SPWF-P resistance.

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Table 1. Mean plant size[z] and mean plant condition[y] of 151 wild melon Plant Introductions (PI) from India.

PI	Size	Cond.	PI	Size	Cond.
116479	3.0	3.0	123504	3.5	3.5
116482	3.0	4.5	123505	2.5	2.0
116487	2.0	2.5	123517	3.0	3.0
116489	2.5	3.5	123680	3.0	3.5
116490	3.5	3.0	123681	3.0	3.5
116659	3.0	2.5	123682*	2.5	4.0
116660	4.0	5.0	123683	3.0	3.0
116661	2.5	3.0	123685	2.5	2.5
116664	3.0	3.5	123687	3.0	3.0
116667	2.5	2.5	123689	3.0	3.5
116736	3.0	2.5	123821	3.0	3.0
116738	2.5	2.5	123822*	3.5	4.0
116824	3.0	2.5	123823	3.0	3.2
116826	3.0	3.5	123824	2.5	3.5
116827	3.0	3.5	124092*	3.5	3.5
116828	3.5	4.0	124096	2.5	2.5
118584	2.0	2.5	124098	2.5	3.5
122847	4.0	3.5	124100	3.5	3.0
123187	2.5	2.5	124101	2.5	2.5
123188	3.0	3.0	124102	2.5	3.0
123493	2.0	2.5	124103*	3.0	3.5
123494	2.5	3.0	124106*	1.5	2.5
123495	2.5	2.5	124107	3.5	4.0
123496	2.5	2.5	124108	2.5	3.0
123498	2.5	3.0	124109*	3.0	4.0
123499	2.0	3.0	124111	2.5	2.5
123500	3.5	3.5	124112	1.5	2.0
123501	2.5	3.5	124113	3.5	3.0
123502	3.5	4.5			

continued

Table 1. (continued)

PI	Size	Cond.	PI	Size	Cond.
124114	3.0	3.5	164585	3.0	3.0
124206	3.0	3.0	164586	3.0	3.0
124208	2.5	3.0	164617	3.0	3.0
124210	2.5	2.5	164635	3.0	3.0
124214	2.5	2.5	164653	2.5	3.0
124429	2.0	2.0	164654	3.0	3.0
124431*	3.5	3.5	164666	2.5	2.5
124436	3.0	3.5	164720	3.0	3.0
124439	3.0	3.0	164723	3.5	3.0
124440*	2.5	2.0	164749*	3.5	3.5
124441	2.0	2.5	164750	2.5	2.5
124443	2.5	2.5	164756	2.5	2.5
124447*	2.0	3.0	164794	3.0	2.8
124449	2.5	3.0	164795	3.0	3.0
124550*	4.5	4.5	164815	3.0	3.5
124552	2.5	3.5	164821	3.0	3.0
124553	4.0	3.5	164825*	3.5	3.5
134196	2.5	3.0	164826	2.0	2.5
134199	2.5	3.0	164856	2.5	3.0
134200	3.0	3.5	165508	2.5	3.0
145594*	4.5	5.0	165513	3.0	3.5
163206	2.0	3.5	165514	2.5	3.0
163208	3.0	3.0	165515*	3.0	3.5
163211	2.5	3.5	165516	3.0	2.8
163212	3.0	3.5	165525*	2.5	3.0
163219	2.5	3.0	166125	1.0	0.8
163220	3.0	3.0	173891	2.0	2.5
164179	2.0	3.0	174814	3.5	3.5
164270	2.5	3.0	174815	3.0	2.8
164320	3.0	3.5	175109	1.5	2.0
164323	3.0	3.2	179666	2.5	2.0
164327	2.5	2.5	179668	2.5	2.5
164329	2.5	2.5	179669*	2.5	2.0
164330	2.5	3.0	179670	3.0	3.0
164331	2.5	3.0	179671*	3.5	2.5
164343	3.0	3.5	179672	2.5	3.5
164400	2.0	3.2	179674	2.0	2.0
164409	3.0	3.5	179677	2.5	2.5
164466	2.5	2.5	179888	2.0	2.5
164479	2.0	2.0	179890*	2.5	2.5
164481	2.0	2.5	179891	3.0	3.0
164487	2.0	2.0	179892	2.5	3.0
164492	2.5	2.5	179893*	3.5	3.5
164493	1.0	2.0	179894	3.0	2.5
164551	3.5	4.0	179895	2.5	3.0
164569	2.5	3.0	179896	3.0	3.0
164583	2.5	2.5	179899	2.5	2.5

[z]Size was rated on a 1 (very small, only a few true leaves) to 9 (completely covering the bed) scale.

[y]Condition was rated on a 1 (dead) to 9 (vigorous, flowers) scale.

*PIs that should be investigated further for SPWF-P resistance.

In Vitro Regeneration in *Cucumis melo* cv. Pusa Madhuras

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Introduction. Most of the cultivated forms of *Cucumis melo* L., such as Pusa Madhuras, are highly susceptible to field conditions at young stage of vegetative growth. Field conditions, namely the biotic factors like Fusarium wilt caused by *Fusarium solani* and cucumber green mottle mosaic virus (CGMMV) are responsible for eradication of cultivated forms (1,2). Thus, it becomes essential to incorporate resistance in cultivated forms through non-conventional means of somaclonal variation, somatic hybridization, or protoplast fusion. Conventional means of interspecific hybridization has strong barriers (3).

Present study is a step towards standardization of tissue culture cycle of various explants of Pusa Madhuras and regeneration of plants in vitro, followed by screening under field conditions against CGMMV.

Materials and Methods. In Pusa Madhuras of *Cucumis melo* L., seeds were surface sterilized with 0.1% mercuric chloride for 7 min. and were germinated under sterile conditions. Explants such as cotyledonary leaves and epicotyl were excised from 9-day-old seedlings and inoculated onto two kinds of basal media such as Nitsch and Murashige and Skoog. De-differentiation medium and differentiation medium were standardized for various hormonal combinations and concentrations. Regenerated plants were screened for CGMMV resistance under natural field conditions.

Results. De-differentiation: Two kinds of explants namely epicotyl and cotyledonary leaves were tried for differentiation. Epicotyl explants with an apical meristem lead to direct regeneration of shoot. Cotyledonary explants exhibit callus induced regeneration of shoot buds and axillary bud induced direct regeneration.

Basal medium, Nitsch, had no effect on epicotyl explants but induced 49.01 ± 3.40% (Table 1) of direct regeneration of shoot(s) and roots in cotyledonary leaves explants.

Murashige and Skoog (MS) basal medium favored 92.00 ± 2.10% callus formation in epicotyl explants. Addition of benzyl amino-purine (BAP) 0.5 mg/1 induced shoot buds in 40.47 ± 4.12% of calli and favored callus formation in 66.66 ± 22.05% of cotyledonary leaf explants. Addition of GA_3 (0.5 mg/1) to MS basal medium also favored callus induced shoot buds formation in 46.66 ± 5.77 percent of epicotyl explants. Addition of zeatin (0.5 mg/1) to MS favored root formation in both epicotyl and cotyledonary explants.

Differentiation medium: Addition of GA_3 (0.5 mg/1) to MS medium induced 41.66 ± 7.21% (Table 2) of shoot buds in MS + 0.5 mg/1 BAP induced callus. Addition of zeatin (0.5 mg/1) + BAP (0.5 mg/1) to MS medium (MBZ) had regenerative effect on MB obtained cotyledonary leaf explant callus. Regeneration of shoot buds was of the order of 0.84 ± 0.25% of callus induced shoot buds (somatic embryoids), 4.20 ± 1.47 percent of direct initiation of shoot(s), 10.08 ± 2.50% of initiation of leaves and shoot elongation.

On the other hand, addition of IAA (1 mg/1) + Kinetin (5 mg/1) to MS medium (MIK) had induction of direct shoot(s) and root(s) in 66.66 ± 7.21% of epitocyl explants and 16.66 ± 8.86 percent of cotyledonary leaf explants.

In conclusion, hormones such as IAA (1.0 mg/1) + Kinetin (5.0 mg/1) with MS basal medium are highly effective in inducing regeneration of shoot buds in epicotyl explant callus obtained on MS + BAP (0.5 mg/1) medium, whereas GA_3 (0.5 mg/1) with MS basal medium is effective for inducing regeneration in cotyledonary leaf explant callus obtained on MS + BAP (0.5 mg/1) medium (Table 3).

Table 1. Effect of various hormones on de-differentiation and differentiation of various explants of Cucumis melo cv. Pusa Madhuras.

Basal Media	Hormonal Supplement	Explant	Callus + Regeneration: Callus Formation (%)	Callus + Regeneration: Callus + Shoot Buds (%)	Callus + Regeneration: Callus + Root (%)	Direct regeneration: Initiation of Root (%)	Direct regeneration: Initiation of Shoot + Root (%)
Nitsch	-	Epicot.	0	0	0	0	0
		Cot. leaf	15.68 ± 3.39	0	0	19.60 ± 3.39	49.01 ± 3.40
MS	-	Epicot.	92.00 ± 2.10	0	0	0	0
		Cot. leaf	18.36 ± 1.32	0	0	0	0
MS	BAP (0.5 mg/l)	Epicot.	9.52 ± 4.12	40.47 ± 4.12	0	0	0
		Cot. leaf	66.66 ± 22.05	0	0	0	0
MS	GA_3 (0.5 mg/l)	Epicot.	0	46.66 ± 5.77	0	0	0
		Cot. leaf	0	0	0	0	0
MS	Zeatin (0.5 mg/l)	Epicot.	0	0	7.1 ± 0.0	46.42 ± 3.21	0
		Cot. leaf	0	0	0	55.55 ± 3.21	0

Table 2. Effect of various hormones on differentiation of MB (MS + 0.5 mg/l BAP) generated callus of various explants of Pusa Madhuras.

De-differentiation Medium	Differentiation Medium	Explant	No Change	Callus + Shoot Buds	Callus + Shoot	Callus + Root	Shoot Buds + Root	Shoot Elongation + Leaf Formation
MS+BAP(0.5 mg/l) (MB)	MS+GA_3(0.5 mg/l) (MGA_3)	Epicot.	75.00±35.35	0	0	0	0	0
		Cot. leaf	58.33±7.21	41.66±7.21	0	0	0	0
	MS+Zeatin+BAP(0.5 mg/l)(0.5 mg/l) (MBZ)	Epicot.	37.30±1.06	0	0	0	0	0
		Cot. leaf	5.88±1.41	0.84±0.25	4.20±1.47	0	0	10.08±2.50
	MS+IAA+Kinetin(1 mg/l)(5 mg/l) (MIK)	Epicot.	33.33±7.21	0	0	0	66.66±7.21	0
		Cot. leaf	83.33±28.86	0	0	0	16.66±8.86	0

Table 3. Summation of effect of hormones on differentiation and de-differentiation in Pusa Madhuras.

De-differentiation Medium	Differentiation Medium (mg/l)	Explant	Regeneration (%)	Callus Formation (%)	No Response (%)
MS+BAP(0.5 mg/l)	-	Epicot.	40.47 ± 4.12	9.52 ± 4.12	50.00
		Cot. leaf	0	66.66 ± 22.05	33.33
MS+GA_3(0.5 mg/l)	-	Epicot.	0	46.66 ± 5.77	53.84
		Cot. leaf	0	0	0
MS+Zeatin(0.5 mg/l)	-	Epicot.	*46.42 ± 3.21(Rooting)	0	46.43
		Cot. leaf	*55.55 ± 3.21(Rooting)	0	44.44
	MB → MGA_3(0.5)	Epicot.	0	75.00 ± 35.35	25.00
		Cot. leaf	*41.66 ± 7.21	58.33 ± 7.21	0
	MB → MS+BA+Zea(0.5)(0.5)	Epicot.	0	37.30 ± 1.06	62.69
		Cot. leaf	15.12 ± 1.74	5.88 ± 1.41	78.99
	MB → MS+IAA+Kin(1.0)(5.0)	Epicot.	*66.66 ± 7.21	33.33 ± 7.21	0
		Cot. leaf	16.66 ± 8.86	83.33 ± 28.86	0

CGC 15:63 (1992)

Further, MIK generated plants hardened for roots on MS + 0.1 mg/l NAA (MN) medium, could survive in sterilized soil for 15-20 days. Appearance of one to two new leaves was noticed in regenerated plants in soil and the plants were found to be sensitive to CGMMV.

Discussion. Pusa Madhuras, a cultivated variety of *Cucumis melo* L. has been found to be responsive to in vitro regeneration. Similar studies have also been reported in other species and varieties of *Cucumis melo* (4,5,6,7,8,9). Somatic embryogenesis has also been noticed in 'Pinyonet Piel de Sapo', 'Amarillo Canario' and 'Amarillo Golda' of *Cucumis melo* (10,11) and in Pusa Madhuras (12).

Regenerated plants resistant to CGMMV and isolated as somaclonal variant would be of immense use in future disease resistance programs.

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An In Vitro Selection Method for a Melon Variety which Regenerates by Direct Organogenesis

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The Israeli F1 hybrid melon cv Galia is an important export crop. We wish to engineer this melon variety with coat protein mediated protection against Zucchini Yellow Mosaic Virus (ZYMV). We have cloned ZYMV (5), and identified and isolated the ZYMV coat protein gene (6). In devising a transformation system an important step is to identify a selection agent which is capable of blocking regeneration in non transformed tissues i.e. which lack an introduced resistance gene. In cucurbits the antibiotic kanamycin has been used successfully as a selection agent at concentrations of 25-100 mg/l (1,3,4,9). Here we report that the responses of cv Galia to kanamycin are atypical of published cucurbit selection methods.

Materials and Methods. Seeds of *Cucumis melo* L. cv Galia were pealed and surface sterilised in 1.2% solution of hypochlorite, 1 drop of Tween 20 per 100ml. Seeds were washed 4X with sterile water, and cut into 4 longitudinally through the embryo, each explant thus being half of a cotyledon with attached embryo fragment. The primary explants were then plated onto autoclaved Murashige and Skoog (8) medium, with 3% sucrose, 8-10 g/l agar, and 1 mg/l benzyl adenine (MSBA1 medium). Plant material was incubated in a growth room at 26 C in continuous light. Explants were transferred to fresh MSBA1 medium with kanamycin at periods up to 7d after primary explant preparation. In a second set of experiments explants were cut in pieces prior to transfer to kanamycin-containing MSBA1 at 5d old (Table 2). Regeneration was scored as the % of explants with shoots or shoot buds after 30d total in culture.

Results and Discussion. The response to kanamycin was independent of age for exposures to kanamycin beginning up to 7d of age, for the concentration range 100-250 mg/l (data not shown). The effect of kanamycin on regeneration also appeared to be independent of concentration within that range (Table 1). Clearly kanamycin has a small effect (17%) in reducing regeneration - statistically significant, but inadequate for a selection system.

However, in a second series of experiments, the antibiotic had a powerful effect when explants were cut into fragments on transfer to MSBA1 with kanamycin (Table 2). This technique is the basis of our selection system for genetic transformation of melon cv Galia.

The recalcitrance of "Galia" melon to kanamycin is probably due to anatomy of regeneration in this variety. The published cucurbit transformation systems (1,3,4,9) all feature organogenesis or embryogenesis via a callus phase or on the edge of the explant, all directly exposed to the selection agent. "Galia", however, regenerates by direct organogenesis across the surface of the explant (2; Gaba, unpublished). The developing buds are therefore protected from the antibiotic by a mass of cotyledon tissue. Such protection is obviated by reducing the size of the explant (Table 2). This technique will be useful for a selection method for the transformation of other cucurbit varieties which regenerate directly on the cotyledon surface, such as some cucumber cultivars (7).

