Cucurbit Genetics Cooperative

17

July 1994

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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
1994-95	$14.00 US*	$24.00 US

*Payment must be by a check drawn on a U.S. bank, or by a U.S. or International Postal Money Order. Checks and Money Orders should be made payable to "Cucurbit Genetics Cooperative." Airmail subscription rates for the CGC Report are also available upon request.

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 1995 Report (CGC Report No. 18) will be mailed in August 1994. Papers should be submitted to the respective Coordinating Committee members by 31 December 1994. The Report will be published by June/July 1995. As always, we are eager to hear from CGC members regarding our current activities and the future direction of CGC.

Gary W. Elmstrom (melon)	Dennis T. Ray (watermelon)
Mark G. Hutton (other genera)	Jack E. Staub (cucumber)
J. Brent Loy (*Cucurbita* spp.)	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 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:	Billy B. Rhodes
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
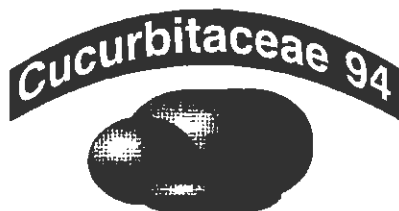
Cucurbitaceae 94

**Evaluation and Enhancement
of Cucurbit Germplasm**

**1-4 November 1994
South Padre Island
Texas**

"Cucurbitaceae '94: Evaluation and Enhancement of Cucurbit Germplasm" will be held 1-4 November 1994 at the Radisson Resort on South Padre Island, Texas USA. It will be hosted by the Texas Agricultural Experiment Station and Extension Service and the USDA-ARS Subtropical Agricultural Research Laboratory.

The purpose of Cucurbitaceae '94 is to provide a forum for the presentation and exchange of scientific information about germplasm evaluation and enhancement research activities on cucurbit crops. All persons engaged or interested in these research areas are invited to participate. (We anticipate that the USA Cucurbitaceae meetings will continue on a four-year schedule, alternating every two years with the Eucarpia meetings in Europe.)

The Cucurbitaceae '94 scientific program will consist of posters, invited talks, and panel discussions on diseases, host-pest interactions, and genetics related to the enhancement of cucurbit germplasm. Molecular and genetic aspects of diseases, germplasm resources, breeding strategies, and the physiology of fruit quality are some of the topics that will be covered. All contributed oral presentations and posters will be published in a proceedings as full papers.

Meetings of the following groups will also take place:

Cucurbit Crop Advisory Committee
Cucurbit Genetics Cooperative
National Melon Research Group
Watermelon Research Group
Squash Breeders Group
Cucumber Breeders Group

Advanced registration (prior to 1 September 1994) is $135 US and includes all meals, social events, and a copy of the proceedings. For more information, contact Jim Dunlap, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596 USA (Phone: 210-968-0641; FAX: 210-968-5585; Internet: *j-dunlap@tamu.edu.*

17th Annual CGC Business Meeting

The 1993 CGC Annual Business Meeting was held on Monday 26 July 1993, in Nashville, Tennessee, in conjunction with the 90th Annual Meeting of the American Society for Horticultural Science. There were 22 CGC members and other interested individuals present. Tim Ng began by reporting on the history of the CGC Report through 1993, including membership statistics, report submissions and publication costs.

Two proposed changes to the CGC By-Laws were discussed, one allowing for the CGC Gene List Committee to expand beyond five members and the other to limit the responsibility for maintaining a stock of back issues of the CGC Report to only the most recent five years. It was agreed that the proposed changes should be considered, and that the next step would be to send a mail ballot to the CGC membership as our the By-Laws.

Other business items included the appointment of Bill Rhodes (Clemson, SC) to replace Warren Henderson as the watermelon individual on the Gene List Committee, and a change of the research report format in the CGC Report from a single-column to multi-columns. Finally, tentative plans were discussed concerning the digitization of back issues of the CGC Report to make them available for distribution on magnetic media (e.g., floppy disk).

Changes to CGC By-Laws

As decided at the 1993 Annual CGC Business Meeting, ballots for proposed changes to two CGC By-Laws were mailed to the membership in the fall of 1993. The first ballot item proposed that the Gene List Committee described in Article III, No. 2, be changed from "consisting of five members" to "consisting of *at least* five members." The second proposed that back issues of the CGC Report be changed from "available indefinitely" to "available *for at least the most recent five years*."

A total of 57 ballots were returned. The change to the Gene List Committee wording was approved (52 yes, 2 no, 3 abstain), as was the change to back issue availability of the CGC Report (54 yes, 3 abstain). These changes to the By-Laws are reflected in this issue (CGC Report 17:164-166; 1994).

CGC Meetings in 1994

CGC will hold two meetings during 1994.

The **annual CGC Business Meeting** will be held in conjunction with the annual meeting of the American Society for Horticultural Science at Oregon State University in Corvallis. The meeting is scheduled for Tuesday, **9 August 1994**, from 2:30-3:30 p.m. in room 212 (MLK Room) of the Memorial Union.

CGC will also meet in Texas at Cucurbitaceae '94 in November (see the notice elsewhere in this issue).

US Cucurbit Crop Advisory Committee Update

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

On July 24, 1993 the CCAC held its 10th meeting in Nashville, Tennessee in conjunction with the Annual Meeting of the American Society for Horticultural Science. Molly Kyle was appointed to the Committee.

The GRIN database was moved to Oracle on a Unix mini-computer in order to facilitate information exchange with other countries. A PC (DOS) version of GRIN was released to users; Macintosh and Windows versions were to be released in late 1993. The top CCAC germplasm priorities were cage isolations for Geneva and Griffin; a gene stock center was second priority. The concept of test arrays has begun to replace that of a core collection for germplasm evaluation of each species. Test arrays could be made for different diseases, insects, etc., or could be customized for any specific purpose. All CCAC germplasm evaluation datasets received to date have been entered into GRIN. Elimination of duplicates from the National Seed Storage Laboratory should be done cautiously: Gary Elmstrom found three accessions of 'Summit' watermelon to differ for a key disease resistant trait. USAID is providing funding to VIR for new/improved storage rooms. The CAC Report is six years old and should be updated. This is especially so for the melon section because the melon collection was moved from Griffin to Ames. The watermelon section was recently completed.

Ten new *C. metuliferus* accessions are available from Ames. Maricopa, Arizona is being evaluated for southern increases of some cucurbits. Steve Kresovich was transferred fron Geneva to Griffin, Georgia which needs more field space, or an alternative site such as Maricopa. Griffin is in the process of increasing 341 *Citrullus* accessions with the help of Joe Norton.

Three germplasm evaluation proposals were recommended for funding in 1994: 1) Evaluation of the U.S. plant germplasm collection of melon (*Cucumis melo*) and squash (*Cucurbita moschata* and *C. pepo*) for resistance to gummy stem blight (*Didymella bryonia* Auersw.) Rehm., Investigators: M.A. Kyle and T.A. Zitter; 2) Evaluation of *Cucumis* sp. germplasm for resistance to zucchini yellows mosaic virus, Investigators: J.D. Norton and G.E. Boyhan, Auburn University; and 3) Genetic diversity in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) accessions, Investigators: J.E. Staub and J.D. McCreight. Two proposals were endorsed for special funding from NPGS: 1) Screening of the watermelon (*Citrullus lanatus*) germplasm collection for low-temperature seed germinability, Investigators: M.R. Hall and R.L. Jarret and 2) Cucurbit gene stock center, Investigator: Todd C. Wehner.

The 1994 CCAC meeting will be in conjunction with Cucurbitaceae '94 on South Padre Island, Texas, November 1-4.

Watermelon Research Group - 1994

The Watermelon Research Group met on 31 January 1993 in Tulsa, OK in conjunction with the Southern Region:American Society for Horticultural Science and the Southern Association of Agricultural Scientists. Approximately 50 people attended the session.

The first half of the meeting was dedicated to brief discussions of general interest. Graves Gillaspie, Southern Region PI Station, reported on over 670 entries screened for resistance to watermelon mosaic virus 1 . Many of them were from Africa and appeared good. Larry Hollar, Hollar Seed Co., requested a discussion on the inheritance of resistance to Fusarium wilt in watermelon and the status of *Fusarium oxysporum* f. sp. *niveum* race 2 in the United States. Ray Martyn, Texas A&M University, indicated that inheritance of race 1 resistance was probably single gene dominance and inheritance to race 2 was unknown, but likely to be multiple gene. He also stated that race 2 was present in several states, but as of yet, has not become a wide-scale problem. Benny Bruton, USDA, ARS, Lane, OK, reported on a new yellow vine disease of squash and melons in the Oklahoma/Texas area. Glen Price, American Sunmelon, expressed interest in the genetics of water-melon rind characteristics and if they could be altered, and also mentioned what he thought was the smallest watermelon - 30 grams! Joe Norton, Auburn University, reported on his program for selecting resistance to ZYMV in squash and anthracnose resistance in watermelon. He also indicated that AU-Producer will grow out ZYMV infection in the greenhouse. Don Maynard, UF-Gulf Coast REC, described several symptoms of unknown etiology on fruits. These included ring spots on varieties with darker rinds, blotchy disease on Crimson Sweet, cross-stitch disease on All Sweet, Sangria, and Fiesta, and white leaf on Tri-X 31 3. Marty Baker, TAES, Overton, TX reported on the problems associated with variation in germination rates of the seedless varieties. Gary Elmstrom, UF-Central Florida REC led a discussion on rind patterns, colors, and shades in watermelon fruit and questioned whether we should attempt to "officially categorize" fruit types for breeders and seedsmen to use.

The second half of the meeting was devoted to a discussion on the watermelon fruit blotch disease. Ron Gitaitis, Coastal Plain Experiment Station, Tifton, GA opened the session with his efforts in developing a selective or semi-selective medium for detecting the bacterium in seed. Rick Latin, Purdue University, followed with his work on the seedborne nature of the pathogen and its spread, and Tom Kucharek, University of Florida, concluded with his research on the spread of the pathogen on foliage in the greenhouse.

A very impressive publication on bacterial fruit blotch of watermelon was distributed. This was written jointly by scientists from the University of Florida, University of Georgia, and Clemson University and supported by eight different seed companies and the National Watermelon Association. Another publication was also announced, the Watermelon Production Guide, which is available through the University of Florida for $7.00.

The last item of business was Gary Elmstrom announcing he was stepping down as Chairman of the Watermelon Research Group. Gary has served as chairman of the group since its inception in 1980 and has been instrumental in making the group what it is today. Ray Martyn has accepted the chairmanship for the next interim.

Next year's meeting will be in Nashville, TN, February 5-9, 1994. The headquarters hotel will be the Opryland Hotel.

The Cucurbit Network (TCN)
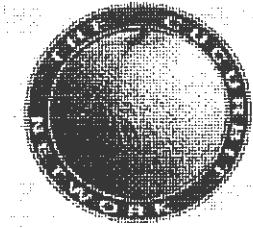
CGC members Deena Decker-Walters, Thomas C. Andres and Terrence W. Walters have recently established The Cucurbit Network (TCN) to facilitate the exchange of ideas among different groups of "cucurbitologists." TCN is targeted towards a broad audience, including growers, enthusiasts, seed companies, pharmaceutical companies, and researchers in other disciplines, many of whom may have valuable cucurbit information or germplasm which they would be willing to share.

Dedicated to promoting the conservation and understanding of the Cucurbitaceae through education and research, TCN publishes a semiannual newsletter called The Cucurbit Network News (TCN News). TCN News strives to give its readership timely information on events and meetings, publications (both book reviews and recent citations), and societies of interest to cucurbitologists. Also featured are short articles on individual cucurbits and cucurbitologists. For example, a section called "Profiles in Cucurbits" discusses lesser-known taxa or lesser-known aspects of the better-known species, and cucurbit researchers and/or enthusiasts are profiled in special "Meet..." articles. The "Bulletin Board" section of the newsletter includes advertising space available to TCN members, including individuals and businesses. Here, one can post requests for plant material or literature, direct items of concern to the cucurbit community, or make inquiries related to current research projects. TCN members are encouraged to become active contributors to TCN News.

Annual membership rates for TCN are $5 for USA, Canada and Mexico ($10 for other countries). For more information, or to subscribe, please contact TCN at P.O. Box 560494, Miami, FL 33256, USA.

Melon, not Muskmelon

At Cucurbitaceae '89 (Charleston, S.C.), Larry Hollar began a campaign to have "melon" replace "muskmelon" as the official U.S. common name for *Cucumis melo* L. This campaign has borne "fruit" in that the Agriculture Marketing Service of the U.S. Department of Agriculture recently proposed an Amendment to Regulations Under the Federal Seed Act to change the kind name of *Cucumis melo* to "melon." "Cantaloupe" and "muskmelon" would be acceptable synonyms of "melon." [The proposed amendment began on page 25706 in Vol. 59, No. 94, of the Federal Register (17 May 1994), with the proposed change to "melon" appearing on 25710. A Public Hearing was held on 8 June 1994, and written comments were solicited up until 8 July 1994.]

Senescence of Note: <u>Thomas Wallace Whitaker</u>

James D. McCreight
USDA-ARS, U.S. Agricultural Research Station, 1636 East Alisal Street Salinas, CA 93905 (USA)
G. Weston Bohn
1094 Klish Way, Del Mar, CA 92014 (USA)

Dr. Thomas W. Whitaker born August 13, 1905 in Monrovia California passed peacefully away November 29, 1993 in La Jolla, California. He was 89 years old. Tom was a seventh generation Californian whose family helped found the city of Los Angeles. He is survived by two children, five grandchildren and eight great-grandchildren.

Tom received his B.S. from University of California, Davis in 1927. He went to University of Virginia as a DuPont Fellow where he earned masters and doctoral degrees in genetics and cytology in 1929 and 1931. A Post-Doctoral Fellow took him to the Bussey Institution at Harvard University through 1933. While there, he associated with many well known geneticists including W.E. Castle and E.M. East. Afterwards, he was Associate Professor of Biology at Agnes Scott College, Decatur, Georgia through 1936. At that time Tom joined the staff at the USDA-ARS, Horticultural Field Station, La Jolla, California. There he carried out research on cytology, genetics and breeding of lettuce (*Lactuca sativa* L.), melons *Cucumis melo* L.) and *Cucurbita* spp., and their lesser known relatives until his retirement in 1973.