Table 1. Kanamycin has little effect on regeneration of explants of melon cv Galia. All treatments for transfer to kanamycin at ages 0-7d have been bulked. Data from 7 separate experiments.

Kanamycin concentration (mg/l)	% regeneration	Standard error of mean	Number of experiments n
0	87	2	7
100	75	3	6
150	77	8	4
200	73	6	3
250	65	11	3

Table 2. Kanamycin has a strong effect when explant size is reduced. Primary explants were cut into fragments on tranfer to MSBA1 with 250 mg/l kanamycin, after 5d on MSBA1. Minimum of 30 explants per treatment.

Primary explant	% of pieces regenerating	
	kanamycin	control
uncut	65	87
cut in 2	0	65
cut in 4	0	48
cut in 8	0	27

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Cold Resistance in Accessions of Watermelon from Zimbabwe

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Watermelon seedlings grown at low temperatures (< 20 C) usually develop a foliar mottle and stunting. Cotyledons appear to be whitish green, while the first leaves develop a 'mosaic', consisting of scattered irregular white-yellow patches. A persistent low temperature is conducive to more prominent foliar symptoms, malformation, and growth retardation. Varietal reaction, however, is also an important factor, hence, some cultivars appear more affected than others by this disorder. Due to this sensitivity, temperature is a major factor in determining watermelon planting dates in warm, as well as temperate regions of the world.

During the winter of 1988, while analyzing for viral resistance a collection of 57 plant introductions (PI's) of *Citrullus lanatus* from Zimbabwe (3,4), we noted that plants of a large number of these lines (67%) were prominently affected by mosaic-like symptoms. Under the same conditions, plants of the remaining lines (33%) developed normal growth. To confirm the apparent resistance to cold temperature (15-20 C), which prevailed at the beginning of our tests, additional seedlings of the same collection and domestic cultivars were exposed to two sets of temperatures, using greenhouses and growth chambers. At 28-30 C, plants of every line grew normally, but at 15-20 C, those of 38 PI lines and 15 domestic and foreign cultivars developed varying degrees of foliar variegation, malformation, and stunting. Under the same conditions, 19 PI lines yielded mostly normal plants.

The availability of cold-resistant watermelons offered the opportunity to elucidate simultaneously the genetics of the mosaic-like disorder and cold resistance. Inheritance studies were based on crosses between the cold-sensitive cultivar New Hampshire Midget (NHM) with the cold-resistant line PP261-1. This was a single-plant selection from PI 482261, which we previously reported to be resistant to the Florida strain of zucchini yellow mosaic virus (ZYMV-FL), and possessing the gene for the isozyme *Pgi-2b* (1,3). Using similar genetic populations we had also found that the resistance to ZYMV-FL in PP261-1 was conferred by the single recessive gene *zym*, not linked to *Pgi-2b* (3). Cold tests were conducted in both an insect-free greenhouse and a growth chamber at 15-20 C. Although complete data will be presented at a later date, our findings can be summarized as follows:

a) All plants of F_1(PP266-1 x NHM) and F_1(NHM x PP261) were normal.

b) Resulting F_2 plants of these crosses segregated in a ratio of 3 normal: 1 cold-sensitive.

c) Plants of F_1 x NHM were all cold-sensitive, whereas those of F_1 x PP261-1 segregated 1 normal: 1cold sensitive.

d) There was no detectable linkage between the factor for cold resistance and *Pgi-2b* or *zym*. However, *Pgi-2b* (= $Pgi\text{-}2^2$) (2) was a valuable marker in determining whether some of the populations resulted from true crosses.

From our findings, it is evident that the mosaic or leaf variegation affecting many watermelon lines is not cytoplasmically inherited. This disorder is induced by the expression of a single

recessive nuclear gene, which is activated by temperatures below 20 C. In the absence of such a factor, plant growth is unaffected by temperature. Because this cold-resistance is inherited dominantly, it can be easily exploited for the development of commercial F_1 hybrids.

The watermelon collection from Zimbabwe offers a range of plant sizes, leaf types, fruit characteristics, and maturity. Most of the cold-resistant lines can be considered as primitive forms (landraces) of cultivated watermelon. They develop very large and vigorous plants, producing fruits with a firm yellowish flesh, and low sugar content. In Zimbabwe, these fruits are used mostly for cooking and for animal feeding. Due to their unusual firmness and low sugar, these fruits can be stored for many months. Young plants of these lines can be used as rootstocks, because of their well developed root system and apparent resistance to soil-borne diseases.

The introduction of cold resistance into commercial cultivars can have great economic advantage for the production of early crops. They could be planted one or two months before the usual planting dates, particularly where heavy frost occurs rarely. Although none of the cold resistant lines is frost-resistant, in two consecutive years, they were only moderately damaged by the first heavy frosts in western New York.

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Blossom-end Rot (BER) and Cracking in Watermelon *Citrullus Lanatus* (Thunb.) Matsum & Nakai)

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Introduction. Watermelon requires light soils for successful production. In heavy clays it faces many problems such as small size, fruit cracking and blossom-end rot (BER). To exploit the possibility for its production in the vast clays of central Sudan, varieties that are resistant to BER and cracking are required.

Materials and Methods. A variety trial was conducted at the University of Gezira, Wad Medani, Sudan in July to October 1987. Soil type of the site was heavy clays representing those of the Gezira area. The experiment layout was a randomized complete block design with four replicates. The trial included four elongated varieties and four round. The elongated varieties were 'Charleston gray', 'Charleston 76', 'Congo', and 'Fair fax'. The round varieties were 'Crimson sweet', Sugar baby' and the local collections 'HSD0177' and HSD0319'. The in-row and between row spacings were 60 cm and 3 meters respectively. Plot size was 6 X 7 m. Observations on percentage of fruits with BER and cracked fruits per plot were recorded.

Results and Discussion:

Table 1. Percentage of fruits with blossom-end rot (BER) and percentage of cracking fruits in different varieties.

Variety	Fruit Shape	Percentage of BER[z]		Percentage cracking[z]	
HSD0177	Round	01.8	d	04.8	d
Sugar baby	Round	07.4	d	22.7	bc
HSD0319	Round	08.5	d	08.3	cd
Crimson sweet	Round	31.6	c	45.3	a
Congo	Long	38.4	bc	24.1	b
Charleston 76	Long	40.4	bc	22.9	a
Fair fax	Long	44.9	ab	11.0	bcd
Charleston gray	Long	53.1	a	18.7	bcd

[z]Means with the same letter(s) are not significantly different at (P=0.001). S.E. for BER=±3.9 and S.E. for cracking =±4.6. Data was subjected to angular transformation.

Varieties tested showed marked differences in BER and cracking, although the percentages of BER were generally higher. Those results support previous findings (1,3) that spherical cultivars usually posses a complete or high resistance to BER and cracking. The round local collections were much better than the introduced round varieties in yield and resistance to cracking and BER. However, the local collections had a relatively lower sucrose percentage and a larger number of seeds per fruit. The round variety Crimson sweet marked susceptibility to cracking and BER was not in agreement with the mentioned established fact about round varieties being resistant.

It has been suggested that the resistance to BER in watermelon is inherited as a monogenic trait (2). Thus a breeding program aiming towards combining the desirable characters of the introduced varieties with the resistance to BER and cracking present in the local collections will be very useful for improvement of watermelon production in the heavy clays of central Sudan.

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Phenotypic Variation of the Spotted Trait in Watermelon

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In many North American localities, a watermelon variant called 'Sun, Moon, and Stars' or 'Moon and Stars' has been maintained by home gardeners. Its name is derived from the yellow spots which form on its stems, leaves and fruit. These spots are noticeable in some cases on the cotyledons. It does not affect flesh color.

Spotted cultivars have generally been maintained locally by individual growers. In 1989, 18 different spotted cultivars were obtained from growers in the states of Alabama, California, Indiana, Louisiana, Michigan, Minnesota, Missouri, North Carolina, Ohio, Pennsylvania and Tennessee.

It was found that pattern of spotting was consistent within each cultivar. Some cultivars had mostly medium (2 - 6 cm) and large (6 - 15 cm) spots while other cultivars had only small (< 2 cm) spots. Some cultivars had numerous spots while others had very few spots. Several cultivars were unstable for inheritance of the spotting character and segregated for presence of spotting, size of spotting, and intensity of spotting. Segregates from one such cultivar are pictured in Figure 1.

It appears that more than one genetic mechanism is responsible for the spotting found in 'Sun, Moon, Stars' and 'Moon and Stars' cultivars.

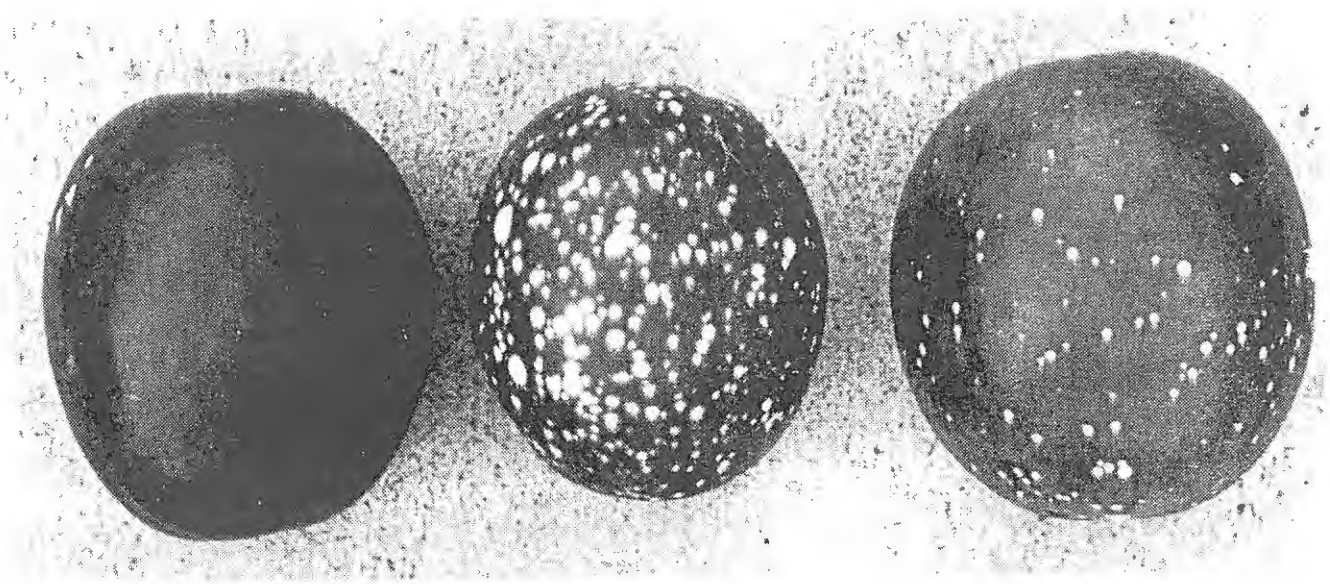

Fig. 1. Segregates from unstable spotted cultivar.

Segregation Data Suggest Male Sterility Gene *ms* and the Spotted Gene *Sp* in Watermelon Are Not in the Same Linkage Group

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Conflicting genetic explanations have been reported for the trait in watermelon characterized by varying sized yellow spots located on both leaves and fruit. A report in 1944 involving the variety 'Sun, Moon and Stars' concluded that the spotted condition was due to a chloroplast deficiency situated in the cell cytoplasm and was not due any nuclear gene (1). A report in 1986 involving a spotted variant labeled 'Moon and Stars' stated that the spotted condition was controlled by a dominant gene which was therein designated *Sp* (2). The pubescent male sterile trait in watermelon has previously been reported as the expression of the single recessive gene *ms* (3).

Male sterile segregates were crossed with two different spotted cultivars, 'Moon and Stars Yellow Flesh' and 'Moon and Stars Red Flesh'. All F1 plants were spotted. Self pollinations were performed on one spotted plant from both F1 populations. F2 segregation ratios are presented in Tables 1 and 2.

Table 1. F2 segregation ratios of the cross of a male sterile segregate of red flesh breeding line 'CRM.28' (*ms ms sp sp*) x 'Moon and Stars' Yellow Flesh (*Ms Ms Sp Sp*)

Phenotypic class	Putative genotype	Number of plants	Expected if 9:3:3:1 segregation
Fertile spotted	(Ms _ Sp_)	22	27.56
Ferile non-spotted	(Ms _ sp sp)	12	9.19
Sterile spotted	(ms ms Sp _)	8	9.19
Sterile non-spotted	(ms ms sp sp)	7	3.06
		Chi-Square = 7.815; 3 d.f. P > 0.05	

Table 2. F2 segregation ratios of the cross of a male sterile segregate of red flesh breeding line 'DJB.29' (*ms ms sp sp*) with 'Moon and Stars' Red Flesh (*Ms Ms Sp Sp*)

Phenotypic class	Putative genotype	Number of plants	Expected if 9:3:3:1 segregation
Fertile spotted	(Ms _ Sp_)	97	89.44
Ferile non-spotted	(Ms _ sp sp)	31	29.81
Sterile spotted	(ms ms Sp _)	29	29.81
Sterile non-spotted	(ms ms sp sp)	2	9.94
		Chi-Square = 7.04; 3 d.f. P > 0.05	

These results verify dominant nuclear inheritence of the *Sp* gene from 'Moon and Stars' and independent segregation of *Sp* and *ms*.

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The effect of the *branch less* gene *b l* on plant morphology in watermelon

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The branch less variant in watermelon (1) was discovered by Xu Liguan in a watermelon field at Shihezi in Xinjiang Province, PRC, in the late 1960s and named *branch less*. The verb and adverb aptly describe this variant that branches less than the normal phenotype. Li Shengxion used it as a parent to breed a new variety, 'Early Branch Less', a monoecious variety with a dark green fruit. We used this variety to study morphology and genetics of the natural variant. This variant has not previously been reported (2).

Parents and progeny: The normally branched breeding line, S173, was the female parent (P_1). The male parent (P_2) is 'Early Branch Less' (EB). Progeny included the F_1 (P_1 x P_2), the F_2 (F_1 selfed), BC_1 (F_1 x P_1), BC_2 (F_1 x P_2). These progeny were generated in Xinjiang Changji Horticultural Farm in 1989-1990.

Field Plots: Seed were planted in the greenhouse at Clemson University on May 5 and transplanted to the field at the Edisto Research and Education Center at Blackville on May 20. Transplants were 5 feet apart on the row and 5 feet apart between rows. Parents and progeny were arranged by order without replication. Fertilizer and spray applications were uniform over the entire field plot area. Number of branches, main stem length, main stem diameter and leaf length and leaf diameter were recorded on June 19, July 3, and July 9.

Results: The morphology of the *branch less* phenotype is compared with the normal phenotype in Table 1.

Table 1. The morphology of EB (P_2), normal line S173 (P_1) and F_1 (P_2 x P_1) hybrid plants in field plots at Blackville, SC in 1991.

	Main Stem			Leaf	
Genotype	Branch No.	Length, m	Diameter, cm	Length, cm	Width, cm
EB (P_2)	4.8±0.68	2.3±0.76	6.7±1.11	23.4±3.35	20.0±3.71
S173 (P_1)	10.9±3.56	2.5±0.50	4.9±0.70	19.5±2.70	16.0±2.04
F_1 (P_2xP_1)	14.5±3.50	3.17±0.71	6.4±0.88	22.5±1.19	18.4±1.06

Values are means of 15 plants ± standard error. The leaf measured was the 10th on the main stem.

Only half as many branches originated from nodes of the *branch less* phenotype as the normal phenotype (Table 1), and these branches originated at the first five nodes. No branches originated from the middle and top stems. The F_1 mean branch number suggests that the branches from the *branch less* genotype are different in origin from the branches of the normal genotype. Thus, the total number of branches on the F_1 is greater than, rather than less than, on the normal parent.

Segregation for number of branches in parents and progeny is given in Table 2. Chi-Square tests were used to determine the goodness of fit observed to hypothetical segregation ratios in the F_2 and BC populations.

Table 2. Branch less segregation in the F_2 and reciprocal backcross populations of the cross branch less x normal phenotype in field plots at the Edisto REC, Blackville, SC, 1991.

Generation	Observed			Ratio:	Chi-Square		
	total	normal	branchless	normal:branch less	Value[z]	df	p
P_1 (S173)	84	84	0	all normal	-	-	-
P_2 (EB)	90	0	90	all branch less	-	-	-
F_1 (P_1xP_2)	84	84	0	all normal	-	-	-
F_2 (F_1 self)	90	66	24	3:1	0.1037	1	0.75-0.50
BC_1 (F_1xP_1)	62	62	0	all normal	-	-	-
BC_2 (F_1xP_2)	66	29	37	1:1	0.9848	1	0.50-0.25

[z]Chi-Square$_{0.05}$ = 3.84

The F_1 plants were normally branched, the F_2 population segregated 3:1 and the BC_2 population segregated 1:1 for normal: branch less, respectively, as expected for single recessive gene control of the branch less character (Table 2). We suggest the symbols, *b l* , be used for the gene to describe the character *branch less*.

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Watermelon Variety Improvement in China

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Watermelons have been grown in China for more than 1000 years. Watermelon is one of the most important economic crops, grown in all parts of China except Qinghai, Tibet plateau and some very cold, northern regions. Watermelon acreages increased rapidly in the last ten years to 1,054,200 hectares in 1989. China is number one in watermelon production and consumption.

Because of the special importance of watermelon, China developed a very large watermelon breeding effort in the last few years. Many agricultural universities, colleges and institutes have watermelon breeding programs. The National Watermelon and Sweetmelon Association (NWSA) of the Chinese Society for Horticultural Science (CSHS) organizes at least one national/regional meeting on watermelon breeding and production each year. The CSHS has a journal called *Watermelon and Sweetmelon of China* edited by NWSA and Zhengzhou Pomology Institute, Chinese Academy of Agricultural Science. These provide a forum for watermelon breeders to present their research and to exchange ideas. We will provide an introduction to recent efforts made by Chinese watermelon research scientists toward watermelon variety improvement.

F_1 Hybrid Development. The advantages of F_1 hybrids of watermelon were realized in China in the late 1970s and early 1980s. Before that time, commercial watermelon varieties were open pollinated varieties developed in China (e.g., 'Zaohua', 'Xingchen Hong', 'Zhongyu 1' and 'Zhengzhou 3') and introduced by Japan (e.g., 'Asahi Yamato' and 'Miyaka') and the United States (e.g., 'Sugar Baby' and 'Charleston Gray') as well as local varieties. The first commercial F_1 may have been 'Xiangmi', which was released by Hunan Academy of Agricultural Science in 1973. However, the first popular F_1 hybrid widely grown in the 1980s was 'Xincheng'. 'Xincheng' was a cross between a selection of a Japanese variety (seed parent) and a selection of 'Charleston Gray'. An F_1 hybrid 'Xin Hong Bao' from Taiwan, which appears to be a cross between a round, light green fruit and a selection of a 'Charleston Gray' type with small seed, has been very popular since 1986. A national group was organized by the NWSA to develop F_1 hybrids of the 'Xin Hong Bao' type. Three F_1 hybrids, 'Luyuan 1', developed by GuangZhou Pomology Institute, Guangdong; 'Jubao 1', released by Hefei Watermelon Research Institute, Anhui; and 'Qihong', developed by Qiqihr Horticulture Institute, Heilongjiang, were recommended after three years' cooperative trials, to be the alternates for 'Xin Hong Bao'. More than sixty F_1 hybrids were released by Chinese breeders in the last few years. According to the NWSA, the six most popular commercial varieties in 1989 were all F_1 hybrids. The American varieties 'Charleston Gray', 'Jubilee', 'Crimson Sweet' and 'Sugarlee' are good parents for generating heterosis in Chinese hybrids.

Efforts were also made to investigate new genes which can be used for hybrid seed production and identification of true hybrids. Two different genic male sterile genes exist in China. The male sterile gene *ms* from G17AB(9) has been inserted into several desirable cultivars and breeding lines and has been used for hybrid seed production by researchers from Shengyang Agricultural Institute, Liaoning. Another male sterile which was found in Xinjiang is less utilized because of the extreme female sterility associated with the male sterility in the homozygous condition. The male sterile mutant , found from a gamma-irradiated line by Professor Jingyi Wu, may be a cytogenetic male sterile (personal communication). The gene *nl* , for nonlobed leaves (1), has been introduced into several genotypes to be used as a seedling marker for identification of true hybrids. Cultigens with elongate fruit are preferred as paternal parents so that true hybrids can be discerned by the shape of the ovary.

Isozyme markers for hybrid identification were investigated in Northwestern Agricultural University, Shaanxi, in 1985-1986 (8). More protein and isozyme markers related to hybrid identification are being assayed by researchers from Beijing Vegetable Research Center and Zhengzhou Pomology Institute, Chinese Academy of Agricultural Science.

Disease Resistance Breeding. Along with the rapid increase of watermelon production, diseases - especially the soil-borne disease, *Fusarium* wilt, has become a serious problem in areas where little crop rotation occurs. Even though anthracnose can reduce yield much more in some seasons and in some areas, *Fusarium* wilt is the most important disease in most areas. A national watermelon disease resistance breeding group consisting of breeders and plant pathologists was organized in 1985. Research confirms that *Fusarium* wilt pathogens in China are race 0 and race 1. Meanwhile, there is also evidence that a new race other than races 0 and 1 may also exist in China (10).

Watermelon disease resistance breeding ia a new subject in China. There is no germplasm with high resistance to *Fusarium* wilt in Chinese varieties (4). Germplasm introduced from Africa demonstrates great resistance, but are too primitive to be used directly (3). Some American varieties - 'Dixielee', 'Sugarlee', 'Charleston Gray 133', 'Crimson Sweet' and 'Calhoun Gray' - show moderate or high resistance depending on the host and the wilt isolate. Breeders are trying to use American germplasm to make resistant F_1 hybrids. However, genetic studies and breeding practices indicate that the inheritance of *Fusarium* wilt is much more complex than simple dominance and recessiveness. Results from Jiansu Academy of Agricultural Science indicate that resistance is determined by several recessive genes (7).

Anthracnose resistance has not been a specific project in many breeding programs. The Department of Horticulture, Northwestern Agricultural University has had a Master's Degree program for studies on watermelon anthracnose since 1986. Resistant germplasm has been identified. Pathogen differentiation studies are underway.

Watermelon fruit blotch is a new bacterial disease in China (5). Researchers from Qiqihr Horticulture Institute and Qiqihr Teachers University are working on pathogen identification and resistant germplasm screening.

Triploid Watermelon Development. Triploid watermelon production is mainly in Southeast China. Most of the seedless watermelons are marketed in Hong Kong. Many of the popular seedless watermelon cultivars are round, dark green fruit with red flesh. Only in the last few years have breeders become more interested in breeding for triploid hybrid production. Many more diploid than triploid hybrids have been released, but there will be more triploid varieties released in the coming years. Colchicine treatment has been the only method used by Chinese breeders for chromosome doubling. Yunhe Wang and Xingrong Cai reported a dwarf, branch less tetraploid recently, which was developed by a series of crosses, selections and finally, chromosome doubling. It is expected that a novel type of dwarf, branch less triploid watermelon will be available in China in the near future.

Mutation Breeding. Gamma-irradiation is used to induce reciprocal translocations to develop diploids with few seeds. This research began in Guangdong Academy of Agricultural Science, Gansu Agricultural University and Northwestern Agricultural University in the late 1970s (2). In many genotypes, high frequency reciprocal translocations can be induced by using 50 kr for seed irradiation and 3 kr for pollen irradiation. Individuals with 55 percent or lower viable pollen from the M_0 (seed) generation or M_1 (pollen) generation were selected for cytological testing and selfing. Several chromosome translocation lines are being evaluated in China.

Irradiation can induce various mutations in watermelon. Researchers from Lanzhou Watermelon and Sweetmelon Institute reported an extremely small seed line 91.5H/309, with a weight of 4.5

g/1000 seeds, selected from gamma-irradiated r23X Jubilee M_9 population. This line is being used as the paternal parent to produce triploid hybrids. Professor Mingzhou Wu noticed a glabrous mutant from gamma-irradiated population, but she was unable to get seed from the mutant. Professor Jingyi Wu got a male sterile mutant from gamma-irradiated germplasm which may be a cytogenetic male sterile because 100% of a testcross population was male sterile (personal communication).

Application of *In Vitro* Culture. Successful anther culture has been reported in China in many crops. The only successful watermelon anther culture was reported by Xue, *et al.* (6). Even though the frequency was low, they did get haploid and then dihaploid watermelon plants from culture. Anthers at the uninucleate stage, 5.0 mm in diameter and flower buds with an obvious corolla were harvested. Calli were induced on MS salts plus 2 ppm BA, 2 ppm Kn, 3% sucrose and 0.6-0.7% agar at pH 5.8. Organogensis was obtained by culturing the hard nodular callus on MS salts plus agar, 5-10 ppm GA_3, 4 ppm BA, 30-40 ppm adenine and 500 ppm lactalbumin hydrolysate. Differentiated organogenesis and shoot development occurred on agar-based medium with MS salts and 2 ppm triaconital. Roots were induced on agar-based medium with 1/2 MS salts and 0.2 ppm IBA, 1 ppm IAA and 1.5% sucrose at pH 5.7.

Efforts toward micropropagation of triploids and chromosome translocation diploid stocks have been made by several labs. Transplanting of triploid watermelon is not yet competitive with direct seeding in China because of the low cost of seed.

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Effect of Pollination Techniques on Fruit Set and Seed Yield of Egusi Melon (*Citrullus colocynthis*)

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Citrullus colocynthis (L.) Schrad, known as egusi melon in Nigeria, belongs to the Cucurbitaceae family (3). It is a prostrate herbaceous perennial, probably an ancestral type of watermelon, and is cultivated extensively for its fruits and seeds (4). The seeds are edible and rich in oil and protein. Unlike the typical watermelon, flowers of egusi melon are smaller, less showy than those of most other species of cultivated Cucurbitaceae (6) and are pollinated by honey bees (5). Egusi is monoecious, and pistillate flowers open early in the morning and are receptive for one day only. Thus, continuous regular fruit set depends upon daily activity of honey bees (1). Little information exists on the effect of controlled pollination and seed production of egusi melon. The objective of this study was to compare effects of controlled or hand-pollination and bee-pollination of egusi melon flowers on fruit set and seed yield.

In May 1992, 150 randomly selected seedlings of egusi melon were transplanted from the greenhouse to a field plot on a Willacy sandy loam soil at the Texas A&I University Research Farm. The plot design was 10 rows of 5 plants with 2.5 m interrow and 5.5 m intrarow spacings with three replications. Prior to anthesis, 50 pistillate flowers were selected randomly from each block and tagged. When newly-opened, the pistillate flowers were pollinated by hand daily at about midday. Another 50 flowers were tagged from each block and allowed to be pollinated by honey bees. Fruits were harvested 110 days after anthesis and the seeds were extracted from each fruit. Data obtained for hand- pollinated and bee-pollinated plants were: number of flowers/plant, number of fruits/plant, fruit size, number of seeds/fruit and seed weights of random 100- seed samples. Seeds were tested for their viability using the tetrazolium method of Moore (2). Analysis of variance was used to partition the variation in fruit set and seed yield into pollination techniques.

Fruit set and seed yield of egusi melon pollinated by hand and bees are presented in Table 1. While no significant differences were found between pollination techniques for number of flowers/plant and number of seeds/fruit, highly significant ($P < 0.01$) differences were found between pollination techniques for number of fruits/plant, seed weight and seed viability. Mean fruit weight and fruit size were significantly ($P < 0.05$) affected by pollination technique.

Based on our results, honey bees appear to be far more efficient pollinators of egusi melon than controlled or hand-pollination method. The percentage of flowers that set fruit under controlled pollination was generally below 20%, while under natural or bee-pollination more than 85% of flowers set fruits. FActors responsible for the inhibition of fruit set in hand-pollinations of egusi melon are not known and are under investigation in our laboratory.

Table 1. Effect of pollination methods on fruit set and seed yield of egusi melon (*Citrullus colocynthis*).

Yield parameter	Pollination Method		Significance[z]
	Hand	Bee	
No. flowers/plant	8	7	ns
No. fruits/plant	1	6	**
Mean fruit wt (kg)	5.2	3.2	*
Fruit diam (cm)	12.4	9.4	*
No. seed/fruit	367	347	ns
Seed wt (g/100)	5.7	19.9	**
Seed viability (%)	10	90	**

[z]ns, * and ** indicate not significant, and significant at the 5% and 1% level, respectively.

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Improved germination rate after 8 years in certain watermelon families

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Rhodes and Love (1) noted that germination rates were severely delayed in some of the crosses among the four genotypes PI 189225 (C), PI 29937 (B) and *Citrullus colocynthis* R309 (D), and 'New Hampshire Midget' (A). Most families from these crosses were observed again in 1991 for germination rates, resistance to anthracnose and resistance to *Fusarium* wilt. This report deals only with germination rates of the three *Citrullus lanatus* genotypes and crosses among these three parents.

After the 1983 study, seed were stored in airtight ammo boxes with silica gel at room temperature until the 1991 study. Germination was scored every day for 33 days. Eight-day germination was considered "normal" and later germination was considered "delayed". Germination at 8 days from the 1991 study were compared with germination data from the 1983 study. Delayed and total germination were also recorded in 1991. In the 1983 study, 288 seed of the parents and 96 seed of the progeny were planted. In the 1991 study, 50 seed of each family were planted in most cases.

Results of these comparisons are given in Table 1. Germination rates are grouped into families from an one or more original crosses.

The germination of New Hampshire Midget in 1991 was much lower than it was in 1983. However, 8-day germination of PI 299379 (B) and PI 189225 (C) was significantly higher 8 years after the original study, and 33-day germination was 83% and 94% for the two PI's, respectively.

Generally, loss of 8-day germination over the 8 year period occurred in the presence of the NHM genome: AxB, A(AxB), A(AxC), and (AxC)A. Gain of 8-day germination over the 8 year period occurred in backcrosses to B: B(AxB), (BxC)B. Gain of 8-day germination over the 8 year period occurred in AxC, CxB, and (BxC)C. After 8 years, 8-day germination percentages increased in parents B and C and in 14 out of 17 populations from these parents.