Tom was twice awarded Guggenheim Foundation Fellowships. He was active in several scientific societies including American Society for Horticultural Science (President, Editor and Associate Editor), American Association for the Advancement of Science, American Genetic Society, American Phytopathological Society, American Plant Society (Executive Secretary), American Society of Naturalists, California Botanical Society, San Diego Society for Natural History (President), Sigma Xi, Society for Economic Botany (President; Distinguished Botanist, 1980), Society for the Study of Evolution, The Torrey Botanical Club and the Torrey Pines Association (President). In addition, he served on the Research Council of the San Diego Zoo and was a Research Associate at Scripps Institute of Oceanography, La Jolla, California.

Tom was a prolific author and wrote about 200 papers including research reports, technical reports, popular articles and (with G.N. Davis) the classic review book, Cucurbits. Tom had great interest in archeological studies of cucurbits. He and his colleagues elucidated evolutionary relationships among various cucurbits including *Cucurbita* and *Cucumis*. He helped develop several landmark varieties and breeding lines, most notably Great Lakes type lettuce and PMR 45 melon.

Tom participated in numerous germplasm collection expeditions. Two of these led to the description of two plant species: *Cucurbita ecuadorensis* Cutler and Whitaker, and an*Amaryllis* species. Two post-retirement expeditions added significant numbers of *Cucurbita* spp. (with Robert L. Knight) from Mexico, and wild lettuce species (*L. saligna* and *L. serriola*) from Turkey and Greece (with R. Provvidenti) to the U.S. National Plant Germplasm System.

Tom was well known and liked for his generosity, kindness and sense of humor. He was blessed with a phenomenal memory for facts and details, and the abilities to speed-read and quickly recall this information. This allowed him to know virtually all of the U.S. plant literature during his pre-retirement years, and remember people's faces and names. The latter ability was especially helpful to his colleagues not so blessed. He was accomplished in many areas of botany and agricultural science, yet he was unassuming and genuinely interested in the work and interests of his colleagues and friends. Tom will be fondly remembered by all who knew him.

A Core Collection for Cucumber: To Be or Not To Be

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

In the recent past there have been discussions among scientists interested in germplasm management regarding the definition and construction of core collections for agronomic and horticultural crop species (Brown 1989a,b; Frankel and Brown, 1984). A core collection might be defined as a subset of a larger array of germplasm accessions (collection). A core collection attempts to maintain the majority of diversity present in a larger (whole) collection, and thus provides for the potential efficient access to the possible range of variation found in the larger collection.

A core collection should be constructed on the basis of unique descriptive determinants (characters or traits) so that the core is clearly defined in quantitative or qualitative terms. Thus, "the core concept entails identifying a range of accessions within a collection, the total of which include, with an acceptable level of probability and with minimum redundancy, most or much of the range of genetic diversity in the crop species and its relatives" (National Research Council, 1991). The U.S. Germplasm Resources Information Network (GRIN) now contains information on the evaluation of about 750 accessions for disease resistance (7 economically important diseases), cold, drought and heat tolerance, yield (as measured by F_1 combining ability), various morphological characteristics (passport and evaluation data), and biochemical variation (14 isozyme loci).

There has been some debate over the applicability of the core concept to the U.S. cucumber collection. Discussions have occurred at a number of U.S. Cucurbit Crop Advisory Committee meetings and at the annual meetings of the American Society for Horticultural Science. It has been thought that large collections such as those of corn, soybeans and wheat might benefit from the use of the use of core collections (National Research Council, 1991). Nevertheless, it has been suggested that the application of a core collection for these crops be handled cautiously. It is feared that the management of the larger collection might be compromised by exclusive maintenance of core collections. Furthermore, core collections, if not properly constructed, might misrepresent the true variability present in the collection.

Hundreds of national and international requests are made to the U.S. National Plant Introduction system each year for seed samples of cucumber accessions. Given that the U.S. cucumber collection is relatively small (1000+ accessions) and that it may be possible to devise a core collection for cucumber, the question is whether such a core should be constructed. Therefore, I conducted a survey in 1992 intended to ascertain the general feeling among U.S. cucumber researchers regarding the potential need of a cucumber core collection. If the feelings were relatively positive my intent was then to gather information which might be useful in constructing a core collection. In order to reach my objectives, I surveyed 54 U.S. cucumber researchers inquiring as to: 1) their depth of knowledge regarding the Core Concept and its applicability for the management of germplasm collections; 2) their feelings regarding the construction of a core collection for cucumber; 3) whether such a core should be based on the frequency of inquiry to the GRIN system and/or regional plant introduction stations for a particular trait; and, 4) what criteria might be used to establish a core in cucumber (given those found presently in GRIN) and how they would prioritize such criteria. This survey was not meant to be comprehensive or definitive, but rather to provide a springboard for further discussion.

There were 24 respondents to my questionnaire (54 surveyed). Of these one indicated that he was not knowledgeable about the Core Concept, 16 felt they were fairly knowledgeable, and seven regarded themselves as highly knowledgeable. While eight respondents felt that the Core Concept was applicable to cucumber, 12 felt it might have value, and three indicated that it would be of no value. Some of their comments are provided in Table 1. Fully 23 respondents felt that the establishment of a core should not be based on the frequency of inquiry to the GRIN system and/or regional introduction stations for a particular trait. The respondents differed in their perception of what criteria might form a core for cucumber (Tables 2 and 3). These results suggest that there is a desire and need for continued discussion regarding the Core Concept as it might apply to cucumber.

Literature Cited

Brown, A.H.D. 1989a. The case for core collections. p. 136-156. In: A.H.D. Brown, O.H. Frankel, D.R. Marshall, and J.T. Williams (ed.). The Uses of Plant Genetic Resources. Cambridge University Press, New York.

Brown, A.H.D. 1989b. Core collections: A practical approach to genetic resources management. Genome 31:818-824.

Frankel, O.H., and A.H.D. Brown. 1984. Plant genetic resources today: A critical appraisal. p. 249-257. In: J.W. Holden and J.T. Williams (ed.). Crop Genetic Resources: Conservation and Evaluation. Allen and Unwin, London.

National Research Council. 1991. Prescription for effectiveness. p. 124- 126. In: Board on Agriculture, National Academy of Sciences. Managing Global Genetic Resources: The U.S. National Plant Germplasm System. National Academy Press, Washington DC.

Table 1. Some comments from respondents to the question of whether the core concept was applicable to cucumber.

In general, my feeling is that it is not appropriate for cucumber at this time. The number of cucumber introductions is quite modest, in comparison with cereals and some other crops, and it should not be too much of a burden to maintain most, if not all, of them. In many cases, we have insufficient information to eliminate a cucumber introduction from the core. An exception is that of duplications. There are many cases known of the same cultivar assigned two or more PI numbers, and of multiple accessions at the NSSL of the same cucumber cultivars. I suggest that all but one of the duplications be eliminated from the core.	Cucumber collection size is marginal, at this time, for full benefit of core concept. The cantaloupe collection probably is large enough. *Cucurbita* species are probably large enough if all are considered together. *Cirtullus* spp. probably is large enough. When other appropriate data are not available, geographic origin has been very useful in developing cores for alfalfa, lentils, chickpeas, and *Phaseolus* beans.
Collections are to be limited both in number and in geographic representation.	Reduction in time required to incorporate PI germplasm into commercial lines by using the most valuable material.
One of the best breeders in Egypt requested 1,000 *C. sativus* accessions and, not having received them, requested my help in getting from PI Station, Ames. I cannot support such a request. A representative subset is what he should get initially.	I would use the germplasm collection in helping to find traits of interest that are not in adapted germplasm, usually this would be rare or unusual traits. Limiting the number of accessions that are very active may limit the utility of the collection.
The core concept could work with large, well-documented collections.	While the core concept may have great applications for agronomic crops, how often do we get requests in cucumber for more than 20 accessions from any one person?
My impression is that for some cucurbits the total number of accessions is not that high compared to wheat or corn. Is the number of cucumber accessions low enough, after deleting duplications, that it could function as a core?	The core collection should be used as a genetic tool or window to view the diversity represented in a given collection. For some traits the whole collection can be screened, while for others, screening a core could provide direction and save resources at the same time. It must be noted, however, that developing a "core collection" for a crop will generate *more* than less germplasm maintenance problems.
Evaluation and utilization would be more efficient and more appropriate plant collecting proposals could be prepared.	In cases where the collection is large and represents a broad spectrum of genetic diversity for the crop and there is a high proportion of duplication, I think the core concept may have merit if *carefully* implemented.
Permits in-depth study of representative cultigens.	I don't see the core collection so much as a management practice. I see the core collection more useful as a tool in evaluation work, seed requests for representative samples of germplasm collections, etc. Many times we receive requests for entire PI collections from people who have little knowledge of the NPGPS. Core collections would be helpful in these situations.

Table 2. Mean and standard deviation of rankings of criteria that might be considered in the establishment of a core collection for cucumber.

	Respondent[1]																				$\bar{X}$	SD
	1	2	3	4	5	6	7	8	9	10	11	12	13	14[3]	15	16	17	18	19	20		
Disease	1	1	1	2	1	4	1	1	1	3	1	3	2	1	3	2	3	1	1	2	1.8	1.0
Cold	5	2	3	4	3	5	5	6	5	3	3[2]	5	5	1	4	3	4	4	.2	5	3.9	1.3
Yield	6	6	5	6	5	6	3	2	6	6		6	4	1	5	1	5	5	5	6	4.7	1.7
Morphological	2	3	4	1	2	2	2	5	3	2		4	1	1	2	6	2	2	3	3	2.6	1.3
Biochemical	3	5	6	3	4	3	6	4	4	2	2	2	3	1	1	4	1	3	4	1	3.1	1.5
Suggest			5				4		2				6	1	6	5			6	4	3.5	1.9
Drought	4																					
Insect		4							3													
Unique trait			2[4]																			
Geographical origin							1			1		1										

[1]Some respondents did not complete question 4.
[2]Respondent stated. "Considering the resources involved, this seems less useful and much more expensive."
[3]Respondent felt that all parameters were equally important.
[4]Trait not specified.

Table 3.   Summary of comments regarding the construction of a core collection in cucumber

Frequency of inquiry to PI stations would reflect past use, especially for PIs referred to in publications. To appropriately define a core one would need complete knowledge of all future needs for a crop. Each user of PI collections asks different questions each time they use the collection. If I'm interested in fruit size, I'll scan descriptor data on fruit size and select from that basis. If I'm interested in disease resistance when the disease only occurs in Nepal, I may only evaluate accessions for Nepal and adjacent regions, etc. For a CAC or other crop experts to define one core collection would mean that the rest of the collection is less important. Unless a committee is set up to define a core for **each** project which would like a core, the core concept cannot work as theorized. Until we know **much** more about each of our collections, probably choosing random accessions evenly distributed over a geographic range will best suffice as cores.	Our experience is that PIs from similar locations are more likely to share an attribute of interest for breeding. Thus, geographical distribution of accessions, and selection within an area based on collection in different habitats, is probably the most efficient way to preserve diversity that we may not be able to identify as yet. I'd suggest also gross morphological differences are much more significant than single gene traits such as white spine. In my opinion, the use of combining ability in this contest is useless. I suggest you redefine your quest as one to develop 'test arrays' of *C. sativus* which would be useful for various breeding/genetic/research purposes. For example, a disease resistance array, an eco-geographic origin array, an American Slicer array, etc. Maximize phenotypic diversity with each array but build around your target objectives. The 'core concept' requires accuracy in assessing *genetic* diversity and I don't believe we're close to knowing that well enough.
Instead of defining core collections it would be more productive to a) eliminate duplications, b) obtain good data on a wide range of high priority descriptors (as determined by CAC), and c) train potential PI users on PI information and GRIN use.	As a way to come up with accessions to include in a Core, I think an advisory group consisting of the NPGS cucumber curator, members of the CCAC, CGC, and other breeders and geneticists should be selected to represent a wide array of perspectives.
Aim should be to maximize diversity. I can visualize disease resistance and stress tolerance as either favorable or unfavorable depending on how they are used.	Should develop data base for cucumber PIs, then analyze diversity. Choose one PI from each group to make 40-80 top cultigens.

A Core Collection for Cucumber: A Starting Point

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James McCreight and I have recently returned from a plant collection trip to India (McCreight and Staub, 1993). This collection trip increased the number of accessions in each of the U.S. cucumber and melon collections by approximately 20%. These accessions are currently being increased at the Regional Plant Introduction Station, Ames, Iowa. After their increase these accessions will be further evaluated for various morphological and biochemical polymorphisms.

Although collection and evaluation efforts such as these are important for maintaining an adequate level of genetic diversity in these crops, the management of the collections will be increasingly difficult given the projected limited resources. It has been the continued desire of the U.S. Plant Introduction System to devise efficient management strategies which enable the use of a wide range of diverse materials in collections. This has led to proposals for the development of core collections. A core collection should not be considered a separate collection, but rather a subset of an existing collection. The purpose of a core collection is to facilitate use and provide efficient access to the potential variation that exists in the whole collection (Brown, 1989a,b; Frankel and Brown, 1984).

It has been suggested in discussions at U.S. Cucurbit Crop Advisory Committee meetings and other U.S. national meetings on germplasm diversity (e.g., meetings of the Genetics and Germplasm Working Group, American Society for Horticultural Science) that the cucumber collection is small and not well characterized. This has led to a general consensus that cucumber would not lend itself to the establishment of a core collection. However, based on the fact that the cucumber collection has now been evaluated for various disease resistances, cold, drought and heat tolerance, yield performance, various morphological characteristics, and biochemical variation, I submit that further discussion on this subject is warranted.

In this edition of the Cucurbit Genetics Cooperative Report, I reported the results of a survey intended to ascertain the general feeling among U.S. cucumber researchers regarding the potential need of a cucumber core collection. This survey suggested that there is a desire and need for continued discussion regarding the Core Concept as it might apply to cucumber. In an effort to stimulate such a discussion, in 1993 I requested that the 24 respondents of the original survey provide me with more information regarding their impressions on which cultigens (adapted or unadapted inbreds, hybrids, populations) might be used to construct a core collection in cucumber if it were deemed appropriate. The results of this survey are presented in Table 1.

For a core collection to be effective it must mirror the genetic diversity found in the whole collection of which it is a subset (National Research Council, 1991). Typically core collections are considered to house no more than 10% of the collection from which they are drawn (Brown, 1989a,b; Frankel and Brown, 1984). I suggest that further discussion as to the potential establishment of a core collection for cucumber be based on these two guiding principles (i.e., potential diversity and size). It is possible that, based on the present genetic information available, cucumber subsets might be constructed which would provide researchers with accessions which, taken collectively, possess genetic diversity for specific characteristics (e.g., disease resistance and environmental stress tolerance).

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Table 1. Cultigens/PIs which warrant consideration for possible inclusion in a core
collection for cucumber.