The results from some of the AxB crosses suggest maternal recessive control of delayed germination in B. The F_1 population germinates at 8 days like parent A. Eight-day germination in the F_2 in '83 is less than parent A or the F_1, suggesting that the population is segregating for delayed germination. When B was the maternal parent in the backcross B(AxB), germination in was delayed in 1983 and 1991.

The results from the AxC crosses are even more complex. The C genome delays germination only in the F_1 and in the backcross C(AxC) in 1983. In 1991, early and total germination are increased in the F_1, but decreased in the backcross A(AxC). However, the reciprocal backcross (AxC)A germinates slowly but completely after 8 years.

These results suggest that different factors retard rapid germination in fresh seed and prolong viability in old seed of PI 299379 and PI 189225, and that these factors deteriorate with time. The deterioration of these germination inhibitors makes it impossible to distinguish them simply by scoring germination after 8 years in families from crosses of the two PI's. The use of fresh seed from new crosses of inbred lines, complete germination data over a 30-day period and biochemical

analysis might lead to an understanding of the inheritance of these factors. These factors may benefit the species by maintaining seed viability over a longer period.

Table 1. Percent germination rates of seed from *Citrullus lanatus* 'New Hampshire Midget'(A), PI 299379 (B) and PI 289225 (C) and F_1, F_2 and BC progeny in 1983 and 1991.

Parent or Progeny	'83 8-day	'91 8-day	'91 delayed	'91 Total
Parents: A	64 a	* 16 a	14 b	30 c
B	1 b	* 31 b	52 a	83 a
C	1 b	* 90 c	4 b	94 a
F_1 (AxB)	68	28	28	56
F_1 (BxA)	65	-	-	-
F_2 (AxB)	51	* 82	6	88
A (AxB)	40	* 22	8	30
(AxB) A	44	ns 56	24	80
B(AxB)	14	* 34	54	88
(AxB)B	38	* 84	4	88
F_1 (AxC)	7	* 73	24	97
F_2	83	* 1	82	83
A (AxC)	80	* 6 (1)	10	16
		23 (2)	37	60
(AxC) A	89	* 34	64	98
C (AxC)	53	-	-	-
(AxC) C	-	54	30	84
F_1 (BxC)	7	ns 4 (1)	4	8
		46 (2)	0	46
F_1 (CxB)	1	* 55	45	90
F_2 (BxC)	0	* 6 (1)	44	50
		12 (2)	14	26
(BxC) B	0	* 54	8	62
C (BxC)	50	* 33 (1)	42	75
		82 (2)	4	86
(BxC) C	12	* 50	18	68

Small letters denote differences between parents. The * denotes differences between 8-day germination in '83 versus '91. The LSD statistic with an alpha of 0.05 was used.

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Use of a Detached Leaf Assay for Race 1 and Race 2 Anthracnose Resistance in a Diallel cross with *Citrullus*

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Love and Rhodes (2,3) studied race 2 anthracnose resistance in populations from a diallel cross among the susceptible watermelon variety 'New Hampshire Midget' and three disease resistant sources: PI 299379, PI 189225 and a *Citrullus colocynthis*, 'R309'. We concluded that resistance in both PI 299379 and PI 189225 was governed by a single dominant gene with modifiers. Suvanprakorn and Norton (4) had reported a dominant gene in PI 189225, PI 271778 and PI 326515. Resistance in 'R309' appeared to be controlled by several dominant genes different from the one in the 4 PI's. Our studies were based on observations of whole plants in the field. This study was undertaken with detached leaves from the remainder of the populations generated in the previous study.

Fifty seed or less from familes from the original study were planted in flats of soilless medium and watered daily. 'Edisto' muskmelon and 'Butternut' squash were also used to differentiate races. The second or third true leaf was detached and inoculated with drops of a 10^6 spore suspensions of anthracnose isolates in order to evaluate isolates. Finally, a single isolate of race 1 and race 2 was replicated by drops on each detached leaf, and lesion size was recorded with a micrometer five days after inoculation. Leaves were kept moist and exposed to fluorescent light in a transparent polyethylene chamber lined with filter paper. Six-week-old seedlings were transplanted into a 15-year monoculture area at the Edisto REC at Blackville. Surviving plants were considered resistant to wilt. Thus, each individual from a specific family was rated for anthracnose lesion size using race 1 and race 2 isolates and survival in the presence of *Fusarium* wilt. *Fusarium* wilt resistance will be discussed separately.

Results of inoculations with race 1 and race 2 are given in Table 1 in terms of leaves with and without lesions, lesion size and the associated sample standard deviation.

The most obvious result from these inoculations is that escapes occurred. 'New Hampshire Midget' is susceptible to both races of anthracnose. Yet, 27% and 55% of the A plants were scored resistant to race 1 and race 2, respectively. Escapes and small populations limit analysis of the data. The reaction of the race 2 susceptible 'Edisto' melon check to our race 2 indicated resistance instead of susceptibility.

Inheritance of race 1 resistance. Incomplete dominance for race 1 resistance is indicated in parents B and C to both races in the F_1 hybrids with A. Both F_1 populations have larger numbers of resistant individuals. Backcrosses to the resistant parent result in a larger number of resistant individuals. The F_2 populations also have more resistant individuals. Dominance for resistance in B and C is indicated in the F_1 hybrids with D. For race 1, backcross populations (BxD)B, B(BxD), C(DxC) and (DxC)C all suggest dominance for resistance in B and C. The best

evidence that B and C are homozygous for the same dominant gene is the virtual absence of susceptible progeny in all the populations from the BxC and CxB crosses.

Backcrosses involving parent A to the resistant parent B or C indicate that one or more susceptible classes are being generated in the presence of the dominant gene. Susceptibility in the presence of the dominant gene is coming from the susceptible parent A because all the progeny of the cross BxC are resistant in contrast to the AxB and AxC progeny. Henderson, *et al.*, (1) has shown that resistance level to anthracnose is affected by gene dosage. F_2 populations of AxB and AxC classes fit 3:1 resistant:susceptible ratios for race 1.

F_2 progeny from the AxD cross suggest that D may have a recessive gene for resistance to race 1; but, because escapes are evident in the parents, it is possible that all the resistant progeny are escapes.

Inheritance of race 2 resistance. Because escapes are evident in the susceptible parent A, it is likely that escapes occurred in the progeny. Dominance is indicated in the F_1 of the cross AxB. Backcrosses to B exhibit more susceptible progeny than backcrosses to A, but the F_2 progeny from the cross AxB fit a 3:1 R:S ratio.

Segregation of all the progeny from the AxC cross suggests a single dominant gene for resistance. The backcrosses to parent A fit a 1:1 R:S ratio, and most of the progeny from the backcross to parent C are resistant. The F_2 progeny also fit a 3:1 R:S ratio.

Virtually all the progeny from the cross BxC are resistant to race 2 as expected from a cross between two homozygous parents. Resistance to race 2 parallels that of resistance to race 1. Similarly, dominance for resistance is indicated in parents B and C when crossed to parent D.

Relationships between resistance and suceptibility and race 1 and race 2 resistance. An attractive hypothesis to explain the presence of the susceptible class in the backcrosses to the resistant parent is a loose linkage between the dominant gene for resistance and another gene which allows the expression of resistance. When the resistant parent is crossed with parent A, crossing over may occur between the dominant gene for resistance and the modifier gene, resulting in two new recombinant gametes that contribute susceptible genes. The large number of individuals susceptible to both races in the backcrosses B(AxB) and (AxB)B support this hypothesis.

In this small data set, the presence and size of a race 1 lesion was positively correlated with the presence and size of a race 2 lesion in all the *lanatus* families. Thus, in crosses with two known race 2 resistant lines, segregation for race 2 resistance paralleled segregation for race 1 resistance.

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Table 1. Number of individuals with (S) or without (R) anthracnose lesions on detached leaves from *Citrullus lanatus*, *Citrullus colocynthis*, *Cucumis melo* and *Cucurbita moschata* drop inoculated with race 1 and race 2 isolates and maintained in a moist, illuminated polycarbonate chamber.

Parents and Checks	Observed: Race 1	Lesion Size[z]	Sample SD[y]	Observed: Race 2	Lesion Size[z]	Sample SD[y]
	R:S[w]			R:S[w]		
New Hampshire Midget (A)	3:8	1.46	0.79	6:5	0.48	0.15
PI 299379 (B)	20:0	0	0	19:1	0.50	0
PI 189225 (C)	15:1	2.60	0	15:1	3.20	0
C. colocynthis 'R309' (D)	1:5	1.02	0.96	2:4	0.90	0.73
'Edisto' muskmelon	1:8	0.57	0.39	9:0	0	0
'Butternut' squash	5:0	0	0	5:0	0	0
Progeny						
AxB	7:3	0.70	1.04	8:2	1.10	1.13
A(AxB)	5:7	1.29	0.94	10:1	0.40	0
(AxB)A	13:13	1.22	1.07	21:5	1.40	0.99
B(AxB)	22:14	1.42	0.79	19:17	2.28	1.27
(AxB)B	40:27	1.15	1.38	55:12	0.60	0.42
(AxB)F2	34:26	0.54	0.48	44:16	0.43	0.29
AxC	11:5	0.55	0.25	13:3	0.40	0.08
A(AxC)	11:6	1.58	0.68	9:8	1.98	1.25
(AxC)A	20:16	2.70	1.18	23:13	1.70	1.05
(AxC)C	29:6	3.52	1.39	33:2	1.55	1.06
(AxC)F2	22:11	1.50	1.57	28:5	2.40	1.76
AxD	6:7	1.57	1.04	10:3	0.40	0.14
(AxD)A	1:0	0	0	1:0	0	0
(AxD)F2	5:37	2.19	0.90	24:18	1.34	0.62
BxC	16:0	0	0	14:2	1.05	0.64
CxB	10:0	0	0	10:0	0	0
(BxC)B	30:2	1.80	2.12	32:0	0	0
(BxC)C	32:0	0	0	32:0	0	0
C(BxC)	39:1	0.90	0	38:2	0.50	0.28
(BxC)F2	30:1	0.5	0	31:0	0	0
DxB	8:6	1.85	2.41	12:2	2.80	3.39
D(DxB)	2:2	1.60	1.84	4:0	0	0
B(DxB)	33:4	3.10	3.54	34:3	2.00	0
(DxB)B	15:1	0.40	0	14:2	1.00	0
DxC	16:0	0	0	16:0	0	0
C(DxC)	17:0	0	0	17:0	0	0
(DxC)C	33:2	1.15	0.21	35:0	0	0
D(DxC)	16:0	0	0	13:3	0.35	0.07
(DxC)D	6:0	0	0	2:4	0.88	0.76

[z]Lesion size in millimeters. [y]Sample standard deviation , using n-1 weighting. [w]Plants with leaves with lesions after inoculation were classified as "susceptible", and the mean lesion size of susceptible plants and the associated SSD are recorded here.

Watermelon DNA Isolation and Amplification Using the Polymerase Chain Reaction

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In recent years molecular genetic maps comprising closely-linked DNA markers have been constructed in several important field crops and horticulture crops (1,4,5). DNA markers linked to traits of interest can facilitate rapid and efficient selection of these traits in breeding programs. DNA markers commonly used are restriction fragment length polymorphisms (RFLP's). More recently, DNA markers based on a modification of the polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD), shows special usefulness in genome mapping because of its speed and simplicity (5,6). RAPD assay is also well suited for DNA fingerprinting and varietal identification as well as isolation of specific DNA fragments. Watermelon is an important crop worldwide. However, genetic studies on watermelon have lagged behind those on other important crops. DNA polymorphism studies have not been reported yet on watermelon. We are now working on identification of DNA markers linked to important watermelon genes. This report includes the procedures we have used for DNA isolation and the conditions for PCR amplification. Related results from this research will be reported separately.

DNA Isolation. The procedure of Dellaporta *et al.* (3), as modified by Culpepper *et al.* (2) was used. Young leaf tissue (ca. 1 gm) was collected from 14 genotypes grown in the greenhouse. These were ground to a fine powder in a precooled mortar and pestle in liquid nitrogen and 0.25 g of water insoluble polyvinylpolylpyrrolidone (Sigma P6755). To the cold mortar, 15 ml of extraction buffer (100 mM Tris.HCl, pH 8.0; 200 mM Na_2 EDTA, pH 8.0; 500 mM NaCl; 10 mM beta-mercaptoethanol) were added, and the mixture was allowed to freeze. As the sample thawed, it was ground again. The slurry was transferred to a 50 ml centrifuge tubes containing 1 ml of 20% SDS and mixed thoroughly but gently. The centrifuge tube was placed in a 65°C water bath for 15 min. Then, 6 ml of cold 5M KOAc was added, mixed and placed on ice for 20 min. Following precipitation of the proteins and polysaccharides, they were removed by centrifugation at 25K x g at 4°C for 20 min. The supernatant was filtered through sterile cheesecloth into a new 50 ml centrifuge tube containing 15 ml of ice cold isopropanol. After gentle, thorough mixing, the tube was kept at a minimum of -20°C for 30 min. The nucleic acid was pelleted at 20K x g for 15 min at 4°C and the supernatant discarded. The pellet was washed once with 80% ethanol to remove excess salt and partially air-dried to remove the alcohol. The pellet was dissolved in 0.6 ml of T_{10} E_1 buffer (10 mM Tris.HCl, pH 8.0; 1 mM Na_2EDTA, pH 8.0) with gentle swirling, transferred to a microcentrifuge "bullet" tube, and spun 5 min at 4°C to pellet insoluble material. The supernatant was removed to a new tube. Sixty µl of 3 M Na0Ac was added, mixed gently and then 400 µl of cold isopropanol was added and mixed gently. The tube was placed at -20°C for 20 min. The nucleic acid was pelleted at maximum RPM (Microcentrifuge Model 235C, Fisher Sci) for 10 min. The supernatant was discarded, the nucleic acid pellet washed twice with 80% ethanol, briefly air-dried to partial dryness and resuspended in 300 ml of TE buffer (pH 8.0) on ice, and then kept in a refrigerator or freezer until used.

Using the above protocol we obtained a DNA yield of 17.5 - 139.2 µg per leaf, depending on leaf size and genotype. The measurement was made on a TKO 100 Dedicated MiniFlurometer (Hoefer Scientific Instruments). This yield was much more than we needed for PCR amplification. The DNA preparation were digested by restriction enzyme Eco R1 for 4 hr and overnight to test the DNA quality for RFLP assay. The DNA was completely digested in both cases. The nondigested DNA check band on the gel did not show degradation. Furthermore, PCR amplification indicated that the DNA could be amplified by arbitrary primers (Fig 1). We think that this protocol is a simple, rapid and appropriate one for watermelon DNA isolation for RFLP and RAPD studies.

PCR Amplification. Amplification reactions were performed in a Perkin-Elmer/Cetus Model 480 DNA Thermal Cycler programmed for 5 min at 94°C followed by 45 cycles of 1 min at 93°C, 1 min at 35°C, 2 min at 72°C, and then followed by 5 min at 72°C. All amplification reactions were in volumes of 25 µl containing 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, dATP, dCTP, dGTP, dTTP (each at 0.2 mM), 0.5 unit of Ampli Taq DNA polymerase (Perkin Elmer/Cetus). In order to optimize the reaction conditions, different $MgCl_2$ concentrations (1.5, 2.0, 3.0 and 4.0 mM), primer concentrations (0.2, 0.5 µM; Operon RAPD primer kit OPC 01) and genomic DNA (15 and 25 ng) were tested. Amplification products were resolved by electrophoresis in a 1.2% agarose gel which was stained with ethidium bromide and photographed. The results from two genotypes (G17AB male sterile and PI 296341) indicated that the proper concentration for $MgCl_2$ was 3.0 mM. $MgCl_2$ at 1.5 mM was too low to amplify the genome DNA of G17AB in the PCR reaction. A higher concentration of primer (0.5 µM) increased the amount of smaller DNA amplification products but decreased the amount of larger DNA fragments during the amplification reaction. No obvious differences were observed for different amounts of genomic DNA (15, 25 ng) on two genotypes (Fig 1). The primer concentration of 0.2 µM and the genomic DNA amount of 25 ng were chosen for PCR amplification in our RAPD marker screening. The RAPD makers were observed on the arbitrary primers (Operon RAPD primer, Alameda, CA 84501) screened in 14 watermelon DNA samples.

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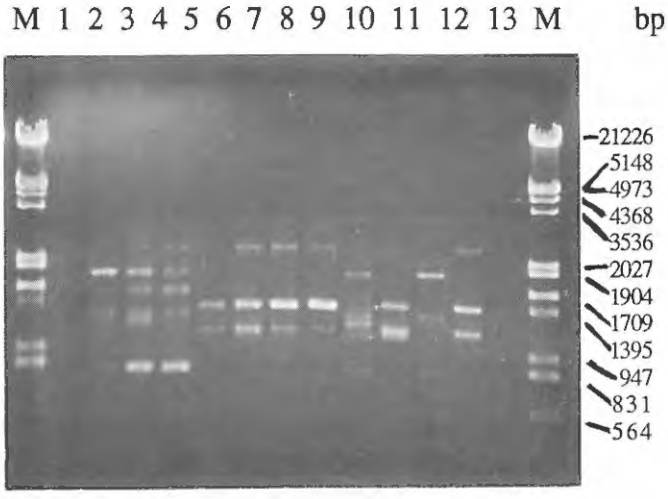

Fig 1. Amplification of two watermelon DNA fragments at different concentrations of $MgCl_2$, primer and different amounts of DNA. Lanes 1, 2, 3 and 4: G17AB at 1.5, 2.0, 3.0 and 4.0 mM $MgCl_2$, respectively, were used. Lanes 5, 6, 7 and 8: PI 296341 at 1.5 mM, 2.0 mM, 3.0 mM and 4.0 mM $MgCl_2$, respectively, were used. Lanes 9 and 10: G17AB and PI 296341 with 0.2 µM primer and 2.0 mM MgCl2 and 15 ng DNA. Lanes 11 and 12: G17AB and PI 296341 with 0.2 µM primer, 2.0 mM $MgCl_2$ and 15 ng genomic DNA. Lanes 1-8 used 0.2 µM of primer and 25 ng of DNA. The primer was OPC-01 with sequence 5' TTCGAGCCAG 3'.

Reinterpretation of Fruit Color Inheritance in *Cucurbita pepo* L.

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Most of the literature describes fruit striping in this species as being due to a single dominant gene, St (1,2,5), which has been found to be part of an allelic series and is more properly designated as $l\text{-}1^{St}$ (6,3). According to the review by Robinson et al. (4, p.564), "Sinnott and Durham [1922] however, concluded that striping is recessive." The summary of this latter paper (7, p.186) actually said that "Plain (solid or self) color of fruit is dominant over striping in some cases but appears to be recessive in others. A single factor difference is involved." Their data are presented in Table 1.

If we ignore cross 21 for the moment, a heterogeneity chi square test on the F_2 data of the other three families for only their striping vs. nonstriping segregation gives us a value for $X_T^2=4.30$ ($p>0.20$), providing an acceptable fit to the hypothesis that striping is segregating as a monogenic dominant trait in all three families, as found by subsequent researchers.

The simplest explanation of cross 21 in light of these results is that it is due to the independent segregation of a dominant inhibitor, I_{mf}, which prevents the production of any color in mature fruit, unlike W in these data. The original cross would be $i_{mf}i_{mf}$ StSt WW yy X $I_{mf}I_{mf}$ stst WW yy; the F_1 $I_{mf}i_{mf}$ Ststt; and the F_2 segregation ratio 13 white, unstriped (I_{mf} St, I_{mf} st, and i_{mf} st) : 3 white, striped (i_{mf} St). The data fit this ratio well ($X^2=0.071$,$p>0.70$). The presumed recessive inheritance of striping in cross 21 has been variously attributed to situations where striping is masked in darkly pigmented fruit (6, p.218) and where dark stripes occur over the main vascular tracts (3, p.493), neither explanation being supported by the original report (7).

If we look at the complete segregation for cross 6, we find a 3:1 segregation for white vs. yellow fruit, but W and St are not assorting independently ($X^2=11.30$ for 9:3:3:1,$p<0.02$), as indeed Sinnott and Durham (7, p.181) suggested: "... the further possibility suggests itself that there may be linkage between the factor for white and the factor for striping." We can calculate a product-ratio value here of 19.0+7.63 crossover units.

Returning to cross 5, we note that the F_2 segregation of white to yellow is an excellent fit to the 15:1 ratio expected if white is determined by two pairs of dominant, duplicate genes, again as the authors suggested, although not accepted by Robinson et al. (4). Moreover, if we compute the overall expected

values for all phenotypes in this F_2, based on the above-calculated linkage of St to one of these putative loci for white, and test these against the observed values, we again get an excellent fit ($X^2 = 1.23$, $p>0.70$).

Although the family sizes are small, they are consistent in supporting all of the conclusions drawn here: striping is due only to a single dominant gene, presumably St (i.e., $l\text{-}l^{St}$); there are two independently assorting dominant genes, W_1 and W_2, for white mature fruit color, neither of which suppresses the expression of St, and one of which is linked to St; and there is a dominant color inhibiting gene, I_{mf}, which is epistatic to St and assorts independently of it.

Table 1. Inheritance of striping in fruit.[z]

Pedigree	Parents	F_1	F_2
21	White, green striped X Plain white	Plain white	Plain white 43; White, green stripes 9
5	White, yellow striped X Plain yellow	White, pale yellow stripes	White, pale yellow stripes 23; Plain white 8; Plain yellow 2
6	Plain yellow X White, yellow stripes	White, yellow stripes	White, yellow stripes 22; Plain white 3; Yellow, stripes darker 3; Plain yellow 6
7	White, yellow striped X Plain yellow	White, pale yellow stripes	White, pale yellow stripes 20; Plain white 13

[z] Data from Table 2 of Sinnott and Durham (1922)

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Impact of Powdery Mildew and Gummy Stem Blight on Collapse of Pumpkins (*Cucurbita pepo* L.)

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Powdery mildew (PM) caused by *Sphaerotheca fuliginea* and gummy stem blight (GSB)/ black rot caused by *Didymella bryoniae* (teleomorph) and *Phoma cucurbitacearum* (anamorph) are important diseases of many cucurbits. Growers in New York and other regions of the northeast have frequently complained about the collapse of pumpkins in the field or during the fall marketing period. Loss of handles (peduncles) at the same time is also a common occurrence. GSB is particularly important because plants already infected with PM are predisposed to more severe GSB (1). There are no reports on the potential interaction of PM and GSB susceptibility leading to collapse of pumpkins caused primarily by black rot. In 1990 and 1991, field studies were initiated to examine the rate, timing, and fungicide combinations necessary to control PM and GSB in pumpkin (cv. Howden). This variety was chosen because market losses occur more commonly with large pumpkins. In 1991 we also had the opportunity to evaluate two segregating pumpkin breeding lines with PM resistance, with Howden used in the backcross. This report summarizes the results of postharvest studies to establish the importance of PM and GSB on crop losses.

Methods. An isolate of *D. bryoniae* was recovered from infected muskmelon leaves showing typical symptoms of GSB in Onondaga County, NY in 1990. This isolate was maintained on V-8 agar plates at 21 C with 14 hr light. The concentration of the spore suspension was adjusted to 6 X 10^4 with nutrient solution (1), and was used to inoculate the segregating pumpkin breeding lines on 24 July 1991. A greenhouse isolate of *S. fuliginea* accidentally infected all cucurbit seedlings transplanted to the field in 1991, thus allowing for early occurrence of PM in plots used for chemical control and evaluation of the segregating pumpkin lines. Both PM and GSB occurred naturally in the plots in 1990. In 1991, the pumpkin plots were on the same land used the previous year to increase the natural occurrence of GSB.

The details of the fungicide trials conducted at Freeville, NY in 1990 and 1991 have been reported (2,3). A total of 48 pumpkins were held in a walk-in cooler set at 55 F for 6 wks in 1990, while in 1991 63 fruit were held for 12 wks.

Two early generation pumpkin lines backcrossed to Howden and segregating for PMR (9-741-2 X 747), referred to as line 741, and (9-746-1 X 747-4), referred to as line 746, were produced. Plants already infected with PM were transplanted to the field on 4 June and subsequently inoculated with the GSB organism on 24 July. Individual plants were rated for PMR segregation, and the severity of disease noted in June and July prior to GSB occurrence. The PM rating scale used was 0 = no colonization, 1 = mild infection on both leaves and stems, 2 = moderate infection, and 3 = severe infection. Individual pumpkins with good appearance and dark green handles were harvested 19 Sept and stored in the same cooler as mentioned above for 11 wks. The organisms responsible for collapse were determined in the laboratory.

Results. Disease pressure was reasonable high in 1990 for both PM and GSB as indicated by mid-season disease ratings (Table 1). Pumpkin collapse began 1 mo into storage and progressed rapidly. Black rot lesions were evident on most collapsing pumpkins, with a water-soaked, circular lesion surrounding the blackening tissue. The inside of the pumpkin was often filled with water. The greatest losses occurred most frequently for those treatments which provided poor control of both PM and GSB. Good control is usually achieved with a broad spectrum fungicide like Bravo and systemic control of PM provided by Bayleton or Benlate.

In 1991, PM pressure was intense owing to the early introduction of inoculum, but because the

season was reasonably dry, GSB was not readily detected in the foliar blight phase. This lack of severe pressure is reflected in the ability to hold most of the stored pumpkins for 11 wk (Table 2). Greatest losses again were sustained in those fungicide treatments which provided less than adequate control of either PM or GSB or both diseases. The product ASC at both rates provided systemic control of PM, giving results comparable to the Bravo + Benlate treatment. Anthracnose, although not as common as black rot, did appear as small sunken spots usually occurring on the side of the rind facing the ground.

In the field it was observed that line 741 was less susceptible to PM, while line 746 showed considerable loss of foliage attributed to both PM and GSB. A good correlation existed between plant susceptibility to PM that resulted in fruit losses primarily due to GSB infections (Table 3). Nine of the 20 fruit discarded from line 746 showed watery collapse of the rind due to black rot. Only three pumpkins were discarded for line 741 with black rot responsible in two cases and anthracnose in the other. The remaining pumpkins were still firm with dark green handles. In all cases, discarded fruit originated from plants which had earlier been rated in the field at 2 or greater for PM severity.

The two separate lines of evidence support the conclusion that susceptibility to PM does predispose plants to greater levels of infection by GSB, and as a result losses in the field or in storage from black rot can be expected. Control of PM through resistance should significantly reduce market diseases and storage losses.

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Table 1. Evaluation of fungicides for powdery mildew (PM) and gummy stem blight (GSB) control in Howden pumpkin, 1990[a]

Treatment & rate/A (spray interval in days)	Disease rating[b] PM	GSB	Storage losses[c]
Bravo 720 2.0 pt (7)	2.8	2.0	2/6
Bravo 720 3.0 pt (7)	2.8	1.3	3/6
Bravo 720 2.0 pt (7) + Bayleton 50 DF 4 oz (14)	2.3	1.0	0/6
Bravo 720 3.0 pt (7) + Bayleton 50 DF 4 oz (14)	2.3	1.3	0/6
Bayleton 50 DF 2 oz (14)	2.3	1.5	5/6
Bayleton 50 DF 2 oz (14) + 0.75 JMS Stylet Oil (14)	3.3	1.2	1/6
Bayleton 50 DF 4 oz (14)	3.2	1.2	3/6
Untreated Check	4.0	1.7	4/6

[a]No significant difference in yield (RCB with 3 replications). PM and GSB occurred naturally in plots.

[b]Foliar rating: 1 = 20% disease; 2 = 21-40% disease; 3 = 41-60% disease; 4 = 61-80% disease; 5 = over 80% disease.

[c]Six pumpkins were selected at random (2 per rep and treatment) and stored at 55°F for 6 wk.

Table 2. Effect of fungicide treatments in Howden pumpkins on handle appearance and storage losses, 1991

Treatment (Spray interval in days)	Handle appearance and comments[a] Firm	Soft	Shr/Disc	Wh. Myc.	Losses[b] and disease
Bravo (7)	1	6	2	(4)	2 (2 BR)
Bravo (7) + Bayleton (14)	4	3	2	(4)	3 (2 BR, 1A)
Bravo (7) + Benlate (7)	5	3	1	(4)	0
Bravo (7) + ASC-low (14)	5	3	1	(3)	1 (BR)
Bravo (7) + ASC-high (14)	3	4	2	(4)	0
$KHCO_3$ + Sun Spray Oil (7)	1	8	0	(7)	4 (2 BR, 1 A, 1 A + BR)
Water Control	3	4	2	(4)	2 (2 BR)

[a]Nine pumpkins selected (3/rep) had good appearance on 9/13/91. Handle readings made 1 mo. after storage at 55°F. Rind appearance was good. Handles noted as firm, soft, shrivelled and discolored, with white mycelia growth noted.

[b]Pumpkins were discarded over a 12 wk period with most occurring on Nov. 15 and Dec. 6 (experiment ended). BR = Black rot caused by *Didymella bryoniae* (Gummy stem blight); A = Anthracnose caused by *Colletotrichum orbiculare*.

Table 3. Reaction of segregating PMR pumpkin lines for powdery mildew and gummy stem blight, 1991

Pumpkin lines	PM damage by hill[a] Rating: 0	1	2	3	No. fruit	Storage results[b]
741 x Howden	10	6	2	3	21	3 discarded (2 BR, 1 A)
746 x Howden	0	15	2	3	20	9 discarded (9 BR)

[a]PM rating 0 = no mildew on leaves or stems, 3 = heavy colonization.

[b]Moved to storage 19 Sept at 55°F. Final readings 6 Dec. BR = Black rot caused by *Didymella bryoniae* (gummy stem blight), A = Anthracnose caused by *Colletotrichum orbiculare*.

Some considerations about expression of marker genes in experiments of genetic transformation in *Cucumis anguria* var *longipes* by coculturing cotyledon explants with *Agrobacterium tumefaciens*.

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Introduction.- *Cucumis anguria* var *longipes* is a wild cucurbitaceous species in which resistance to 'yellowing virus disease', cucumber green mottle mosaic virus, powdery mildew (*Erisiphe cichoracearum* DC ex Merat), nematodes (*Meloydogine sp.*) and bean spider mite (*Tetranychus urticae* Koch.) have been described (5, 9, 10, 11; for a revision see 14). The introduction of these genes in the genetic background of melon (*Cucumis melo* L.) would represent an important improvement of this crop species. Since for some of these plagues or diseases there are not sources of resistance within the species and because of the existence of incompatibility barriers for the sexual cross with the wild species, the most feasible alternative is the somatic hybridization by protoplast fusion. But for the efficient application of this technique it is necessary to have a good method for the selection of the somatic hybrids among the parental plants. So, the use of *Agrobacterium tumefaciens* strains as a biological vector for introducing marker genes in the wild species would facilitate that selection.