Cultigen/PI	Reason for inclusion
163213	anthracnose resistance
163217	anthracnose resistance
164743	low reducing sugar concentration
165499	disease resistance
165509	disease resistance
173889	disease resistance, _Bt_ gene
175120	multiple tolerance or resistance
175686	low soluble solids
175697	fruit pH of 5.9
179676	multiple disease resistance, downy mildew, good fruit type
181755	cold germination
183967	multiple & sequential fruiting character, hardwickii
188749	triazine tolerance
188807	vigor, wilt and multiple disease resistance, triazine tolerance
197085	multiple disease resistance
197087	anthracnose, downy, powdery mildew resistance, India
197088	multiple disease resistance
200815	wilt resistance
200818	wilt resistance, bacterial wilt resistance
211983	cold germination
211984	amiben tolerance
212233	powdery mildew resistance
212599	cold germination
220791	cold germination
220860	multiple disease resistance, gynoecious character
222243	cold germination
227207	multiple disease resistance
227208	multiple disease resistance
234517	powdery mildew resistance
249561	disease resistance
249562	disease resistance
257486	low soluble solids
263079	cold germination
264668	amiben tolerance, low soluble solids
267087	cold germination
267746	biochemical variation
267747	Alko bush cucumber
267942	multiple disease resistance
269480	biochemical variation
275411	fruit pH of 6.9
279465	disease resistance, angular leaf spot, 'Natsufushinari'

283902	low soluble solids
285603	low reducint sugar concentration
288238	powdery mildew resistance; comes from 'Yomahi'
306179	cold germination
308915	dwarf (short internode)
308916	dwarf (short internode)
321006	disease resistance
321007	disease resistance
321008	disease resistance
321011	'Taichung Mou Gua', virus resistance
351139	hermaphroditic
351140	hermaphroditic
355052	best alpar from Israel
356809	hermaphroditic
360939	'kora', European pickle, prolific
369717	androecious
372893	bitterfree
390244	atrazine tolerance
390258	powdery mildew resistance
390260	triazine tolerance
400270	'Kyoto Three Feet'
418962	powdery mildew resistance, downy mildew resistance
418964	powdery mildew resistance, downy mildew resistance
418989	cold tolerant
419009	cold tolerant
419017	heat resistant
426169	powdery mildew resistance
426170	powdery mildew resistance
432860	biochemical variation
432895	powdery mildew resistance
451975	powdery mildew resistance, downy mildew resistance
451976	powdery mildew resistance, downy mildew resistance
458845	biochemical
Addis	disease resistance, long pickle
Arkansas Little Leaf	sequential fruiting, multiple branching
ASHE	
Ashley	classic variety, old slicer
Chinese Long	CMV, PRV, WMV, ZYMV resistance
Chipper	disease resistance, fruit quality, fruit shape, old pickle
Clinton	pickle, dark green fruit, small seed cavity, disease resistance
Coolgreen	Beit Alpha
Delcrow	late slicer
Dual	long pickle
Delikatess	Germany
Galaxy	old pickle
Gemini	Clemson University

Gy 54/57	disease resistance, gynoecious slicing hybrid parent, GCA*
Gyn 3	disease resistance, gynoecious
Gyn 14	disease resistance, gynoecious character, GCA
Gyn 4	disease resistance, high yielding
Gyn 5	disease resistance
Gyn 57u	gynoecious slicer
Hokus	
Homegreen #2	GSB resistant**
Lemon	genes _m_,_l_, and _yg_
M 21	disease resistance, determinate gene, fruit quality, GCA, dwarf
M 27	disease resistance, dwarf
Marketmore	slicer, non-bitter, uniform color, classic for type & disease resistance
Marketmore 76	classic for type & disease resistance, northern slicer
Marketmore 76F	disease resistance
Marketmore 80F	disease resistance, gynoecious slicer
Marketmore 83	slicer representative
Marketmore 85	dwarf slicer
Marketmore 87	disease resistance
Marketmore 88	disease resistance, slicer
Marketer	Important sw check; induced resistance, classic variety
Minn. Dwarf XII	_de_
Model	classic variety
MSU 713-5	disease resistance
National pickling	historically important
Ohio MR 17	historically important
Palmetto	Clemson University
Pixie	Clemson University, bloater susceptible
Poinsett	dm resistance; classic for type & disease resistance
Poinsett 76	classic for type & disease resistance, southern slicer
Poinsett 83F	gynoecious slicer
Poinsett 87	disease resistance & scab, CMV, and TLS***
Poinsett 88	disease resistance, slicer
Polaris	dm resistance, Clemson Univ.
Regal	high yield, long pickle type
Slice	GSB resistance
SMR 18	classic variety, disease resistance, vigor, cold tolerance, GCA
Spacemaster	slicer, determinate vine, dwarf slicer
Spartan Salad	PMR and some DMR and CMV
Straight 8	old susceptible slicer
Stono	Clemson University
Summit	old southern pickle
Sumter	old southern pickle
Supergreen-butalpha	Beit Alpha type
Tablegreen 65	mosaic resistance
Tablegreen 72	old slicer

Telegraph	parthenocarpic
Tiny dill	dwarf pickle
TMG-1	CMV and virus resistance
Tokyo Long Green	virus resistance
W1082HP	hermaphrodite, parthenocarpic, pickle
W2757	multiple disease resistance pickle; combining PMR with TLS
Wautoma	northern pickle
White wonder	white slicer
Yomaki	homozygous F/F
Zeppelin	schalgurken

* GCA = General combining ability for yield as measured by F_1 performance.
** GSB = Gummy stem blight resistance
*** TLS = Target leaf spot resistance

A Set of Cucumbers to Represent the American Market

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I often receive requests for a set of cultigens (cultivars, breeding lines and plant introduction accessions) that represents the diversity of the American cucumber market. The cultigen set might be used for testing herbicides, starting a breeding program, or for basic research where a representative sample is needed for proper extrapolation to the whole population. It is difficult to define a set of cultigens that represent all of the diversity, but after many genetic studies I have settled on a primary and a secondary set that serve the purpose. The primary set would suffice if only a small sample can be grown, but the secondary set should be added for better representation. For breeding, the cultigens should be chosen that represent the types needed.

The cultigens presented here are a subset of those recommended to expand the USDA cucumber germplasm collection (1). I have classified them by fruit type, sex expression, region of adaptation, and anthracnose resistance (Table 1). Fruit type is American pickle or slicer type, and there are also some round- fruited and Beit Alpha (middle-eastern) types that have useful traits. Sex expression is gynoecious, monoecious or hermaphroditic. Region is the general area of the U. S. used for breeding and testing. Usually, southern cultigens have white spined fruits and resistance to anthracnose, while northern cultigens have black or white spined fruits and resistance to scab and cucumber mosaic.

Perhaps other researchers can suggest representative sets that work better, or that cover different market areas of the world.

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Table 1. A set of 12 primary and 28 secondary cucumber cultigens that represent the American market[z].

Cultigen	Type	Sex	Region	AR	Notes
Primary (12 cultigens)					
Coolgreen	Beit Alpha	Mon	N/A	S	Susceptible to most diseases
Gy 14	Pickle	Gyn	South	R	Common inbred
Gy 57u	Slicer	Gyn	South	R	Common inbred
H-19	Pickle	Mon	South	R	Little leaf
M 21	Pickle	Mon	South	R	Dwarf-determinate
Marketmore 76	Slicer	Mon	North	S	Common inbred
Poinsett 76	Slicer	Mon	South	R	Common inbred
Straight 8	Slicer	Mon	North	S	Standard of 1940s
Sumter	Pickle	Mon	South	R	Standard of 1980s
Tablegreen 72	Slicer	Mon	North	S	Standard of 1970s
WI 2757	Beit Alpha	Gyn	North	R	Multi-disease resistant
Wisconsin SMR 18	Pickle	Mon	North	S	Standard of 1960s
Secondary (28 cultigens)					
Addis	Pickle	Mon	South	R	Long fruits
AR 75-79 (Little John)	Pickle	Mon	South	R	Little leaf
Armstrong E. Cluster	Round	Mon	N/A	S	Early flowers
Chipper	Pickle	Mon	South	R	Blocky fruits
Clinton	Pickle	Mon	South	R	Blocky fruits
Delcrow	Slicer	Mon	North	S	Late yielding
Early Russian	Pickle	Mon	North	S	1854 cultivar
Gy 3	Pickle	Gyn	South	R	Common inbred
Gy 4	Pickle	Gyn	South	R	High yielding gynoecious
Homegreen#2	Slicer	Mon	North	S	Gummy stem blight resistant
Lemon	Round	Herm	North	S	Hermaphroditic
LJ 90430	Round	Mon	N/A	S	Wild type
M 27	Pickle	Mon	South	R	Dwarf-determinate
Marketmore 80 Bw	Slicer	Mon	North	S	Common inbred
Marketmore 85	Slicer	Mon	North	S	Small dwarf
Marketmore 86	Slicer	Mon	North	S	Medium size dwarf
Model	Pickle	Mon	South	S	Standard of 1940s
MSU 713-5	Pickle	Gyn	North	S	First gynoecious inbred
National Pickling	Pickle	Mon	North	S	Standard of 1930s
Producer	Pickle	Mon	South	S	Standard of 1940s
Redlands Long White	Slicer	Mon	North	S	White fruits
Slice	Slicer	Mon	South	R	Multi-disease resistant
Spacemaster 80	Slicer	Mon	North	S	Dwarf-determinate
Spartan Salad	Pickle	Mon	North	S	Powdery mildew resistant
SR 551	Pickle	Mon	North	S	Scab resistant pickle
TMG-1	Trellis	Mon	N/A	S	Virus resistant
Wautoma	Pickle	Mon	North	R	Multi-disease resistant
White Wonder	Slicer	Mon	North	S	White fruits

[z]Fruit type is American pickle or slicer, round-fruited, or Beit Alpha (middle-eastern).
Sex expression is gynoecious (Gyn), monoecious (Mon) or hermaphroditic (Herm).
Region is north or south U.S. or not applicable (N/A).
Anthracnose resistance (AR) is resistant (R) or susceptible (S).

Cucumber Cultivar Cluster Analysis

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Cultivar genetic clustering resulting from differences in trait expression can be the basis for parental selection in hybrid breeding programs. With a prerequisite of fitting cucumber types to local consumer customs, it is expected that heterosis can occur between cultivars which are the most different. Such differences among traits can be selected from corresponding categories according to the principle of complementary traits.

Method. An experiment was conducted at the Horticulture Station of Northwestern Agricultural University during the summer of 1991. Fifteen cultivars and inbreds possessing contrasting trait differences were evaluated in a two-direction randomized block design with 3 replications. The cultivars and inbreds used were: 1) 58-663; 2) Jin 4-3-1; 3) 'Jinan'; 4) Heidan-1; 5) 121713; 6) 3511; 7) Changmi-1; 8) 'Mai'; 9) 'Pingli'; 10) Yue 82; 11) Shifeng- 8; 12) Jin 1717; 13) 'Baitoushuang'; 14) Jiyu-2; and, 15) 'Najingci'. Twenty- two quantitatively inherited traits were evaluated during the cucumber growing season. The traits evaluated were: average yield per plant (x_1); number of harvested fruits per plant (x_2); average fruit weight (x_3); early yield per plant (x_4); number of harvested fruit per plant in the early stage (x_5); average fruit weight in the early stage (x_6); number of leaf at first harvesting stage (x_7); leaf area at the first harvesting stage (x_8); the node position of the first pistillate flower (x_9); number of leaves at the end of growth season (x_{10}); number of nodes at the end of growth season (x_{11}); number of nodes at the end of growth season (x_{12}); total number of effective branches (x_{13}); the days from sowing to the first pistillate flowering plant in the population (x_{14}); the days from sowing to the pistillate flowering of 50% of the plants (x_{15}); fruit length (x_{16}); fruit volume (x_{17}); fruit diameter (x_{18}); average fruit developing average rate (x_{19}); downy mildew resistance (x_{20}); developing relative fruit rate at mid-growth period (x_{21}); and developing relative fruit rate at early stage (x_{22}).

After comparing several methods of estimating genetic distance, Mahalanobis distance (pivotal condensation) was selected for cluster analysis. Genetic distance estimates were clustered using each of the following methods: 1) nearest-neighbor method; 2) furthest-neighbor; 3) median; 4) centroid; 5) group-average; 6) flexible group-average; 7) flexible; and, 8) squares sum of dispersion. The results of clustering were contrasted and analyzed in order to compare clustering methods and to determine the most representative traits for each cultivar group.