In this work, the results of a preliminary study about the infectivity of a bacterial strain (LB4404) carrying the binary plasmid pBI121 with two marker genes (ß-D-glucuronidase and neomycin phosphotransferase II) and the attainment of several *Cucumis anguria* plants expressing both markers, are described.

Material & Methods.Plant material.- Seeds of *Cucumis anguria* var *longipes* L-2 (accession No. GBNR 1751, kindly supplied by Dr. Den Nijs, I.V.T., Holland) were decoated and surface sterilized by immersion in 50% commercial bleach (50 g/l active chlorine) for 25 min, followed by three rinses with sterile distilled water. Germination was carried out on MG medium (15) under photoperiod (16 h light, cool fluorescent tubes Gro-lux, Sylvania, 90 $\mu E\ seg^{-1}m^{-2}$), at 27°C. Cotyledon from these plantlets on the appearance of the first true leaf were used as explant source as previously described for melon (13).

Agrobacterium tumefaciens. Strain LBA4404 (kindly supplied by Dr. A. Granell, IATA, Valencia, Spain) carries rifampicin resistance in their genophore and the pBI121 binary plasmid (8), a pBIN19 (1) derivative, with two genetic markers of bacterial origin not found in plants: that of ß-D-glucuronidase (GUS, EC 3.2.1.31), a hydrolase active against a wide number of ß-glucuronides, which under the control of the CaMV35S promoter is able to express in plants, and that of neomycin phosphotransferase II (NPTII, from transposon Tn5) which inhibits the action of the aminoglycoside antibiotics by phosphorilation and, under the nos promoter, confers resistance to kanamycin in plants.
Bacterial growth was carried out in Luria Broth (12) suplemented with 50 mg/l rifampicin (Rifaldin, Merrell Dow Pharm. Inc.) and 100 mg/l kanamycin (Sigma) at 28°C in darkness, overnight, in an orbital shaker at 250 rpm.

Sensitivity of explants to kanamycin.- In this work, the culture media and incubation conditions applied to the explants in order to obtain plant regeneration from them were the most suitable choosen from a previous study (data not shown) that are summarized as follows:

A- Organogenic medium: NB 0.1/1.0 Cu CM consisting of basal medium (13) suplemented with 0.1 mg/l NAA (naphthalene acetic acid), 1.0 mg/l 6BA (6-benzyl-adenine), 1.0 mg/l SO_4Cu, 10% (v/v) coconut milk, and 20 g/l sucrose.
B- Shoot-developing medium: the organogenic zones appeared in the preceding medium were excised from the callus and subcultured in the shoot-developing medium NB 0.01/0.1 Cu (14).
C- Rooting medium: well developed shoots were rooted in medium MB3 (15).

To determine the suitable dose of kanamycin for the selection of transgenic plants this antibiotic was added to the explant morphogenetic culture medium at four concentrations: 100, 200, 500 and 750 mg/l. Incubation was done in the same growth chamber already described for seed germination.

Variables measured were callus growth and organogenic response, expressed as percentage (± SE) of explants giving unorganized growth and organogenic structures, respectively, with readings taken after 21 and 28 days of culture.

Transformation.- In order to check if an increase in the initial number of potentially transformable cells produced an increase in the transformation efficiency, two kind of cotyledon explants were used in this experiment: those directly taken from the plantlets and those precultured in a callus promoter medium (NB 2.5/1.0) consisting of basal medium suplemented with 2.5 mg/l NAA, 1.0 mg/l 6BA, 20 g/l sucrose and 8 g/l agar (Technical No. 3, Oxoid). 200 explants were used for bacterial transformation and 60 for control in both kind of explants.

Infection of the explants was made in a relatively short time (≈ 7 min) with a mixture 1:1 (v/v) of the morphogenetic liquid medium NB 0.1/1.0 Cu CM and an overnight culture of *Agrobacterium* . After infection, explants were dried off with sterile filter paper and cocultured onto solid morphogenetic medium for 2 days at 28°C in darkness. After coculture the explants were taken out of the medium, washed in M&S salt solution containing 500 mg/l cefotaxime (Claforam, Roussel) to remove the bacteria, dried on filter paper and put onto selective solid morphogenetic medium containing 100 mg/l kanamycin as the selective agent and 300 mg/l cefotaxime to prevent the bacterial growth.

After 7 days in this medium (to check the no apparition of bacterial growth and the absence of necrosis in the explants) they were transferred every 14 days to fresh medium to permit the callus growth. Morphogenetic calli grown in this medium were selected and transferred to shoot developing medium NB 0.01/0.1 Cu maintaining the selective agent (100 mg/l kanamycin) and the bacterial controlling antibiotic (300 mg/l cefotaxime). Later subcultures on this medium without the selective agent were also carried out to check its influence on the development of shoots. Shoots were transferred to basal medium with 300 mg/l cefotaxime for rooting.

Test for GUS expression.- Plants regenerated from organogenic calli grown with kanamycin and also developed in the selective medium were analyzed for the expression of GUS gene according to a modification of Jefferson's procedure as follows: 10 mg pieces of leaves were lysed and homogenized with 100 µl extraction buffer (50 mM sodium phosphate, 10 mM EDTA-Na, 0.1% Triton X-100 and 10 mM ß-mercaptoethanol, pH 7.0) in ice-cooled baby Eppendorf. Samples of 40 µl were incubated with 60 µl substratum (1 mM 4-methyl-umbelliferyl-ß-D-glucuronide, Sigma, in extraction buffer) at 37°C in darkness for 20 min. Fluorescence was observed with a transilluminator under long wave UV light.

Results & Discussion.

1.- Sensitivity to kanamycin.- Experiments about kanamycin sensitivity of cotyledon explants of *Cucumis anguria* var *longipes* indicated that a concentration of 100 mg/l was selective. The initial growth, although in the 31 % of the explants, was extremely low (almost nill) and it was finally inhibit by the antibiotic. The morphogenetic response was nill from the beginning and was not observed either after 28 days in culture. Concentrations of 200 mg/l and higher were absolutely deleterious for growing the explants.

2.- Transformation.- Since the explants (both direct and precultured) did not present bacterial growth or necrosis, on day 7 they were trasferred to fresh medium for an additional 14 days, and subcultured again for another 14 days. The frequencies of calli with organogenic response grown from those explants are shown in Table 1.

Table 1- Percentage (±SE) of explants giving calli with organogenic response in medium NB 0.1/1.0 Cu CM with 100 mg/l kanamycin after coculture with *Agrobacterium.*

	Not precultured (direct)		Precultured	
Culture(days)	Control	*Agrob.*	Control	*Agrob.*
21	0,0±0,0	10,3±2,3	0,0±0,0	2,5±2,3
35	1,7±1,7	21,1±3,1	0,0±0,0	4,9±1,5

Both kind of explants experienced a progressive increase of morphogenetic response through the successive subcultures. But this increase was significantly higher in the non precultured explants (21%) than in those precultured for 6 days in the callus-promoter medium (5%). This difference could be due to the distinct differentiation pattern of the cells belonging to both kind of tissues, together with the different expression of the genes GUS and NPTII according to the type of promoter which they carry. This fact has already been reported by Jefferson et al., (8) in their work on tobacco when they found significant differences between the different cell types and even among the cell cycle phases in their response to transformation with *Agrobacterium* . De Kathen and Jacobsen (4), working with *Pisum sativum* also found different infectivity of the bacterial strain depending on the organ or tissue of the plant.

All the organogenic calli formed either in the first (21 days) or in the second (35 days) subcultures were transferred to the developing medium NB 0.01/0.1 Cu with 100 mg/l kanamycin. Nevertheless, in partial experiments, some of them were transferred to medium without selective agent and no differences were found in the subsequent development of shoots, suggesting that the initial calli, selected for its growth in the presence of kanamycin, came from transformed cells.

All shoots developed in this medium were transferred to the rooting medium MB3 with 100 mg/l kanamycin. Although in previous experiments with tobacco (data not shown) the transgenic plants were easily rooted in this medium, in this case the plants of *Cucumis anguria* had a very low growth and after 20-30 days had not rooted. Since the NPTII gene is under the **nos** promoter, it is possible that, according to the above mentioned authors, we had a differential expression of this gene between plant species or even within the different tissues of our species, it being very low or almost nill in its root primordia, making them sensitive to the antibiotic. Therefore, we had to root the plants in medium without kanamycin and we checked the presence of the reporter gene (GUS, which is under the control of the **35S** promoter with a wider range of expression in the whole plant), in almost all the regenerated plants. The results of this study is summarized in Table 2.

Table2 - Partial and total number of organogenic calli, regenerated plants and GUS-assayed plants coming from the transformation of cotyledon explants (direct or precultured) of *Cucumis anguria* var. *longipes.*

	After 21 days		After 35 days		Total	
	Direct	Precult.	Direct	Precult.	Direct	Precult.
Selected calli	25	4	21	4	46	8
Regenerated plants	107	13	198	62	305	75
GUS-assayed	95	5	153	57	248	62
GUS^+ plants	3	0	4	3	7	3

From the 400 (direct+precultured) explants put in coculture with the bacteria, a total of 54 (13.5%) were selected for its growth in the presence of kanamycin. From these calli a total of 380 plants were regenerated in selective medium containing kanamycin and 310 (81.6%) were assayed for the GUS test, giving a positive result with only 10 (3.22%). The origin of these 10 plants corresponds to 7 out of the 54 selected calli (12.96%), so the final yield was 7 transformed calli out of the 400 explants treated (1.75%). The differences found in the percentages of expression of both markers (particularly the low percent of plants GUS$^+$) could be due to several reasons. Thus, Horsch et al (6) highlighted the need to be careful when transformation is applied to plants other than tobacco or petunia, because of the possible apparition of 'scapes' (shoots that grow in the presence of kanamycin but that do not contain the foreign DNA) due to loss of expression of the gene (or even loss of DNA) during the development of shoots, or to a incomplete selection by cross protection of wild-type cells by transformed cells nearby.

On the other hand, Jansen and Gardner (7), working with petunia, observed great differences between the transient and the stable expression of the markers, the former being1000 times higher than the latter. It is possible that the extraordinary morphogenetic potential of our species results in the development of transiently transformed green shoot-buds (resistant to kanamycin) which, in fact, are not stable transformants and which, therefore, maybe do not express GUS activity at the level of mature plants. For their part, Colby et al. (2,3) reported that transformation in *Vitis* only took place in cells adjacent to the regeneration zones of the calli. For the same reasons it is also possible that, in our case, non-regenerative cells were transformed and, by cross protection, the contiguous non-transformed regenerative cells became plants apparently resistant to the antibiotic.

Lastly, although the position of both markers in the T-DNA is very close, the lack of correlation between the expression of such genes has been suggested by van Wordragen et al. (16) in their work on *Chrysanthemum* , where they found that the levels of expression of GUS and NPTII varied among calli and not all of those presenting kanamycin resistance exhibited GUS activity.

Conclusions.- *Agrobacterium tumefaciens* strain LBA4404 with the pBI121 plasmid has been shown to be infective in cotyledon explants of *Cucumis anguria* var. *longipes*. Apparently, the cell type or its state affects such infectivity as shown by the different number of calli selected for its growth in the presence of kanamycin depending of whether the explants have been precultured or not. On the other hand, growth and regeneration capabilities in the presence of kanamycin does not seem to be a suitable index of the transformation efficiency in this species, while the expression of GUS activity in the regenerated plants appear to be as more reliable or advisable index.

At present, experiments aimed at the molecular detection of NPTII gene, either in plants GUS$^+$ or in those not expressing this marker, are in progress. The results will help to clear up if the plants showing an apparent resistance to kanamycin but without GUS activity are true scapes or if a differential expression of both genes (or even a partial loss of DNA fragments) has occurred. Other experiments addressed to increasing the transformation rates in this and other *Cucumis* species are also being carried out. However by the moment, the plants already obtained in this work are going to be used as genetic markers in experiments of protoplast fusion *C. melo* (x) *C. anguria*.

Additionally, this transformation technology applied directly to the cultivated species should give us a powerful tool for the introduction of known valuable traits (such as virus or herbicide resistance, male sterility, etc.) in this group of important crop species.

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Gene List for *Cucurbita* spp.

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Concern has been expressed (26) about the use of the "+" symbol for the normal allele of cucurbit genes. The truly wild type does not exist for most cultivated cucurbits as it does for *Drosophila.* A wild or feral taxon closely related to *Cucurbita pepo*, such as *C. texana* or *C. fraterna*, would be inappropriate as a progenitor or wild type species for *C. maxima, C. moschata*, or other *Cucurbita* species. The cucurbit gene rules are based (31) on those for the tomato, which also lacks a wild type to typify the normal alleles for each gene. Tomato geneticists have selected the cultivar 'Marglobe' to represent the normal type, but the diversity of cucurbit cultivars precludes selecting only one type to represent the normal. No one cultivar for example is representative of summer squash, winter squash, pumpkins, 'vegetable spaghetti', and gourds of *C. pepo*. Instead of selecting one cultivar to represent the normal or "+" allele for each gene, it is left to the common sense of the researcher to distinguish between the normal and mutant alleles of a gene he or she names, and to choose a gene symbol corresponding with the name for the mutant allele. The "+" allele need not necessarily signify the phenotype of a putative wild ancestor, but preferably it should be that of a majority of the cultivars. Although the use of "+" is recommended, the use of upper and lower case for the first letter of symbols for dominant and recessive alleles at the same locus is acceptable.

Updated gene lists have recently been published for cucumber (29), melon (CGC 13:58-68, 1990), and watermelon (CGC 14:129-138,1991). Lists for known genes for *Cucurbita* were published previously (4,5,6,31). In the interest of updating and collecting information on the genetics of *Cucurbita* in one place, the following is a complete list of known genes. In **bold characters** are the genes which are maintained by the curators or which are very common in cultivars or collections. In light type are those genes which have been lost or are not yet in the curator's collection. We hope to continue this practice and publish a complete list for the *Cucurbita* spp. every four years. Researchers are encouraged to send stocks for genes not listed in bold characters, new genes, and article reprints containing descriptions to the gene curators.

Please inform the *Cucurbita* gene curators (R.W. Robinson or Mark Hutton) of any omissions or errors in the following list of *Cucurbita* genes.