Results. Comparisons among different methods of clustering of cultivars and inbreds indicated that the spatial arrangement obtained by the different methods were not similar. Moreover, differences that were observed were mainly in the pattern of the merging of large genetic distances between cultivar and inbreds. The results of different clustering methods can be divided into three types:

The clustering features of the nearest-neighbor method was similar to that of the centroid method (Fig. 1). Fifteen cultivars and inbreds were clustered based on smallest to largest genetic distance, and the clustering core was not distinct. This is especially true of cultivars and inbreds which were not related (i.e. large genetic distance) such as
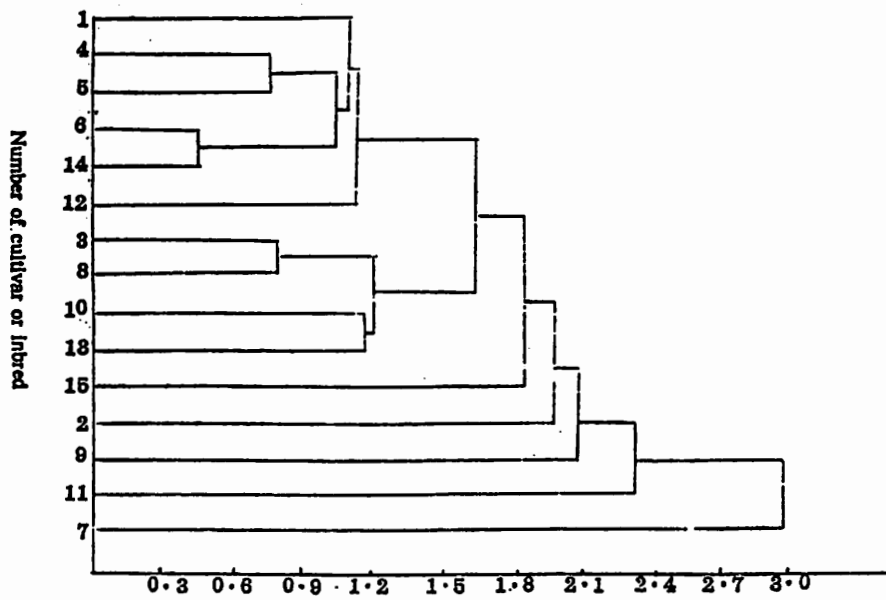
Figure 1. Clustering tree by the nearest—neigbour method
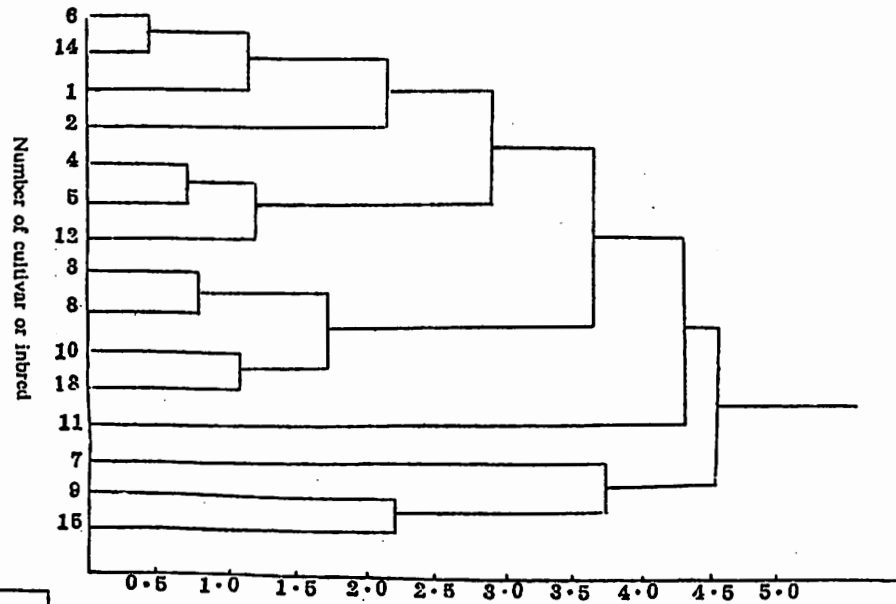
Figure 2. Clustering tree by the furthest—neigbour method
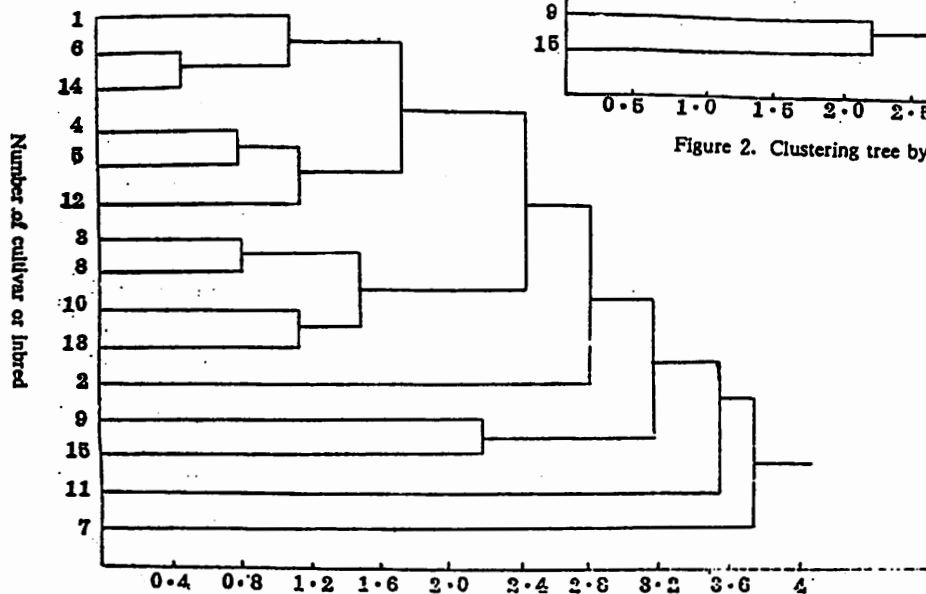
Figure 3. Clustering tree by the group—average method

'Nanjingci' (#15), Jin 4-3-1 (#2), 'Pingli' (#9), Shifeng-8 (#11), and Changmi-1 (#7).

The clustering features of the furthest-neighbor method and squares sum of dispersion methods were similar and clustering cores were distinct (Fig. 2). All cultivars and inbreds were affected mainly by early maturity and high productivity. Jin 4-3-1 (#2), 3511 (#6), 58-663 (#1) and Jiyu-2 (#14) all possessed high productivity and downy mildew resistance and were classified into one group. Changmi-1 (#7) and Pingli (#9), which possessed early maturity, formed one clustering core.

The clustering results of group-average (representing a flexible method) and median methods were similar (Fig. 3). Clustering cores were obvious when the genetic distances were short as depicted by the furthest-neighbor method. When genetic distances among cultivars and inbreds were larger, the nearest- neighbor method was not to be found an adequate classifier and groups were merged one by one.

In summary, the clustering of 15 cultivars and inbreds by the furthest- neighbor method fit the synthetical expression of traits, and therefore can be used as a guide for the selection parents in a hybrid breeding program. Six groups of cultivars and inbreds were analyzed further, and representative traits and common features were found (Table 1).

Discussion. Parental selection is the key for cross-breeding. Cultivars or inbreds with distinctly different genetic distances are often selected for crossing. Thus, it is necessary to select the most appropriate methods for estimating genetic distance. Different methods directly affect the clustering of germplasm. It is regarded that the Mahalanobis distance estimated by pivotal condensation is a good predictor of early yield and productivity. This genetic distance estimate showed cultivar differences for quantitative traits related to yield in this study. Thus, the use of this method of genetic distance estimation coupled with an appropriate clustering method can be an important guide to the selection of parents in a plant improvement program.

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Table 1. Cultivar clustering and representative traits.

Group	Cultivar or inbreds	Representative traits	Typical features
1	3511, Jiyu-2, 58-663, Jin 4-3-1	$X_1, X_2, X_{10}, X_{16}, X_{17}$	Much more number of leaves in growth season, long and large fruit, more fruits per plant, high total production.
2	Heidan-1, 121713, Jin 1717	X_3, X_6, X_{21}, X_{22}	Light fruit weight, fruit developing quickly, not highly productive (between high productive type and early maturity type).
3	Jinan, Mai Jue 82, Baitou shuang	X_7, X_8, X_{11}	Large number and index of leaf area in early stage, nutritive growth luxuriant, not good at later stage.
4	Pingli, Nanjingci	$X_{15}, X_{20}, X_4, X_5, X_{14}$	Early maturity, not resistant to downy mildew, more harvested fruit and high early yield, typical of early maturity cultivars.
5	Shifeng-8	X_4, X_5, X_{15}, X_{14}	Early pistillate flowering, fairly early maturity and high production.
6	Chang mi-1	$X_1, X_2, X_4, X_5, X_{13}$	Many harvested fruits, and effective branches, the typical of early mature cultivars.

The Xishuangbanna Gourd (*Cucumis sativus* var. *xishuangbannesis* Qi et Yuan), a Traditionally Cultivated Plant of the Hanai people, Xishuangbanna, Yunan, China

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Introduction. Xishuangbanna gourd (*Cucumis sativus* L. var. *xishuangbannesis* Qi et Yuan) is a special type of cucumber traditionally cultivated by the Hani people on the mountainous area at about 1000 meters above sea level. The variety has not been introduced for cultivation in other regions. With many national ethnic minorities and rich in resources, the Xishuangbanna autonomous region lies in the southern area of Yunan Province, China. This region is located 2110'-2240' N and 9955'-1015' E, and has an area of 19,220 square kilometers, with a tropical monsoon climate (Pei Shengji, 1982). Hani is an ethnic minority region with a population of about 10,000 who live in the mountains about 800 meters above sea level. They have their own languages but do not communicate by written word. Traditionally, they have their own ways of cultivating and utilizing the Xishuangbanna gourd. It is commonly called 'Shihuo' and has many regional types (e.g. Cattle shihuo, Ivory shihuo, and Round shihuo). The Hani people intercrop gourd plants with dry rice, because they think the two crops are interdependent.

We made two on-site investigations in the Xishuangbanna region in October 1990 and September-October 1991. We observed that plants of Xishuangbanna gourd grow vigorously and produce fruit of special shapes. We also found that the plants were resistant to blight, and therefore this gourd has potential for use as ethnic plant germplasm.

Characteristics. Intercropped with dry rice, Xishuangbanna gourd is sown in April and harvested from July to November. The plants of Xishuangbanna gourd grow more vigorously than the common cultivated cucumber (*C. sativus*), having primary stems 6-7 meters long and 20-40 lateral branches. The plants can reach a whole vine length of around 8 meters with 900 nodes, and bear about 10 mature fruits, with a yield of 10-20 kilograms per plant. Plants are relatively resistant to common diseases.

The first pistillate occurs beyond the fifteenth node. Often two female flowers or a mixture of one female and one male flower occur at a node. The frequency of female flowers is 10-30% less than the common cultivated cucumber. The tops of mature fruits of some Xishuangbanna types have a distinct projecting navel, which is characteristic of hermaphrodite flowers (Fig. 1).

Xishuangbanna gourd consists as a population with a variety of fruit shapes and rind colors. The mature fruits, each weighing an average 2-3 kilograms, have the smooth rinds without thorns. The fruit shapes of the Xishuangbanna gourd can be divided into three types: 1) long and narrow, 2) column-like, and 3) round. The long and column-like types are distributed in Jinhong County, Monghai County and Mongna County while the round type is grown mainly in Jinhong County. The length:diameter ratio of all three types is 3:1. There are three rind colors: orange, light yellow and white. Some orange column types have distinct net veins on the rind (Fig. 2). In contrast, some young round types have veins similar to those of muskmelon. While fruits of Xishuangbanna gourd normally have 3 to 5 carpels (Fig. 3), round fruits always have 5 carpels. The placenta of immature fruits has a light yellow color. This coloration can change to orange in some types, while the nearby pulp remains yellow in color.
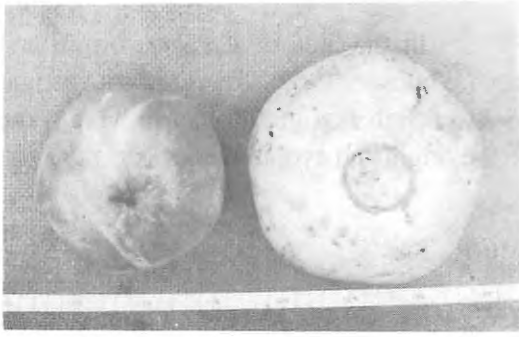
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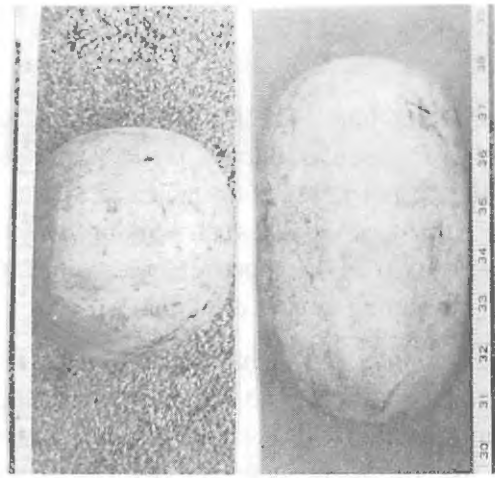
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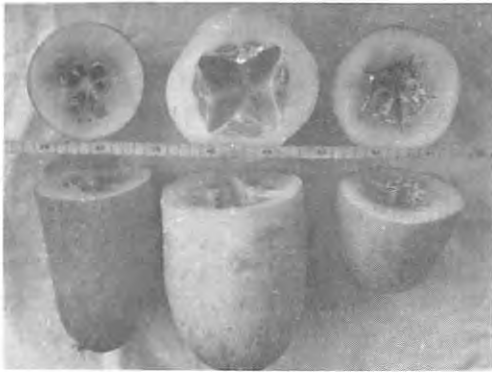
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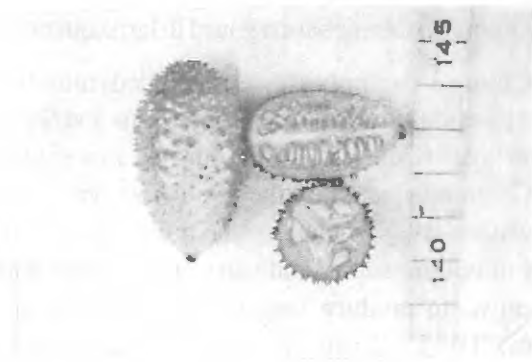
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Figure 1.   Fruit navels of some types of Xishuangbanna gourd.
Figure 2.   Netted veins on the fruit of orange column-shaped Xishuangbanna gourd types.
Figure 3.   Carpels of some types of Xishuangbanna gourd.
Figure 4.   Fruits of *C. hystris* Chakr.

Mode of Use. The fruits of Xishuangbanna gourd can be used as follows: 1) sliced and spiced, and then eaten raw as vegetable; 2) as fruits to quench thirst, especially when working away from home; and, 3) as the appetizers with wines when eaten raw during or after drinking.

Discussion. As mentioned above, the Hanai people have virtually no farming system and no common method of growing vegetables. However, they like the Xishuangbanna gourd very much, possibly because it requires no special care to produce a relatively high yield. Furthermore, this variety has a long harvest span and good postharvest storage characteristics. Because of its delicious taste, Dai and Han have recently begun consuming Xishuangbanna gourd in large quantity.

Chinese cucumbers were divided into two types (horticultural groups): North China and South China types (Cui, 1991). But thus far, few studies have been conducted on the Southwest type. The population of Xishuangbanna gourd has seven pairs of chromosomes and can cross-fertilize with *C. sativus* to produce fertile F_1 progeny (Qi and Yuan, 1983; Yang, 1991). Nevertheless, Xishuangbanna gourd is distinctly different from *C. sativus* in several morphological characteristics, but similar to *C. melo* in fruit shape and color, fruit navel display, carpel and rind structure. It appears that Xishuangbanna gourd is an intermediate type between *C. sativus* and *C. melo* in morphological characteristics and therefore may be of great value for investigating the origin and evolution of *Cucumis*.

Although many types of wild diploid species of cucumber have been found in South and East Africa (Meeuse, 1962; Jeffery, 1967), Cucumis originated in India or China (Harlan, 1975). Recently, cytogenetic, physiological and biochemical studies showed that *C. melo* is more primitive than *C. sativus* (Singh, 1990). During our investigation, we found a wild cucumber type (*C. hystric* Chakr.) called 'Mice gourd' (Fig. 4) to which little attention has been paid. It has 12 pairs of chromosomes and tastes similar to *C. sativus*. Since Xishuangbanna

gourd exists in the area of *Cucumis* origin and diversity (Whitaker, 1962), the further investigation of Xishuangbanna gourd may provide some keys to the origin and evolution of this genus.

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Cucumber (*Cucumis sativus* L.) Mutations III. Young Yellow Leaf Mutant

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Our collection of chemically induced cucumber mutants presented hitherto in the Cucurbit Genetics Cooperative (CGC) Report and in the proceedings of the 1992 Eucarpia Cucurbitaceae meeting includes a mutant with yellow stem and leaf petiole (Rucipska et al., 1992a), short petiole (Rucipska et al., 1992b), and divided and gingko leaves (Rucipska et al., 1992c). We would like to add a new mutant to the series, namely, yellow young leaves and stem. As with previous mutants, this mutant was obtained by Dr. B. Kubicki some twenty years ago and was maintained by self-pollination.

Methods. In order to determinate the inheritance of young yellow leaves, a standard genetic analysis was made. Among the generations analyzed was an Mutant x B (Borszczagowski inbred line) combination. This cross was made because of the difficulties of obtaining viable seeds.

Results. The young yellow leaves of a stem mutant can be distinguished at the cotyledon and first leaf stage. Cotyledons are yellow and green, and the first leaf is yellow. The growth of mutant plants depends upon the intensity of light during growth (i.e., the less intensive the light, the better the plant growth). For instance, a few cloudy days in the spring are enough to change the young yellow mutant plants to green. Intensive sunlight gradually causes some mutant plants to become yellow to white-yellow. Mutant plants remain viable because their older leaves (nearly half of the plant) are green. Usually, mutant plants are smaller than the standard type (B line from which they were induced), and possess less abundant female flowers (seeds). Pollen stainability is between 50 to 90%, and thus it is difficult to cross mutant plants with other "normal" plants. Genetic analysis of the mutant indicates that the trait of young yellow leaves is governed by one recessive gene, which we propose to designate yl.

In the list of cucumber genes (Pierce and Wehner, 1989), as well as in the latest issues of CGC, there were numerous descriptions of chlorophyll deficient mutants. Some of the presentations were very short and incomplete. For cotyledon mutants (Iida and Amano, 1991), it was impossible to determine whether or not any of the mutants are identical with the one we now present in this paper. We provide the first description of the young yellow leaf mutant and its genetic analysis. The data presented in Table 1 confirms the recessive character of the gene.

The chloroplast ultrastructure of the mutant was studied and described previously (Palczewska et al., 1983). It was found that in yellow leaves there were plastids with a varying degree of defective characteristics (i.e. reduction and disorganization of the thylakoid system). Normal chloroplasts were also observed. The character of young yellow leaves was precisely determined from morphological and cytological observation, and can be used for further genetic and physiological investigations (e.g., those related to light intensity).

Table 1. Inheritance of Young Yellow Leaves (*yl*) in cucumber.

Generation	No. observed Normal	No. observed Mutant	No. Expected Normal	No. Expected Mutant	Ratio tested	X^2	P value
P1 (normal)	42	0	42	0	1:0	---	---
P2 (mutatant)	0	42	0	42	0:1	---	---
F1	40	0	40	0	1:0	---	---
F2	185	61	184.5	61.5	3:1	0.005	0.005
F1 x P1	170	0	170	0	1:0	---	---
F1 x P2	87	77	82	82	1:1	0.610	0.05

Figure 1. A cucumber plant possessing young yellow leaves.
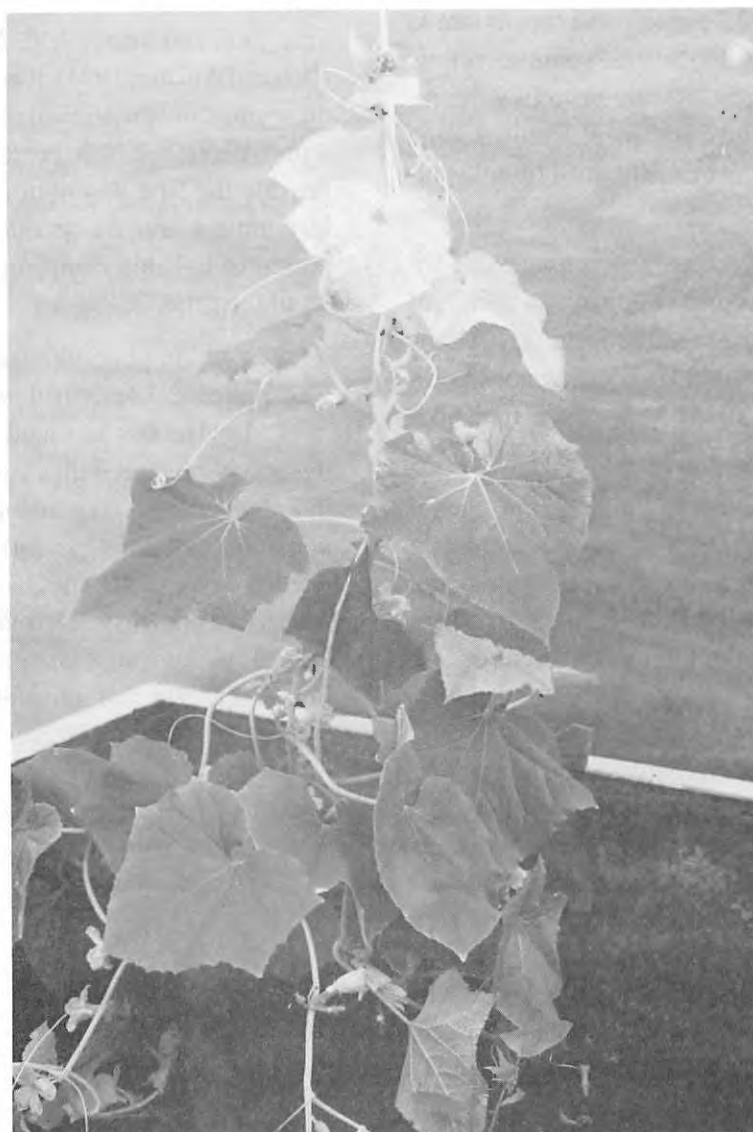
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Figure 2. A comparison of a young yellow (far right) and older green mutant leaves and standard leaf.
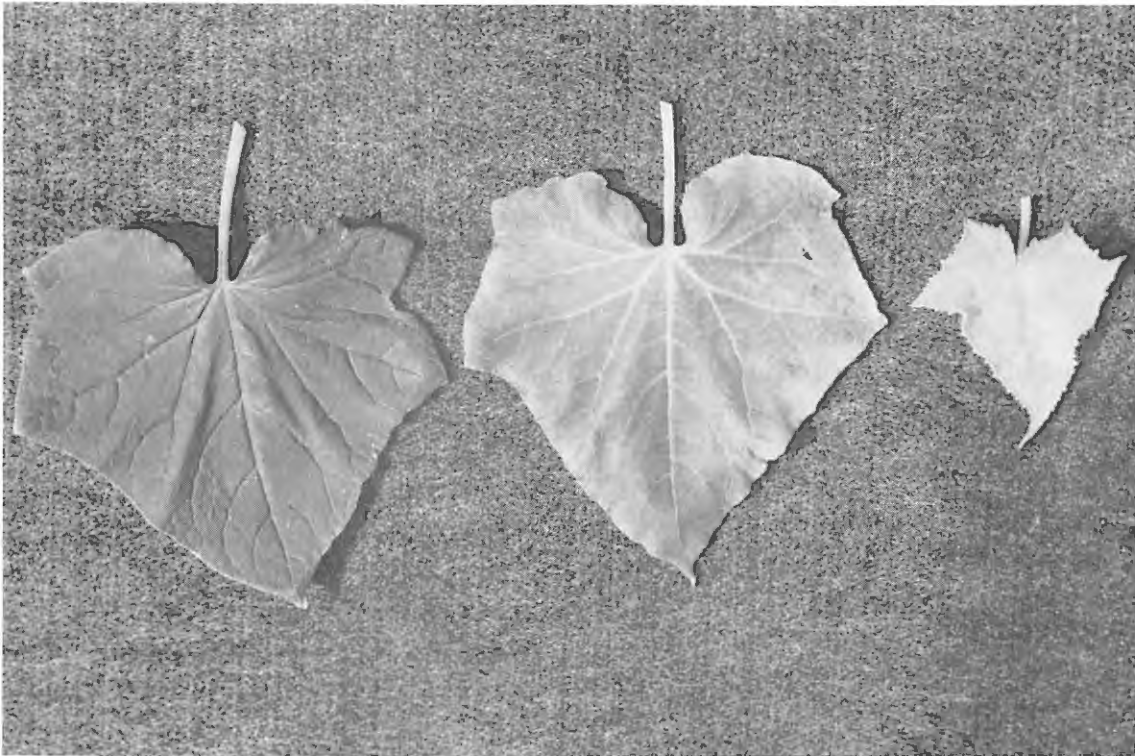
Some Morphological Parameters Involving the Mechanism of Early Yield in Cucumber

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With the development of a market economy in China, increased attention is now being given to early maturity breeding. In the past, only a few traits were selected during hybrid development. There was little attention paid to the selection of multiple traits. Theoretical studies in tandem and multiple trait selection have been proposed but not well documented. Traits related to early yield were regarded as being directly correlated in cucumber and other crops (Yuhuai, 1985; Li and Li, 1985). However, evaluations were subjective in nature. Canonical correlation analysis was used in this study to identify character groups which affect early yield. The aim of this paper is to identify components of early yield in cucumber.

Method. An experiment was conducted at the Horticulture Station of the Northwestern Agricultural University. Twenty-four varieties and inbreds were evaluated in a randomized block design with 3 replications. Ten plants of each plot were randomly chosen to evaluate quantitative traits during the early growth period. The traits were divided into four groups according to biological significance. These groups include: 1) early yield component (YC) = fruit length (YC_1), number of harvested fruits per plant (YC_2), and fruit weight (YC_3); 2) morphological traits (MT) = the node position of the first pistillate flower (MT_1), pistillate flower density (main vine) (MT_2), number of pistillate flower (main vine) (MT_3), and number of staminate flowers (main vine) (MT_4); 3) growth period factor (PF) = the days from sowing to the first pistillate flowering plant in the population (PF_1), the days from sowing to pistillate flowering of 50% of the plants (PF_2), the days from sowing to first staminate flowering plant in the population (PF_3), the days from sowing to staminate flowering

of 50% of the plants (PF_4); and, 4) yield physiological basis (PB) = leaf area per plant (PB_1) and leaf number per plant (PB_2). A genotype correlation matrix was used for canonical correlation analysis. These correlation coefficients were tested by the Bartlett method.

Results. The results (Table 1) show that the canonical correlation coefficient of early yield with yield components (yield physiological basis and trait groups) were extremely significant. Data indicated that these two trait groups directly affected early yield (i.e., more than 97% of the total genetic correlation). This proved that yield components have a direct relationship to early yield. Although the physiological basis of yield correlated significantly with early yield, the coefficient accounted for only 48.6% of total genetic correlation.

In order to determine the interaction between trait groups, their interrelationships were studied further. Data (Table 2) indicated that all first canonical correlation coefficients of yield components (YC) and the other three trait groups examined, and the correlation of morphological traits (MT) with the growth period factor (PF), were highly significant. Each of them accounted for 78, 76, 93, and 60 percent of total genetic correlation information, respectively. The correlation between such trait groups can be summarized by analyzing the structure of four pairs of canonical variables.

The formation of the first canonical variable (FFCV) of yield components (TC) with morphological traits (MT) indicated that they were affected mainly by the action of pistillate flower density (MT_2) and the number of pistillate flowers (MT_3) to the number of harvested fruit (YC_3).

Table 1. Canonical correlation analysis between early yield and character groups in cucumber.

Character group	Canonical correlation coefficient[1]	R²	X²	d.f.	Significant
YC	0.98639**	0.97297	59.57437	3	0.000
MT	0.56015	0.31377	6.02460	4	0.197
PF	0.52194	0.27242	5.08362	4	0.278
PB	0.69743**	0.48641	11.32761	2	0.003
YC + PB	0.98731**	0.97478	57.04573	5	0.000
YC + MT + PF + PB	0.99732**	0.99465	60.13021	13	0.000

[1]*p =0.05, **p=0.01.

Table 2. Canonical correlation analysis between early character groups

First group variable	Second group variable	Canonical correlation coefficient[1]	X²	d.f.	Significance	R²	Generalized correlation coefficient
YC	MT	λ_1=0.83628*	21.20462	12	0.047	0.69936	p = 0.41911
		λ_2=0.38116	3.17704	6	0.785	0.14528	
		λ_3=0.23096	0.82230	2	0.663	0.05334	
YC	PF	λ_1=0.85349*	23.38360	12	0.025	0.72845	p = 0.38519
		λ_2=0.45633	3.82895	6	0.700	0.20824	
		λ_3=0.14674	0.32650	2	0.849	0.02153	
YC	PB	λ_1=0.90294**	28.08585	6	0.000	0.95023	p = 0.47830
		λ_2=0.25336	1.06147	2	0.588	0.06419	
MT	PF	λ_1=0.88451*	31.04834	16	0.013	0.78236	p = 0.34480
		λ_2=0.60495	8.93689	9	0.443	0.36596	
		λ_3=0.37992	2.32996	4	0.675	0.14434	
		λ_4=0.06927	0.06974	1	0.792	0.00480	
MT	PB	λ_1=0.55136	6.59391	8	0.581	0.30400	p = 0.36911
		λ_2=0.24711	0.97664	3	0.807	0.06107	
PF	PB	λ_1=0.54710	7.10821	8	0.525	0.29931	p = 0.41361
		λ_2=0.31270	1.59492	3	0.661	0.09773	

[1]*p=0.05, **p=0.01.

FFCV of yield components (YC) with growth period factors (PF) showed that moving up the time of pistillate flowering (PF$_1$, PF$_2$) would result in an increase in the number of fruits and lowering of fruit weight. FFCV of morphological traits (MT) with growth period factors (PF) showed that postponing the time of pistillate flowering and moving up the time of staminate flowering would increase the node position of first pistillate flower and increase the number of staminate flowers.