Gene Symbol / Preferred Synonym		Character	Species	Reference
a		*androecious.* Produces only male flowers	*pepo*	21
Aat-mb		***Aspartate aminotransferase - microbody* isozyme**	***maxima* x *ecuadorensis***	**51**
Aat-m	***Aat-m1***	***Aspartate aminotransferase mitochondria* isozyme-1**	***maxima* x *ecuadorensis***	**51**

Gene Symbol Preferred	Synonym	Character	Species	Reference
Aat-m2		*Aspartate aminotransferase mitochondria* isozyme-2	*maxima* x *ecuadorensis*	51
Aat-p2		*Aspartate aminotransferase plastid* isozyme-2	*maxima* x *ecuadorensis*	51
Acp	*Acp-1*	*Acid phosphatase* - isozyme-1	*maxima* x *ecuadorensis*	51
Acp-2		*Acid phosphatase* - isozyme-2	*maxima* x *ecuadorensis*	51
Aldo-p		*Aldolase - plastid* isozyme	*maxima* x *ecuadorensis*	50
B_1		*Bicolor-1.* Precocious yellow fruit pigmentation; modified by *Ep-1, Ep-2* and *Ses-B*	*pepo*	41,42
B_2		*Bicolor-2* (precocious yellow fruit)	*maxima*	44
Bi		*Bitter* fruit. High cucurbitacin content in fruit	*pepo*	2,16,52
bl		*blue* fruit color. Incompletely recessive to green	*maxima*	18
Bu		*Bush* habit. Short internodes; dominant to vine habit in young plant stage but recessive at maturity	*pepo, maxima*	8,40,47
cr		*cream* corolla. Cream to nearly white petals for *cr/cr*, yellow for *cr/+*, and orange for *+/+*; derived from *C. okeechobeensis*	*moschata*	34
cu		*cucurbitacin* content. Reduced cucurbitacin content	*pepo*	39
D		*Dark* green stem. Dominant to light green stem	*pepo*	15
Di		*Disc* fruit shape. Dominant to spherical	*pepo*	46
Ep-1		*Extender of pigmentation-1*; modifier of B_1	*pepo*	45
Ep-2		*Extender of pigmentation-2*; modifier of B_1	*pepo*	45
Est		*Esterase* isozyme	*maxima* x *ecuadorensis*	49
Fr		*Fruit fly* (*Dacus cucurbitae*) *resistance*	*maxima*	25
G	*a,m*	*Gynoecious* sex expression	*foetidissima*	9,14
Gal	*Gal-1*	*ß-galactosidase* isozyme-1	*maxima* x *ecuadorensis*	51
Gal-2		*ß-galactosidase* isozyme-2	*maxima* x *ecuadorensis*	51
Gb		*Green band* on inner side of base of petal; dominant to no band	*pepo*	10

Gene Symbol Preferred	Synonym	Character	Species	Reference
gc		*green corolla.* Green, leaf-like petals	*pepo*	48
Got	*Got-1*	*Glutamine-oxaloacetate* isozyme-1	*maxima* x *ecuadorensis*	51
Got-2		*Glutamine-oxaloacetate* isozyme-2	*maxima* x *ecuadorensis*	51
Gpi-c	*Gpi-c1*	*Glucosephosphate isomerase cytosolic* isozyme-1	*maxima* x *ecuadorensis*	51
Gpi-c2		*Glucosephosphate isomerase cytosolic* isozyme-2	*maxima* x *ecuadorensis*	51
Gr	*G*	*Green rind.* Dominant to buff skin of mature fruit	*moschata*	32
Hi		*Hard rind inhibitor*	*maxima*	17
Hr		*Hard rind*	*pepo*	23
i		*intensifier* of the *cr* gene for cream flowers	*okeechobeensis*	34
I-T		*Inhibitor* of the *T* gene for trifluralin resistance	*moschata*	1
Idh	*Idh-1*	*Isocitrate dehydrogenase* isozyme-1	*pepo*	7,20
Idh-2		*Isocitrate dehydrogenase* isozyme-2	*pepo*	7,20
Idh-3		*Isocitrate dehydrogenase* isozyme-3	*pepo*	7,20
l	*c*	*light* fruit color. Uniform light intensity of fruit pigmentation; modified by *St*	*pepo*	15,27,41
l-2		*light* pigmentation of fruit - 2	*pepo*	27
Lap		*Leucine aminopeptidase* isozyme	*maxima* x *ecuadorensis*	49
lo	*l*	*lobed* leaves; recessive	*maxima*	11
Lo-2		*Lobed* leaves -2; dominant	*equadorensis*	17
lt		*leafy tendril.* Tendrils with laminae	*pepo*	36
ly		*light yellow* corolla. Recessive orange yellow	*pepo*	36
M		*Mottled* leaves. Silver gray areas in axils of leaf veins	*pepo, maxima, moschata*	3,35,38
Mdh-m	*Mdh-m1*	*Malate dehydrogenase mitochondria* isozyme-1	*maxima* x *ecuadorensis*	51
Mdh-m2		*Malate dehydrogenase mitochondria* isozyme-2	*maxima* x *ecuadorensis*	51
Mdh-c2		*Malate dehydrogenase cytosolic* isozyme-2	*maxima* x *ecuadorensis*	51

Gene Symbol Preferred	Synonym	Character	Species	Reference
ms-1	*ms1*	*male sterile-1*. Male flowers abort before anthesis	*pepo*	13
ms-2	*ms2*	*male sterile-2*. Male flowers abort	*pepo*	13
n		*naked* seeds. Lacking a lignified seed coat	*pepo*	16,37
Per	*Per-1*	*Peroxidase* isozyme-1	*maxima* x *ecuadorensis*	51
Per-3		*Peroxidase* isozyme-3	*maxima* x *ecuadorensis*	51
Pgi	*Pgi-1*	*Phosphoglucase isomerase* isozyme-1	*pepo*	7,20
Pgi-2		*Phosphoglucase isomerase* isozyme-2	*pepo*	7,20
Pgi-3		*Phosphoglucase isomerase* isozyme-3	*pepo*	7,20
Pgm-c2		*Phosphoglucomutase cytosolic* isozyme-2	*maxima* x *ecuadorensis*	51
Pgm-p		*Phosphoglucomutase plastid* isozyme	*maxima* x *ecuadorensis*	51
Pm		*Powdery mildew*-resistance. Resistance to *Sphaerotheca fuliginea*	*lundelliana*	30
r		*recessive* white. White fruit color	*pepo*	15
Rd		*Red* skin. Red external fruit color; dominant to green, white, yellow and gray	*maxima*	22
ro		*rosette* leaf. Lower lobes of leaves slightly spiraled	*pepo*	23
s		*sterile*. Male flowers small, without pollen; female flower sterile	*maxima*	19
Ses-B		*Selective suppression* of gene *B* 1	*pepo*	43
Skdh		*Shikimate dehydrogenase* isozyme	*maxima* x *ecuadorensis*	51
Sod	*Sod-1*	*Superoxide dismutase* isozyme-1	*maxima* x *ecuadorensis*	51
St	l^{st}	*Striped* fruit. Longitudinal stripes on fruit, conspicuous if *l* but inconspicuous if l^+	*pepo*	35
T		*Trifluralin* resistance. Dominant to susceptibility to the herbicide; modified by *I-T*	*moschata*	1
Tpi-c2		*Triosephosphatase isomerase cytosolic* isozyme-2	*maxima* x *ecuadorensis*	51
Tpi-p2		*Triosephosphatase isomerase plastid* isozyme-2	*maxima* x *ecuadorensis*	51
v		*virescent*. Yellow-green young leaves	*maxima*	12

Gene Symbol / Preferred Synonym	Character	Species	Reference
W	***White* fruit. Dominant to green mature fruit, partially epistatic to *Y***	***pepo***	**47**
Wf	***White flesh.* Dominant to cream flesh color**	***pepo***	**47**
Wt	***Warty* fruit. Dominant to smooth**	***pepo***	**47**
Y	***Yellow* fruit color. Dominant to green**	***pepo***	**47**
Ygp	***Yellow-green placenta.* Dominant to yellow placental color**	***pepo***	**10**
ys	*yellow seedling*. Lacking chlorophyll; lethal	*pepo*	23
zym	***zuchinni yellow mosaic* virus resistance**	***ecuadorensis, moschata*; not yet established if these genes are allelic.**	**24,28,33**

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It is hoped that scientists will consult the above list as well as the rules of gene nomenclature for the Cucurbitaceae (5, 31) before choosing a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature were adopted in order to provide guidelines for the naming and symbolizing genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

Gene List Committee:

Cucumber:	T.C. Wehner
Muskmelon:	M. Pitrat
Watermelon:	W.R. Henderson
Cucurbita spp.:	R.W. Robinson
	M.G. Hutton
Other Genera:	R.W. Robinson

Corrigenda to the 1991 Watermelon Gene List (CGC 14:129-137)

Warren Henderson, North Carolina St. University, Raleigh, NC 27695-7609 USA

The following corrigenda deal with three genes (*Wf*, *y*, y^o) which determine flesh color in watermelon.

Gene symbol		Character	Reference[z]
Preferred	Synonym		
Wf	*W*	*White* flesh. *Wf* is epistatic to the second gene *b* (or *C*?) which conditions yellow (Canary yellow?) and red flesh. *Wf_B_* and *Wf_bb* are white fleshed, *wf wf B_* is yellow fleshed, and *wf wf b b* is red fleshed	33
y	*r*	*yellow* flesh ('Golden Honey' type). Recessive to *Y* (red flesh)	8,24,27
y^o		*orange* flesh (from 'Tendersweet Orange Flesh'). Allelic to *y*. *Y* (red flesh) is dominant to y^o (orange flesh) and *y* (yellow flesh); y^o (orange flesh) is dominant to *y* (yellow flesh)	8

[z]As referenced in the bibliography for the 1991 Watermelon Gene List (CGC 14:129-137).

The major changes are:

1. Relationships at the *y* locus are allelic (rather than epistatic as previously listed).
2. The *red* and *rd* gene synonyms for *y* have been dropped completely until their status becomes clearer. Navot et al. (J. Hered. 81:162-165, 1990) considered *red* recessive, whereas at the *y* locus red (*Y*) is dominant; they did not describe the phenotype of the dominant allele (to *red*) and only stated that its inheritance was complex.
3. *Wf* appears to be epistatic to a second locus, other than *y*. This locus is probably *B* (or maybe *C*) as suggested by Shimotsuma (Jap. J. Breeding 13:235-240, 1963).

Stocks and Germplasm Desired or For Exchange

Request from the CGC Gene Curators

CGC has appointed Curators for the four major cultivated groups: cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), and *Cucurbita* spp. A curator for the Other Genera category is needed. Anyone wishing to take on this responsibility should contact the CGC Chair.

Curators are responsible for collecting, maintaining and distributing stocks of the known marker genes upon request. Members are requested to forward samples of currently held marker gene stocks to at least one of the respective curators: cucumber (Jack E. Staub, Todd C. Wehner), melon (James D. McCreight, Michel Pitrat), watermelon (Gary Elmstrom, E. Glen Price, Billy B. Rhodes) and *Cucurbita* spp. (Mark G. Hutton, R.W. Robinson).

The CGC Gene Curators for the *Cucurbita* spp. are in particular requesting the following gene stocks:

Gene	Character	Species	Reference[z]
a	*androecious*. Produces only male flowers	*C. pepo*	21
lt	*leafy tendril*. Tendrils with laminae	*C. pepo*	36
ly	*light yellow* corolla. Recessive to orange yellow	*C. pepo*	36
ms-1	*male sterile-1*. Male flowers abort before anthesis	*C. pepo*	13
ro	*rosette* leaf. Lower lobes of leaves slightly spiraled	*C. pepo*	23
ys	*yellow seedling*. Lacking chlorophyll; lethal	*C. pepo*	23

[z]As referenced in the 1992 *Cucurbita* spp. Gene List bibliography (CGC 15:102-109).

Individuals with the above gene stocks are requested to send samples to:

Mark G. Hutton
PetoSeed
RR2, Box 80-A Slade Lane
Bridgeton, NJ 08302 USA

R.W. Robinson
Dept. Horticultural Science, Cornell University
New York State Agricultural Experiment Station
Geneva, NY 14456 USA

Other Requests

Edward Croom (University of Missippi, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University, MS 38677 USA) is requesting accessions of *Siraitia* and *Thladiantha* (any species) to study their biodynamic properties and potential. He would be willing to discuss exchange or purchase of the germplasm.

Gene Nomenclature for the Cucurbitaceae

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

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

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

The same symbol shall not be used for nonallelic genes of different *Cucurbita* species. Allelic genes of compatible species are designated with the same symbol for the locus.

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

Mochizuki, Tatsuya Kurume Br, Natl Res Inst Veg Ornam Plant & Tea, 1823 Mii- machi, Kurume, Fukuoka 830, Japan.

Monteiro, Antonio A. Section of Horticulture, Inst. Superior de Agronomia, Techn. Univ. Lisbon, Lisbon, Portugal. Tel: 351-1-3638161. FAX: 351-1-3635031. Melon protection & cultivation in adverse environments.

Moraghan, Brian J. Asgrow Seed Co., P.O. Box 667, Arvin, CA 93203. Tel: (805) 854-2360. Melon and watermelon breeding and disease resistance.

Morelock, Ted Dept. Horticulture & Forestry, University of Arkansas, Fayetteville, AR 72701. Tel: (501) 575-2603.

Muhyi, Rejah I. Petoseed Co., Rt. 4, Box 1255, Woodland, CA 95695. Tel: (916) 666-0931.

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

Murdock, Brent A. Clemson University, Department of Horticulture, Clemson, SC 29634-0375. Tel: (803) 296-1871. FAX: (803) 656-4960. Watermelon breeding; genetic improvement of neglected tropical vegetables.

Nagai, Hiroshi Instituto Agronomico, Cx Postal 28, 13.100-Campinas, Sp., Brazil.

Navazio, John Dept. Hort., 1575 Linden Dr., University of Wisconsin, Madison, WI 53706.

Tel: (608) 238-8567. Breeding for stress tolerance, postharvest physiology, improved cultivars and inbreds.

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

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

Niego, Shlomo Plant Genetics, The Weizman Institute of Science, Rehovot, Israel.

Niemirowicz-Szczytt, Katarzyna Ul. Nowoursynowska 166, Dept. Genetics and Plant Breeding, 02-766 Warsaw, Poland. Tel: 430982. Breeding of cucumber, melon, watermelon & squash. Downy mildew res., wide crosses, tissue culture, haploids.

Norton, J.D. Department of Horticulture, Auburn University, Auburn, AL 36849. Tel: (205) 844-3031. FAX: (205) 844-3131. Multiple disease resistant melon and watermelon.

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

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Owens, Ken PetoSeed Company, Inc., Rt. 4, Box 1225, Woodland, CA 95695. Tel: (916) 666-0931. Cucumber breeding.

Paris, Harry Division of Vegetable Crops, Agric. Research Org., Newe Ya'ar Expt. Station, P.O. Haifa, Israel. Tel: 972-4-894516. Breeding and genetics of cucurbits.

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Poli, Virgil Stauinea de Cercetari Legumicole, Isalnita-Craiova, Romania.

Poostchi, Iraj 97 St. Marks Road, Henley-on-Thames RG9 1LP, England.

Price, E. Glen American Sunmelon, P.O. Box 153, Hinton, OK 73047. Tel: (405) 542-3456. Seedless watermelon; polyploidy, genetics, breeding, cytogenetics.

Provvidenti, Rosario Department of Plant Pathology, NYAES, Cornell University, Geneva, NY 14456-0462.

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Robledo, C. V.R.E.D., Centre de Recherches, Mas d'Aptel, 30510 Generac, France. Tel: 66 01 89 07.

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Roig, Luis A. Departamental Biotechnology, E.T.S. Ingenieros Politecnica, Camino de Vera 14, 46022 - Valencia, Spain.

Rosario, Ted Petoseed - Texas, P.O. Box 532, Donna, TX 78537.

Rovelo, Claudia Horticulture Research Laboratory, Coll. Agriculture, Box 156, Texas A&I Univ., Kingsville, TX 78363.

Rumsey, Anthony E. New World Seeds Pty Ltd., P.O. Box 18, Dural 2158, 22-24 Crosslands Road, Galston, N.S.W., Australia.