Discussion. It is not certain that all characters related to early yield affect early yield directly. Some of the characters will affect early yield indirectly through the others. If one considers that all characters which affect early yield directly, one can find the important characters which directly affect the early yield. The canonical correlation analysis of characters lays a foundation for the study of early yield mechanisms. The correlation of canonical variances is caused by the linear correlation of characters. There may be a more complicated linear correlation in the character groupings generalized in the correlation among traits.

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Analysis of Component Traits for Early Yield in Cucumber

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Early maturity and high yield is an important objective in cucumber (*Cucumis sativus* L.) breeding programs. The early yield of cucumber is complicated by its quantitative nature and is affected by many other horticultural traits (e.g., epistasis, pleiotropy). The study of horticultural traits which affect early yield can help plant breeders recognize the component factors of early yield and provide for theoretical guidance in breeding. Thus, an experiment was designed to analyze the relationship between early yield and 15 horticulturally important traits in cucumber using stepwise regression. It was hoped that an optimum set of functions could be identified for early yield and its component traits.

Methods. Experimental plant material consisted of 41 F_1 hybrids and 4 cultivars (numbered 1-45). Field work was conducted at the vegetable station, Northwestern Agricultural University, China, from early April to mid-June, 1991. Seeds were sown in plastic tunnels and arranged according to number on April 6. There were 30 plants in each plot, spaced on 0.6m row centers and positioned 0.4m apart in each row (4.4m^2 area). Ten plants in each plot were sampled for observation and measurement.

Sixteen traits observed were: 1) days from sowing (dfs) to first staminate flowering (x_1); 2) dfs to first staminate flowering on 50% plants (x_2); 3) dfs to first pistillate flowering (x_3); 4) dfs to first pistillate flowering on 50% plants (x_4); 5) node of first pistillate (x_5); 6) leaf area index (x_6); 7) fruits set on main vine (x_7); 8) pistillate flowers on main vine (x_8); 9) fruiting percentage on main vine (x_9); 10) fruit branches per plant (x_{10}); 11) total branches per plant (x_{11}); 12) fruiting branch percentage (x_{12}); 13) mean pistillate flowers per node on main vine (x_{13}); 14) fruits harvested in early stage (x_{14}); 15)

mean weight in *jin* per fruit (x_{15}); and, 16) early yield in *jin* (y).

Multiple stepwise regression was performed using early yield as the dependent variable and other traits as independent variables using the ANALYST program. Standard determination coefficient, R^2 80%, was used in the analysis.

Results. The F value in the first step (regression) was used as the critical value (i.e., F=2.05) while regressing 15 independent variables. In each step, an independent variable whose F value was not significant and was the least value in partial regression test was eliminated. Then the next regression was performed until all remaining variables had significant F values. According to this principle, x_1, x_7, x_6, x_5, x_8, x_{13}, x_{10}, and x_{11} were were eliminated in order from the first step to the eighth. At the ninth step an optimum regression equation was identified whose F value distribution of independent variables is shown in Table 1 as:

$$y = -21.36513 - 0.4417x_2 - 0.4147x_3 + 1.51552x_4 - 0.05135x_9 + 0.00869x_{12} + 0.20941x_{14} + 5.72047x_{15}$$

Data suggest that cucumber early yield was mainly composed of x_2, x_3, x_4, x_9, x_{12}, x_{14} and x_{15}. In the regression interval, the y value varied directly to x_{14} and x_4, and inversely to x_2.

The coefficient of determination, R^2, was used as a critical value for evaluation of the regression equation's value over sampling dates. It was calculated that R^2 was 0.83 when the regression equation was constructed at the ninth step [i.e., square sum of y variation determined by the 7 independent variables (Uy/x) was 83% of square sum of the total y variation (y^2)]. This value was more than the expected standard R^2 (80%). Thus, the regression equation used accurately estimated sample dates.

Table 1. F value of regression parameters in the ninth step during the determination of early yield in cucumber.

Variable	x_2	x_3	x_4	x_9	x_{12}	x_{14}	x_{15}
F value	12.8132	2.4694	12.9687	3.3389	2.1608	94.9782	4.4819

Table 2. F test of optimum regression equation in early yield determination of cucumber.

Variance	Ss	df	MS	F	Critical value
Regression	427.46362	7	61.06622	26.78780	$F_{0.01}(7,37) = 3.18$
Residual	84.34624	37	2.27963		
Total	511.80981	44	11.63204		

From Table 2 it can be seen that the linear relationship between seven horticultural strains and early yield was significant in the equation. Therefore, the relationship between cause and effect, as expressed by the regression function, was creditable.

The standard errors of the regression estimate was $S(y_0) = 1.50984$. When the confidence coefficient $(1-\alpha)$ was 0.95, then $\Delta = t_{0.05}(n-3)S(y_0) = 2.0211 \times 1.50984 = 3.05160$. The interval estimate of the mean value of $y(E)$ was $y_0 \pm 3.05160$.

The standard error of observation $(S^*)(y_0) = 3.40887$. When the confidence coefficient is $1-\alpha = 0.95$, then $\Delta = t_{0.05}(n-3)S(y_0) = 3.40887 \times 2.20211 = 6.88967$. The interval estimate of the sample value of $y(y^*)$ was: $y_0 \pm 6.88967$.

Discussion. Seven traits remained in the regression equation and eight traits were eliminated. The traits which were eliminated could only be thought of as not significant in their linear relationship with early yield. Their relationship to other traits could be interpreted (e.g., x_3 should have certain relationship to x_5, and x_{12} contained x_{10}. Such complicated relationships can best be understood by path analysis.

Considering the effect of the environment on trait expression, one should analyze the heritability and genotype correlation between the seven traits. Such a treatment would characterize their genetic basis.

The optimum regression equation was based on the mean of the observed value for each trait. Thus, only when a trait value is near to its mean can correct predictions be made. If the trait value is not close to its mean, prediction errors cannot be avoided.

The experiment used only early maturing hybrids and cultivars. If middle and late maturing cultivars were evaluated, the main component of traits for early yield in cucumber would be better understood.

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Fruit Firmness and Quality of Parthenocarpic Versus Nonparthenocarpic Pickling Cucumber Cultivars.

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Parthenocarpic pickling cucumbers may be advantageous to conventional non-parthenocarpic cultivars due to their higher fruiting capacity and ability to produce fruit under poor pollination conditions (Baker et al., 1973; Pike and Peterson, 1969; Zwinkels, 1986). Texture or firmness of pickling cucumbers is used as a criterion of quality. Parthenocarpic pickling cucumbers have rated lower in texture than nonparthenocarpic types (Longan, 1971; Pike and Peterson, 1969). In Europe, parthenocarpic pickling cucumber cultivars tend to soften and become hollow when preserved (Fritz and Weichmann, 1987). The objective of this research was to investigate the effect of genetic and non-genetic parthenocarpy on firmness and fruit quality of pickling cucumber.

Methods. Six genetically parthenocarpic and six nonparthenocarpic pickling cucumber cultivars were chosen for study. Seed was supplied by European (Nunhems, Bejo Zaden) and American (Sunseeds) seed companies and included firm and soft cultivars. Cultivars were gynoecious and either smooth (European type characterized by many small warts and spines) or spined (American type characterized by few large warts and spines). A split-plot design was used with cultivar as the main plot factor and presence or absence of row cover as the subplot factor. Main plots were arranged in a complete random block design with three blocks and bordered with a determinate pickling cucumber hybrid. The subplot factor was randomly assigned. Row covers were applied after flowering began and open and old flowers were removed. Drip irrigation was used. Three weekly harvests were taken. Harvests were initiated when the first oversize fruit (5.1 cm in diameter) was present. Fruit were hand harvested and graded by diameter size as recognized by the Pickling Cucumber Improvement Committee. Ten No. 2 fruit (2.7 - 3.8 cm in diameter) were randomly selected for evaluation. Data from the three harvests were pooled for a total of 30 fruit measured per plot. Whole fruit and mesocarp firmness was measured with a U.C. Firmness Tester (FPT) with a 7.9 mm round tip and 2.0 mm flat tip plunger, respectively. Whole fruit measurements were taken in the center of the fruit with one side of the fruit flush with the pressure tester and the skin intact. A two cm wide slice of each fruit was used for measuring mesocarp firmness. Slices were uniformly taken from the stem end of the fruit adjacent to the FPT puncture. Mesocarp measurements were made by puncturing the fruit wall equidistant from the pericarp and endocarp. Brinestock samples were sent to Steinfeld's in Portland, Oregon, for preservation.

Results. Parthenocarpic cultivars were significantly softer than nonparthenocarpic cultivars (Tables 1 and 2). Cultivars within the parthenocarpic and nonparthenocarpic types were significantly different from one another (Tables 1 and 2). Fruit produced in the presence of row covers were significantly firmer than those which were produced in the absence of row covers (Tables 1 and 2). Cultivars which were firmer under the row cover tended to be firmer in the absence of row cover (Table 2). Fruit carpel separation and placental hollows occurred most frequently in genetically nonparthenocarpic cultivars grown under row covers (Table 3). Brinestock firmness comple-

Table 1. Analysis of variance for whole fruit and mesocarp greenstock firmness of six genetically parthenocarpic and six genetically nonparthenocarpic pickling cucumber cultivars grown in the presence or absence of floating row covers at Brooks, Oregon, 1992.

| | | Firmness (N) | | | |
| | | Fruit | | Mesocarp | |
Variable	df	MS	F	MS	F
Block (Bl)	2	5.54	0.52^{NS}	4.19	0.26^{NS}
Parthenocarpy (P)	1	94.57	8.85^{**}	976.56	59.45^{****}
Cultivar (C)/P	10	124.05	11.61^{****}	116.82	7.11^{***}
Error (a)	22	10.68		16.43	
Row cover (R)	1	104.27	12.26^{**}	924.71	58.58^{****}
P x R	1	2.18	0.26^{NS}	109.47	6.95^{*}
C/P x R	10	8.71	1.02^{NS}	7.84	0.50^{NS}
Error (b)	24	8.51		15.79	
Total	71				

$*,**,***,****,NS$ Significant at 0.05, 0.01, 0.001, 0.0001, or nonsignificant, respectively, by F tests.

Table 2. Mean whole fruit and mesocarp firmness before and after processing of six genetically parthenocarpic and six genetically nonparthenocarpic pickling cucumber cultivars grown in the presence or absence of floating row covers at Brooks, Oregon, 1992.

	Firmness (N)							
	Greenstock				Brinestock			
	Row Cover		No Cover		Row Cover		No Cover	
Cultivar	Fruit	Meso.[z]	Fruit	Meso.	Fruit	Meso.	Fruit	Meso.
Genetically parthenocarpic								
Nun 0136	87.6	7.27	82.7	6.97	77.0	8.57	73.8	8.14
Alstar	81.8	6.99	77.4	6.17	67.2	8.81	61.8	7.70
Anka	77.4	6.73	75.2	6.42	57.8	8.08	56.0	7.36
Parmel	76.5	6.87	76.5	6.44	63.2	7.29	57.8	6.89
Arena	74.3	6.49	74.7	6.23	59.2	7.71	60.0	7.06
Adonis	72.9	6.58	71.2	5.90	63.2	6.93	54.7	6.67
Mean	*78.4*	*6.82*	*76.3*	*6.36*	*64.6*	*7.90*	*60.7*	*7.30*
Genetically nonparthenocarpic								
Furax	87.6	8.20	81.2	6.87	-	-	70.3	8.53
Calypso	85.8	8.40	81.8	7.65	-	-	75.6	8.75
Parker	79.6	8.31	81.4	7.26	-	-	68.5	8.50
Stimora	79.6	7.46	78.3	6.54	70.7	9.28	63.6	8.09
Ilonca	78.7	7.24	76.1	6.31	63.6	7.86	55.2	7.27
Alert	74.3	7.15	70.7	6.42	57.4	7.47	63.2	7.45
Mean	*80.9*	*7.79*	*78.3*	*6.84*	-	-	*66.1*	*8.10*

[z] Meso. = mesocarp.

Table 3. Placental hollows[z] (PH) and carpel separation[y] (CS) as a percent of the total number of fruit of six genetically parthenocarpic and six genetically nonparthenocarpic pickling cucumber cultivars grown in the presence or absence of floating row covers at Brooks, Oregon, 1992.

Cultivar	Row Cover		No Cover	
	%PH	%CS	%PH	%CS
Genetically parthenocarpic				
Nun 0136	8	0	10	0
Alstar	4	0	10	2
Anka	12	3	12	2
Parmel	21	6	29	6
Arena	32	5	20	0
Adonis	19	5	14	4
Mean	*16*	*3*	*16*	*2*
Genetically nonparthenocarpic				
Furax	20	9	20	2
Calypso	38	0	17	1
Parker	66	26	22	2
Stimora	31	5	19	2
Ilonca	44	6	21	2
Alert	58	18	26	3
Mean	*43*	*11*	*21*	*2*

[z] Placental hollows (PH) = one or more holes present in the mesocarp.

[y] Carpel separation (CS) = separation of fused carpels forming a hollow through part or the entire length of the fruit.

mented greenstock firmness for both cultivar and row cover treatments (Table 2).

Discussion. The effect genetic parthenocarpy had on firmness in this study, though significant in the analysis of variance, remains inconclusive due to the inherent nonrandom sampling of the twelve cultivars. Pollination appears to affect fruit firmness regardless of whether or not the cultivar is genetically parthenocarpic. Pollination had a greater softening effect on nonparthenocarpic cultivars than on genetically parthenocarpic cultivars. The effect of the row cover treatment on firmness, if any, could not be separated from the nonpollination effect. Fruit produced in the absence of row cover may be parthenocarpic or pollinated. The firmest fruits, in addition to possessing the highest frequency of fruit defects, were genetically nonparthenocarpic and produced under the row cover. This interaction between row cover and cultivar type is difficult to explain since the row cover and nonpollination effect are confounded. However, one could hypothesize that pollination had a larger influence on fruit quality in genetically nonparthenocarpic types than genetically parthenocarpic types. A study to further investigate the effect of pollination on firmness of parthenocarpic and nonparthenocarpic cultivars was conducted in 1993 and included four treatments: row cover, no row cover, screen cage, and screen cage with honeybees.

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Inheritance of Fruit Firmness in Genetically Parthenocarpic Pickling Cucumbers.

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Fruit firmness in genetically nonparthenocarpic cucumber has been reported as a highly heritable (h = 0.42-0.80) quantitative trait with additive gene effects accounting for 99% of the total genetic variation (Peterson et al., 1978; Smith et al., 1978). The number of genes involved is unknown and maternal effects have not been important (Peterson et al., 1978). Studies on the inheritance of fruit firmness in cucumber have varied in experimental design, germplasm, and methods for firmness evaluation (Peterson et al., 1978; Smith et al., 1978). Reports on the inheritance of fruit firmness in parthenocarpic germplasm are not available. The objective of this research was to investigate the inheritance of fruit firmness in genetically parthenocarpic pickling cucumber.

Methods. A complete M x N mating pattern (Simmonds, 1979) between five nonparthenocarpic monoecious and four parthenocarpic gynoecious inbred lines was used to estimate general combining abilities (GCA) for fruit firmness in 1992. Parent lines with extreme differences in fruit firmness were chosen. Nonparthenocarpic lines were used as males and parthenocarpic lines were used as females to produce 20 F_1 hybrids for evaluation. The male and female parents with the highest and lowest GCA, respectively, were chosen for population development and included the male parents 'Clinton' and 'Armstrong Early Cluster' (AEC), and female parents W744 and WI1983.

Five families were created by crossing each parthenocarpic female with the nonparthenocarpic male parents and by crossing the two male parents together. Reciprocal F_1, F_2, and backcross generations were produced and, with the original parents

and F_1's, were evaluated for fruit firmness in 1993 at Brooks, Oregon, using a complete randomized block design. Firmness measurements were made on No. 2 fruit (2.7 - 3.8 cm in diameter), one fruit per plant. Whole fruit and mesocarp firmness was measured with a U.C. Firmness Tester (FPT) with a 7.9 mm round tip and 2.0 mm flat tip plunger, respectively. Whole fruit measurements were taken in the center of the fruit with one side of the fruit flush with the pressure tester and the skin intact. A 2.0 cm wide slice of each fruit was used for measuring mesocarp firmness. Slices were uniformly taken from the stem end of the fruit adjacent to the FPT puncture. Mesocarp measurements were made by puncturing the fruit wall equidistant from the pericarp and endocarp.

Results. Whole fruit firmness was inherited in a quantitative fashion (Table 1). Inheritance appears additive, though some dominance for firmness may be present as F_1's consistently exceeded midparent values (Table 1). Maternal effects may be present in the 'Clinton' x AEC (P2 x P1) and W744 x 'Clinton' (P3 x P2) families as firmness was higher in F_1's with 'Clinton' as the female (Table 1). Mesocarp firmness was also inherited quantitatively and appears completely additive with no maternal effects (Table 1).

Discussion. Three principal tissues contribute toward overall texture and firmness of cucumbers: exocarp (skin), mesocarp (carpel wall), and endocarp (seed cavity). Each tissue contains an inherent firmness which contributes directly to overall fruit firmness. A tissue may also contribute indirectly to fruit firmness through size, as thickness in the exocarp and mesocarp or diameter as in

Table 1. Whole fruit and mesocarp firmness of two genetically nonparthenocarpic pickling cucumber inbreds (P_1, P_2), two genetically parthenocarpic (P_3, P_4) inbreds, and five families developed from the respective inbreds.

| | | Firmness (N) | | | |
| | | Fruit | | Mesocarp | |
Population	N	Mean	SD	Mean	SD
Armstrong Early Cluster (P_1)	5	53.8	1.3	6.9	0.53
Clinton (P_2)	17	80.1	7.6	8.7	0.97
W744 (P_3)	17	66.7	6.7	7.0	0.67
WI1983 (P_4)	19	75.6	7.6	8.2	0.56
P_2 x P_1	23	82.3	8.0	8.3	0.97
P_1 x P_2	23	74.3	7.6	8.0	0.68
(P_2 x P_1) x P_1	56	67.6	8.9	7.6	1.17
(P_2 x P_1) x P_2	51	81.8	8.5	8.5	1.18
F_2	123	72.9	9.3	7.9	1.33
P_3 x P_1	23	64.9	8.0	6.7	0.73
P_1 x P_3	19	64.5	8.5	7.1	1.00
(P_3 x P_1) x P_1	49	60.1	8.9	6.6	1.00
(P_3 x P_1) x P_3	56	70.3	8.5	8.0	0.92
F_2	166	69.8	9.8	7.0	1.20
P_4 x P_1	25	71.2	8.0	7.0	0.75
P_1 x P_4	31	73.4	4.9	7.8	0.94
(P_4 x P_1) x P_1	46	67.2	11.1	6.9	1.30
(P_4 x P_1) x P_4	-	-	-	-	-
F_2	147	71.2	8.9	7.2	0.93
P_3 x P_2	14	75.2	7.6	8.6	0.84
P_2 x P_3	20	83.6	7.1	7.9	0.97
(P_3 x P_2) x P_2	51	79.6	8.5	8.6	0.93
(P_3 x P_2) x P_3	52	72.1	8.5	7.7	0.78
F_2	118	77.0	8.5	8.4	0.95
P_4 x P_2	23	81.4	7.6	8.4	0.81
P_2 x P_4	39	82.7	8.0	8.4	0.82
(P_4 x P_2) x P_2	47	85.0	7.1	9.0	0.71
(P_4 x P_2) x P_4	59	76.1	6.7	8.2	0.86
F_2	131	77.4	8.5	8.5	1.10

the endocarp. Fruit size and harvest date may also affect firmness. Samples were uniform for fruit size, but time of harvest varied by plot. Therefore, whole fruit firmness evaluations include all the tissue and harvest date components. The mesocarp firmness component does not appear responsible for the suspected maternal effects in whole fruit firmness. Future analysis may reveal that reciprocal cross differences for whole fruit firmness are not significant, or that another component which was or was not included in this study is responsible for the apparent differences. Dominance as a variance component in whole fruit firmness of parthenocarpic pickling cucumber will also be of interest.

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Chilling Resistance of Five Cucurbit Species

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Genetic resistance to chilling in cucurbits would be useful for protection of seedlings against spring frost. Previous research on cucumber (*Cucumis sativus* L.) indicated that it is chilling susceptible, but that there are genetic differences for response to low temperature. We were interested in the relative resistance of other cucurbits, and whether they had the same response to the test method developed for cucumber (Smeets et al., 1991; Smeets and Wehner, 1988, 1989, 1990, 1991a,b; Wehner and Bunch, 1989) using the Phytotron controlled environment facility (Downs and Thomas, 1983) at North Carolina State University.

The objective of this study was to determine the general range of resistance of five cucurbit species using methods developed for testing cucumber seedlings. This preliminary survey will be used to design a more informative and larger study.

Plants of five species were tested: *Cucumis melo* 'Cordele', *Cucumis sativus* 'Gy 14', *Citrullus lanatus* 'Crimson Sweet', *Cucurbita pepo* 'Autumn Gold', and *Luffa aegyptiaca* 'Fletcher'. Seeds were planted in 57 x 57 mm peat pots on 4 August, 1993. They were grown for 16 days under 9 hour daylength in growth chambers at 30/260C. On 20 August (2nd to 3rd true leaf stage) the seedlings were subjected to a chilling treatment of 3, 5, 7 or 9 hours at 4C at a light intensity of approximately $500 \, \mu mol \bullet M^{-2} \bullet s^{-1}$ PPFD 400 to 700 nm. Plants were rated on 25 August using a 0 to 9 scale (O=no damage, 1-2=trace, 3-4=slight, 5-6=moderate, 7-8=advanced, 9=dead). The experiment design was a single replication of 9 plants per species.

The 3 hour duration produced little or no damage on the five species tested except for cucumber and luffa, which had moderate damage (Table 1). At 5 hours, melon also had moderate damage, and foliar damage ratings increased with chilling duration as expected. With 7 hours of chilling, the mean was closest to the midpoint of the 0 to 9 scale, and the range from susceptible to resistant species was as large as it got. Thus, the 7 hour chilling treatment is probably the best for screening for genetic differences in the five cucurbit species. The 7 hour duration also provided the best test conditions for evaluation of cucumber cultivars.

Based on this preliminary test, 'Crimson Sweet' watermelon and 'Autumn Gold' squash were most resistant, and 'Cordele' melon, 'Fletcher' luffa and 'Gy 14' cucumber were most susceptible. Future studies could be run to identify chilling resistant accessions using the test methods described here with a 7 hour chilling duration.

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Table 1. Chilling damage in five cucurbit species[z].

Crop (species)	Chilling damage (rating)				
	3 hours	5 hours	7 hours	9 hours	Mean
Watermelon (*Citrullus lanatus*)	0	1	3	3	1.8
Squash (*Cucurbita pepo*)	0	1	2	6	2.2
Melon (*Cucumis melo*)	2	5	6	7	5.0
Luffa (*Luffa aegyptiaca*)	5	4	6	7	5.5
Cucumber (*Cucumis sativus*)	4	6	7	7	6.0
Mean	2.2	3.4	4.8	6.0	4.1
Range	5	5	5	4	4.8

[z]Damage rated on a single replication of 9 plants per chilling duration. Rating was 0-9 (O=none, 1-2=trace, 3-4=slight, 5-6=moderate, 7-8=severe, 9=dead).

Selection and Characterization of Salt-tolerant Cucumber (*Cucumis sativus* L.) Plants using *in vitro* Culture

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Cucumber is a species for which methods of regeneration of *in vitro* cultures have been elaborated (Malepszy, 1988; Malepszy et al., 1986). Tissue culture provides opportunities for selecting for resistance to different types of biotic and biotic agents. Thus far, mutants resistant to *Fusarium oxysporum* f. sp. *cucumerinum* (El-Kazzaz, 1990; Malepszy and El-Kazzaz, 1990) and herbicides, metribuzin or linuron (Hasegawa, 1980), have been identified in suspension and callus culture. However, suspension culture variants resistant to streptomycin could not be regenerated (Malepszy, 1988). Tolerance to salinity is a trait that can have practical implications for cucumber breeding. No individuals tolerant to salinity have been found among *Cucumis* species (Anastasio et al., 1988). The following work attempts to obtain such forms using NaCl selection in tissue culture.

Methods. The highly inbred (S15) monoecious cucumber line of the variety 'Borszczagowski' (line B) was used for the experiments. The first and second leaves from 14 to 21 day-old seedlings were used as a source of explants, according to Malepszy (1988).

The culture medium used was based on Murashige and Skoog (1962) salts and vitamins enriched with 0.6 mg x dm^{-3} 2,4-D, 0.4 mg x dm^{-3} 2iP, 30 mg x dm^{-3} sucrose, 250 mg x dm^{-3} edamine and 8 g x dm^{-3} agar (medium CSO). Callus pieces (~50 mg) were plated on culture media containing: 0, 10, 50, 100, 125, 150, 175 and 200 mM NaCl. A minimum of three replicates were made. Callus growth after 4 to 6 weeks of culture was established. Fresh weight of the calli on three different NaCl concentrations (0, 125 and 150 mM) was measured after 2 and 4 weeks. The data are presented in Figure 1 as the average of 4 replicates.

For selection of resistant variants, CSO medium was supplemented with two NaCl concentrations [125 mM (medium CS125) or 150 mM (medium CS150)] according to given selection parameters (Figure 2). Ten leaf discs (5 mm in diameter) were plated on one Petri dish supplemented with saline medium. In the first case, selection was accomplished by isolating some small pieces of callus (~2 mm^2) after two weeks of culture from independent sites of an explant. Callus pieces were placed directly on the selection media or after one week preculture on control medium. In the second case, selection was initiated by isolating sectors of callus (i.e., yellow-green or mixed colors distinguishable from the remaining necrotic zones) after six to eight weeks of culture with or without a one week of preculture. The selected calli were taken through passages on salinized media, and the control line (line B) was transferred to NaCl - free medium. Each passage lasted 3 to 4 weeks.

Between the third and fourth passage after selection initiation, the selected calli were transferred on a different regeneration media without (R) or supplemented with two different NaCl concentrations (62.5 mM and 100 mM). The medium was the same as CSO, except that it lacked hormones. The control line was regenerated on medium R simultaneous with the production of selected calli lines. The calli were subcultured at two week intervals. Embryogenic regions and those regions upon which organogenesis occurred were chosen for transfer to fresh media. Once recovered, plants reached 5 to 7 cm in height at which time they were transferred to small pots containing a mixture of peat substrate and perlite, and placed in a growth room for 7 to 10 days to

Fig.1 Fresh weight of cucumber callus line B after 2 and 4 weeks of culture.