Sadik, Sidki 7321 Harps Mill Road, Raleigh, NC 27615.

Scheirer, Douglas M. Nestle USA/Libby Division, P.O. Box 198, Morton, IL 61550. Tel: (309) 263-2651. Processing pumpkin; breeding and cultural practices.

Schnock, Martin G. Norsingen, Fridolin-Mayer-Strasse 5, D-7801 Ehrenkirchen, Fed. Rep. Germany. Tel: 07633-13095.

Schroeder, Robert H. Harris Moran Seed Co., R.R. 1, Box 1243, Davis, CA 95616. Tel: (916) 756-1382. FAX: (916) 756-1016. Cucurbit genetics and breeding; germplasm evaluation and utilization.

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Semillas Fito, S.A., c/. Selva de Mar, 111, 08019 Barcelona, Spain.

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Song, Jin-Soo Plant Breeding & Res. Inst., Nong-Woo Seeds, 387-2 Sasa-2Ri, Panwol, Whasong, Kyonggi, 445-820, Republic of Korea. Tel: 0345-80-0466.

Staub, Jack E. USDA-ARS, Dept. Horticulture, Univ. Wisconsin, Madison, WI 53706. Tel: (608) 262-0028. Cucumber breeding & genetics, physiology, biochemical genetic markers, evolution, environmental stress.

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Taurick, Gary Ferry Morse Seed Company, P.O. Box 392, Sun Prairie, WI 53590. Tel: (608) 837-6574. FAX: (608) 837-3758. Population improvement and hybrid development for cucumber and summer squash.

Teppner, Herwig Karl-Franzens-Universitat Graz, Institut fur Botanik, Holteigasse 6, A-8010 GRAZ, Austria.

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Thomas, Paul PetoSeed Co., Inc., Rt. 4, Box 1255, Woodland, CA 95695. Tel: (916) 666-0931.

Tolla, Greg Campbell Research & Development, Napoleon, OH 43545. Tel: (419) 592-8015. Development of pickling cucumber varieties.

Trulson, Anna Petoseed Co., Inc., Rt. 4, Box 1255, Woodland, CA 95695.

Unander, David P.O. Box 168, Downington, PA 19335. Tel: (215) 873-9131. FAX: (215) 728-3574. Virus resistance, anti-viral natural products, vegetable breeding.

Vakalounakis, Demetrios J. Plant Protection Institute, P.O. Box 1802, Heraklion, Crete, Greece.

van Deursen, S. Sluis & Groot Research, Blaker 7, 2678 LW de Lier, The Netherlands.

van Kooten, Hank Bruinsma Selectiebedrijven B.V., P.O. Box 24, 2670 AA Naaldwijk, The Netherlands.

van Leeuwen, Loes Sementi Nunhems, Via Ghiarone, 2, 40019 S. Agata Bolognese, Italy.

Vecchio, Franco Pioneer Hi-Bred Italia SpA, via Provinciale 42/44, 43018 Sissa (PR), Italy.

Ventura, Yaacov Hazera Ltd., Breeding Department, Mivhor Farm, Post Sde Gat 79570, Israel.

Walters, Terrence Fairchild Tropical Garden Science Center, 11935 Old Cutler Road, Miami, FL 33156. Tel: (305) 665-2844. FAX: (301) 665-8032.

Warid, Warid A. Paseo de las Fuentes No. 18, Col. Valle Verde, 83200 Hermosillo, Sonora, Mexico.

Watterson, Jon PetoSeed Company, Inc., Rt. 4, Box 1255, Woodland, CA 95695.

Wehner, Todd C. Department of Horticultural Science, Box 7609, North Carolina State University, Raleigh, NC 27695-7609. Tel: (919) 737-3167. FAX: (919) 737- 7747. Cucumis genetics and breeding; yield, earliness, quality, disease, cold tolerance.

Wessel-Beaver, Linda Department of Agronomy & Soils, College of Agriculture, Univ. Puerto Rico, Mayaguez, PR 00708. Tel: (809) 832-4040. Pumpkin & squash breeding; disease resistance; insect resistance.

Whitaker, T.W. 2534 Ellentown Road, La Jolla, CA 92037. Tel: (714) 452-0690.

Whiteaker, Gary Canners Seed Corp., 221 East Main Street, Lewisville, ID 83431. Tel: (208) 754-8666. FAX: (208) 754-8669.

Williams, Tom V. Rogers NK Seed Co., 10290 Greenway Road, Naples, FL 33961. Tel: (813) 775-4090. FAX: (831) 774-6852. Watermelon breeding.

Winkler, Johanna Saatzucht Gleisdorf, Ges.m.b.h. & Co. KG, Am Tieberhof 33, 8200 Gleisdorf, Austria.

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Yan, Yin Institute of Vegetable & Flower, Chinese Academy of Agricultural Science, Beijing 100081, P.R. China.

Yang, Si-Lin Dept. Horticulture, Southwest Agric. Univ., Bei-bei, Chong-qing, Si-chuan 630716, P.R. China. Ethnobotany, crop evolution, genetic resources (wild & cultivated) of Asian Cucumis, Benincasa, Momordica.

Yorty, Paul Rogers NK Seed Co., P.O. Box 104, Twin Falls, ID 83303-0104. Tel: (208) 733-0077. Cucurbit breeding.

Yukura, Yasuo 46-7, 3-Chome, Miyasaka, Setagaya-Ku, Tokyo, Japan.

Zhang, Jiannong Melon Research Institute, Gansu University of Agriculture, Lanzhou, Gansu, 730070, P.R. China.

Zhang, Xingping Department of Horticulture, Clemson University, Clemson, SC 29634-0375.

Zhao, Yanru Beijing Vegetable Research Center, P.O. Box 2443, Beijing 100081, P. R. China.

Zink, Frank Department of Vegetable Crops, University of California, Davis, CA 95616. Tel: (916) 795-4967. Plant breeding and disease resistance.

Zitter, Thomas Cornell Univ., Dept. Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-5908.

Zÿlstra, Sierd Centre for Plant Breeding Research CPO, Postbox 16, 6700 AA Wageningen, The Netherlands. FAX: 08370-16513. Breeding for resistance in Cucumis sativus; genetic markers (RFLP's, isozymes).

Geographical Distribution of CGC Members in the USA

Alabama
Dane, Fenny
Norton, J.D.

Arkansas
Morelock, Ted

Arizona
Ray, Dennis

California
Bohn, G.W.
Burkett, Al
Chen, N.C.
Chung, Paul
Close, Timothy J.
DeVerna, J.W.
Ikeda, Satoru
Ladd, Krystyna M.
McCreight, J.D.
Moraghan, Brian J.
Muhyi, Rejah I.
Owens, Ken
Pierce, Lawrence
Pierce, Vickie
Schroeder, Robert H.
Stern, Joseph
Stevens, M. Allen
Thomas, Paul
Trulson, Anna
Watterson, Jon
Whitaker, T.W.
Wyatt, Colen
Zink, Frank

Colorado
Hollar, Larry A.

Florida
Crall, J.M.
Decker-Walters, Deena
Dumlao, Rosa
Elmstrom, Gary
Eyberg, Dorothy A.
Knerr, Larry D.
Meadows, Mike
Miranda, Baldwin
Walters, Terrence
Williams, Tom V.

Georgia
Groff, David

Hawaii
Chen, Fure-Chyi
Sekioka, Terry T.

Idaho
Love, Stephen L.
Whiteaker, Gary
Yorty, Paul

Illinois
Juvik, John
Scheirer, Douglas M.
Skirvin, Robert M.

Indiana
Eigsti, Orie J.
Nourizadeh, Saeid
Oh, Dae-Geun

Iowa
Drowns, Glenn
Reitsma, Kathy

Kansas
Clayberg, C.D.

Kentucky
Callaway, M. Brett

Maine
Merrick, Laura C.

Maryland
Kirkbride, Joseph H. Jr.
Ng, Timothy J

Michigan
Grumet, Rebecca
Quemada, Hector

Mississippi
Croom, Edward M.

Missouri
Layton, Jeanne G.

Nebraska
Coyne, Dermot P.

New Hampshire
Carle, R. Bruce
Loy, J. Brent

New Jersey
Hutton, Mark
Shifriss, Oved
Snyder, Jim

New Mexico
Maiero, Marisa

New York
Carey, Edward E.
Gilbert, Raphael
Ibrahim, Aly M.
Kyle, Molly
McArdle, Richard
McFerson, James R.
Munger, H.M.
Provvidenti, Rosario
Robinson, R.W.
Zitter, Thomas

North Carolina
Barbercheck, Mary
Denlinger, Phil
Henderson, W.R.
Sadik, Sidki
Wehner, Todd C.

Ohio
Tolla, Greg

Oklahoma
Price, E. Glen

Oregon
Di Nitto, Louis
Gabert, August C.

Pennsylvania
Unander, David

Puerto Rico
Wessel-Beaver, Linda

South Carolina
Murdock, Brent A.
Nugent, Perry
Rhodes, Billy B.
Thomas, Claude E.
Zhang, Xingping

Texas
Dunlap, James R.
Kuti, Joseph O.
Mackay, Wayne A.
Rosario, Ted
Rovelo, Claudia

Wisconsin
Baker, L.R.
Havey, Michael J.
Katsiotis, Andreas
Lower, Richard L.
Navazio, John
Simon, Philipp W.
Staub, Jack E.
Taurick, Gary

CGC Members Outside of the USA

Argentina, Republica
Rodriguez, Jose Pablo

Australia
Herrington, Mark Edward
McGrath, D. J.
Rumsey, Anthony E.

Austria
Teppner, Herwig
Winkler, Johanna

Belgium
Goblet, J. P.

Brazil
Maluf, Wilson Roberto
Nagai, Hiroshi
Tasaki, Seikoh

Bulgaria
Alexandrova, Maria

Canada
Punja, Zamir K.

China, Peoples Republic of
Cui, Hongwen
Lin, Depei
Ma, Dewei
Ma, Kechi
Ming, Wang
Wu, Mingzhu
Yan, Yin
Yang, Si-Lin
Zhang, Jiannong
Zhao, Yanru

China, Republic of
Hung, Lih

Colombia
Jaramillo-Vasquez, Juan

Denmark
Kampmann, Hans Henrik

Egypt
Aboul-Nasr, Mohamed Hossam
El-Doweny, Hamdy Hassan Ali
Hassan, Ahmed Abdel-Moneim
Hassan, Mohamed Nabil

England
Leaver, Chris
Poostchi, Iraj

France
Ben Tahar, Sofia
Chambonnet, Daniel
Charpiot, Bernard
Dumas de Vaulx, Robert
Gautier, Graines
Gonon, Yves
Ignart, Frederic
Lecouviour, Michel
Picard, Florence
Pitrat, Michel
Risser, Georgette
Robledo, C.

Germany
Schnock, Martin G.
Tatlioglu, Turan

Greece
Fanourakis, Nicholas E.
Vakalounakis, Demetrios J.

Hungary
Milotay, Peter

India
Jain, Jaagrati
Peter, K.V.
Seshadri, V.S.

Israel
Cohen, Yigal
Davidi, Haim
Gaba, Victor
Herman, Ran
Nechama, Shulamit
Niego, Shlomo
Paris, Harry
Ventura, Yaacov

Italy
de Groot, Erik
Lari, Andrea
van Leeuwen, Loes
Vecchio, Franco

Japan
Fujieda, Kunimitsu
Hagihara, Toshitsugu
Han, Sang Joo
Iida, Akira
Iida, Shuichi
Kamimura, Shoji
Kanno, Tsuguo
Kuginuki, Yasuhisa
Mochizuki, Tatsuya
Oizumi, Toshikatsu
Oridate, Toshiroh
Shiga, Toshio
Shintaku, Yurie
Yamanaka, Hisako
Yukura, Yasuo

Jordan
Kaswari, Mahmoud

Korea, Republic of
Kwack, Soo Nyeon
Lim, Haktae
Om, Young-Hyun
Park, Hyo Guen
Song, Jin-Soo

Mexico
Cano Rios, Pedro
Ortega, Sergio Garza
Steta, Mario
Warid, Warid A.

Peru
Holle, Miguel

Poland
Niemirowicz-Szczytt, Katarzyna

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Monteiro, Antonio A.

Romania
Poli, Virgil

Saudi Arabia
Al-Sulaiman, Abdul Mohsen I.

Spain
Ayuso, Ma Cruz
Corella, Pilar
Gómez-Guillamón, Maria Luisa
Kraakman, Peter
Nuez, Fernando
Roig, Luis A.
Semillas Fito, S.A.,

Sudan
El Jack, Ali Elamin
Omara, Sadig Khidir

Sweden
Lehmann, Louis Carl

Thailand
Maneesinthu, Likhit

The Netherlands
Beekman, A.G.B.
Boorsma, P.A.
de Ruiter, A.C.
Groenewal, Irma
Hertogh, K.
Klapwijk, Ad. A.
Reuling, G.
van Deursen, S.
van Kooten, Hank
Zÿlstra, Sierd

Covenant and By-Laws of the Cucurbit Genetics Cooperative

ARTICLE I. Organization and Purposes

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

ARTICLE II. Membership and Dues

The membership of the CGC shall consist solely of active members; an active member is defined as any person who is actively interested in genetics and breeding of cucurbits and who pays biennial dues. Memberships are arranged by correspondence with the Chairman of the Coordinating Committee.

The amount of biennial dues shall be proposed by the Coordinating Committee and fixed, subject to approval at the Annual Meeting of the CGC. The amount of biennial dues shall remain constant until such time that the Coordinating Committee estimates that a change is necessary in order to compensate for a fund balance deemed excessive or inadequate to meet costs of the CGC.

Members who fail to pay their current biennial dues within the first six months of the biennium are dropped from active membership. Such members may be reinstated upon payment of the respective dues.

ARTICLE III. Committees

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

ARTICLE IV. Election and Appointment of Committees

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

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

In the event of death or retirement of a member of the Coordinating Committee before the expiration of his/her term, he/she shall be replaced by an appointee of the Coordinating Committee.

Members of other committees shall be appointed by the Coordinating Committee.

ARTICLE V. **Publications**

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

ARTICLE VI. **Meetings**

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

ARTICLE VII. **Fiscal Year**

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

ARTICLE VIII. **Amendments**

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

ARTICLE IX. **General Prohibitions**

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

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

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

ARTICLE X. **Distribution on Dissolution**

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

Approvals:

W. Bemis

J. D. Norton

R. W. Robinson

W. R. Henderson

M. L. Robbins

R. L. Lower

Cucurbit Genetics Cooperative

Financial Statement

31 December 1991

Balance (31 December 1990)			**$5,197.58**
Receipts:			
	Dues & CGC back issue orders	$1,897.00	
	Cucurbitaceae '89 back issue orders	$20.00	
	Interest on savings	$251.20	
	Total receipts		$2,168.20
Expenditures:			
	CGC Report No. 14 (1991)		
	Printing	$1,517.12	
	Mailing	$524.85	
	Reprint CGC back issues		
	CGC Rept. No. 3 (1980)	$364.77	
	CGC Rept. No. 8 (1985)	$384.20	
	CGC Rept. No. 10 (1987)	$377.27	
	Call for papers (Report No. 15)	$97.25	
	Miscellaneous (envelopes, postage, etc.)	$64.54	
	U.S. FDIC bank fees	$9.09	
	Total expenses		$3,339.09
Balance (31 December 1991)			**$4,026.69**