Leaf explant → 1 week of culture without pressure

I and II

I and II

→ Subcultures on salinized media (125 and 150 mM) → without pressure

→ with 62.5, 100, 125 mM NaCl
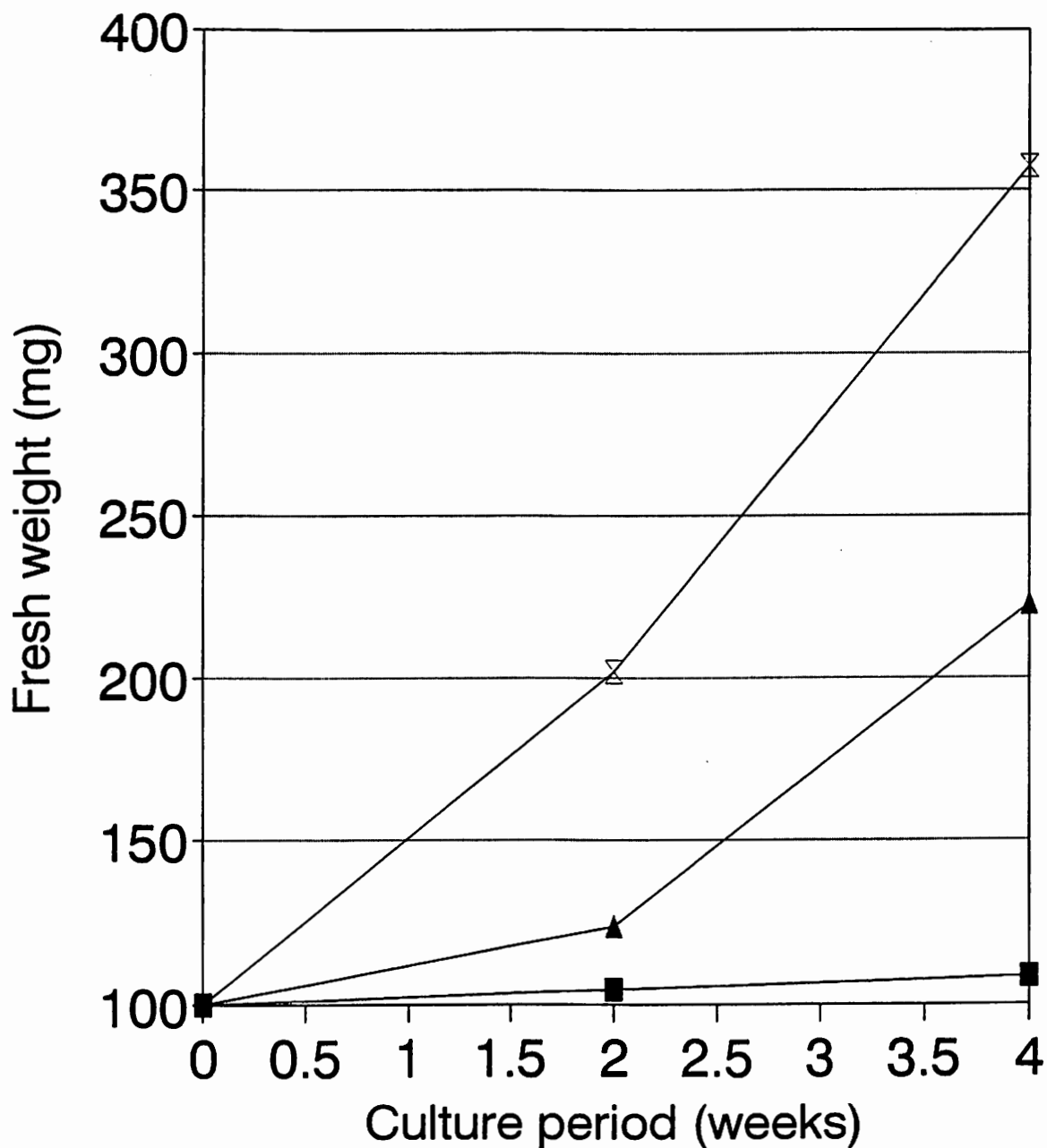
Callus culture   Regeneration
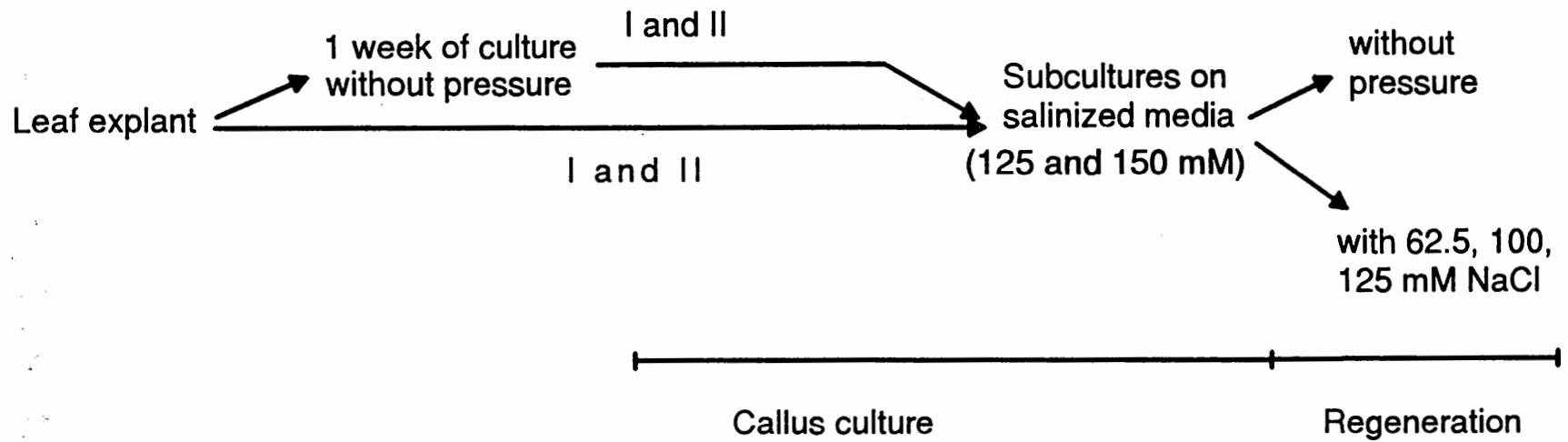
I - two weeks of NaCl pressure
II - 6-8 weeks of NaCl pressure (125 and 150mM)

Fig. 2 The ways of selection of cucumber callus resistant to NaCl.

acclimate. After acclimation, plants were transferred to a greenhouse.

The influence of saline conditions on: 1) callus induction of R1, R2 and F1 plants; 2) the relationship between photosynthetic parameters (efficiency of photosynthesis, stomatal conductance and efficiency of transpiration); and, 3) R2 seedling growth was investigated. Six to twelve leaf explants from each of two selected R1 plants (53.8.3 and 61.6.3) or five from each of 18 to 20 of the control line (line B), F1 generations (line B x 53.8.3 and 53.8.3 x line B), and R2 generation (self pollination of 53.8.3 plant) were plated on NaCl-free medium (CS0) and saline conditions (CS125). After three passages (four weeks each) the growth of calli was estimated. R3 and F1 seeds were germinated at 25C in the dark on filter paper soaked with tap water. Three days later the germinated seeds were transferred into light (16 h photoperiod) and transplanted to a hydroponic system (half-strength of Murashige and Skoog salts). NaCl was absent or added at 75 mM to the nutrient solution.

A Li-6200 Portable Photosynthetic System was used to measure photosynthetic parameters (efficiency of photosynthesis, stomatal conductance and efficiency of transpiration). This system allowed for measurement of a leaf attached to a plant. The leaves (first, second and in one case third) of F1 seedlings treated with 75mM NaCl were selected when they were almost fully expanded. Measurements were performed on the same leaves immediately after the transfer to saline or unsaline conditions (time t_0), and after 3 (experiment I) or 5 (experiment II) days after NaCl treatment (time t_1). Two leaves of each of two seedlings were included for photosynthetic measurements.

In another experiment, seeds were germinated for 36 hours in distilled water. Seven to ten seeds were used in each treatment. Seeds were sown in sand thoroughly soaked with salinized (125 mM NaCl) or unsalinized nutrient solution consisting of half-strength MS salts. The trials were covered with rain-out shelters to avoid unwanted precipitation. Every two days plants were supplemented with an equal amount of unsalinized nutrient solution. The measurements of plant length were performed after two weeks.

Results. In the initial callus growth experiment at NaCl concentrations up to 200 mM, the degree of salt stress was estimated based on visual symptoms. Although a strong inhibition of callus growth occurred at 125 mM, total suppression was observed at or above 175 mM. Starting from 125 mM callus greened rapidly (chlorophyll sectors distinguishable from the remaining necrotic zones). These green sectors never appeared in control callus. Figure 1 shows the growth of callus of line B at the different NaCl concentrations (0, 125 and 150 mM). After 2 weeks of culture at 125 mM or 150 mM, less of an increase in fresh callus weight was observed when compared with control (0 mM). After 4 weeks of culture the callus exhibited a significant increase in fresh weight when grown in the absence of NaCl when compared to the remaining two concentrations. Differences in the increase of fresh callus weight between all three concentrations were significant (analysis of variance).

For selecting salt tolerant cells two NaCl concentrations were chosen (125 mM and 150 mM). NaCl resistant variants with independent sectors of growing callus were primarily considered as a selection method (Figure 2). Initially, the number of initial resistant variants was less dependent on the method of selection. The NaCl concentration had a significant influence on growth but only in combinations without preculture (Table 1). In the eighth passage the number of variants decreased to zero in five out of eight combinations. After 11 weeks of culture only two variants in combination with preculture were maintained. Both the color and rate of growth were not homogenous. The occurrence of variously colored calli (from dark-green through yellow-green to yellow) and two-fold increases in fresh weight after two weeks of culture were noted. Until the eighth passage, the largest number of resistant variants were observed in the second method of selection, especially when the preculture was applied.

Table. 1. The influence of selection method on the number of primary NaCl-tolerant cucumber
          cells variants.

Selection Methods[1]	Medium[2]	Initial no. of explants	No. of primary tolerant variants	Calli/ explant	No. of variants still tolerant after passages		
					4	8	11
I	CS125	60	56	0.93	20	0	0
	P+CS125	72	78	1.08	28	0	0
	CS150	40	15	0.38	11	0	0
	P+CS150	50	36	0.72	11	2	2*[3]
II	CS125	73	46	0.63	14	2	0
	P+CS125	56	52	0.93	30	30	2**[4]
	CS150	88	23	0.26	4	0	0
	P+CS150	44	37	0.84	3	0	0

[1]I = 2 weeks of NaCl-pressure; II = 6-8 weeks of NaCl-pressure.

[2]P = 7 days of preculture on NaCl-free medium.

[3]* After this passage calli died.

[4]** Calli survived 15 passages.

Table 2. The reaction of leaf explants (determined as capable of callus growth) on CS0 and CS125 media after 4 weeks of culture.

| Line or cross | No. of plants | No. of plants with callus regenerating explants on media | |
		CSO	CS125
line B	8	8	0
F1[Bx53.8.3]	12	11*	6
F1[53.8.3xB]	8	8	3
R2[53.8.3 self]	20	15*	6

* Diminished through infections.

Table 3. The characteristics of photosynthesis in cucumber plants grown in nutrient solution for 3[I] or 5[II] days.

| Line or cross | NaCl conc. | A^1 [μmolCO$_2$/m^2/s] | | B [cm/s] | | C [MH$_2$O)/m^2/s] | | D [ppm] | |
		t_0	t_1	t_0	t_1	t_0	t_1	t_0	t_1
line B	0	11.80	9.11	0.27	0.22	5.09	3.06	198	201
[I]	75	10.91	10.02	0.32	0.12	5.31	2.71	226	137
R3[53.8.3]	0	5.68	7.14	0.28	0.18	5.23	3.65	255	215
[I]	75	4.66	7.35	0.27	0.13	4.43	3.76	256	197
line B	0	5.66	2.66	0.14	0.13	2.74	1.90	208	271
[II]	75	4.79	1.84	0.14	0.03	2.89	1.56	221	211
F1[53.8.3xB]	0	5.37	3.74	0.30	0.30	2.83	2.56	251	268
[II]	75	5.17	3.81	0.19	0.05	3.56	0.93	243	164

A^1 = efficiency of photosynthesis, B = stomatal conductance, C = efficiency of transpiration, and D = intercellular CO_2 concentration.

Two types of conditions were tested for regeneration: NaCl-free medium and medium supplemented with NaCl. It was proven that regeneration in the presence of NaCl was impossible even at lower concentrations. Plants were regenerated from compact structures (yellow or yellow-green callus). Symptoms of callus destruction became visible after three weeks of culture on R medium. Of 51 calli plated on R medium, only two showed further development and initiation of regenerating sectors. These sectors were subcultured on fresh R medium. After five weeks, the regeneration of shoot-like structures was observed. Later, plantlets with leaflets were also observed. Finally, after eight weeks of culture on medium R, 15 plants were regenerated. Only four of these survived transfer to soil and two adapted to greenhouse/field culture conditions. They were designated 53.8.3 and 61.6.3. Plant 61.6.3 differed phenotypically from the control plant; its growth was not more than 13 cm in height. Although one female flower appeared on this plant, fruit were not produced. Viability of pollen was apparently lower (53%) relative to the control and plant 53.8.3. No regenerants from initially resistant callus were derived from selection method 1.

The stability of the selected trait was tested by comparing the calli induced from control and those which resulted from tolerant plants. Leaf explants of Rl plants were capable of developing callus on media (CSO and CS125). On the CS125 medium, callus survived at least three passages and calli assumed a yellow coloring (plant 53.8.3). However, the callus of plant 61.6.3 apart from its color (dark-green) also differed from the others in its reaction under unsalinized conditions. It survived on NaCl-free medium for only two passages. In contrast to some explants of Fl and R2 plants, leaf explants of control plants (line B) did not regenerate callus on CS125 medium during four weeks of culture (Table 2). In the self pollinated progeny of regenerant 53.8.3 (R2) somewhat resistant plants were recorded (30%). However, in the Fl generation approximately 50% of the resistant plants were independent of the cross direction.

The aim of the experiments presented in Table 3 was to examine the effect of salt stress on photosynthesis. The data presented show that only the leaves of control plants treated for 5 days had decreased CO_2-assimilation rates. The intercellular CO_2 concentration measured was in the range of 137 to 271 ppm, and the lowest concentration was sufficient for efficient photosynthesis. An addition of 75 mM NaCl to the nutrient solution resulted in stomatal closure in leaves of control and Fl plants. The transpiration of Fl plants was affected by salt amendments only in one case.

The growth characteristics and segregation data for F2 plants are shown in Table 4. Growth in 125 mM NaCl decreased the height of salt tolerant plants by about 30% relative to the control (O mM NaCl). The growth of sensitive plants under salt stress was totally inhibited. The pattern of segregation in both F2 crosses showed that 40% of the F2 seedlings were tolerant to the salt concentrations administered in this study.

Discussion. Developing crops with enhanced salt tolerance is an important current goal in our laboratory. No real source of salt tolerance has been found within *Cucumis* (Anastasio et al., 1988; Shannon and Francois, 1978). Experimental data presented by Shannon and Francois (1978) support the idea that only a partial degree of tolerance is available in the genetic pool of *C. melo*. One possibility for recovering lines with increased salt tolerance through selection *in vitro*. It is known that salt resistance is not stable (Hasegawa et al., 1980). Therefore, an analysis of the resistance mechanism on cellular level has been evaluated using resistant cells obtained from stepwise selection after a long culture period (Hasegawa, 1980; Watad et al., 1983, 1985). No plants were regenerated and analyzed in these experiments. In the present study two methods of selection were applied - one with short term (2 weeks) and the other with long term (6-8 weeks) salt exposure. Both approaches used two NaCl concentrations. The lower salinity levels decreased fresh callus weight (~80%), and higher levels were lethal. Similar selection methods were done by Narayanan

Table 4. Seedling tolerance to NaCl within F2 cucumber progeny.

Line or cross	NaCl concent [mM]	No. seedlings tested	Avg length of seedlings [cm]	Tolerant to sensitive seedlings ratio	Percent of tolerant seedlings
line B	0	10	3.62	----	----
	125	10	0.00	0	0
F2[Bx53.8.3]	0	7	5.22	----	----
	125	10	3.40*	4/6	40
F2[5.38.3xB]	0	7	3.81	----	----
	125	10	2.25*	4/6	40

*Average length of tolerant variants.

and Rangasamy (1989). In the present study plants were regenerated only from calli selected after long term exposure to salt. However, the number of resistant calli at the last selection step was very low regardless of selection method. Therefore, it is concluded that selection efficiency was dependent on the selection method.

The conditions for cucumber *in vitro* regeneration are well known (Malepszy, 1988). However, under salinity stress some differences were found. First, the selected callus was green. This condition was never observed under normal conditions. Second, regeneration was not possible on the saline medium since the concentration was two times lower than that used in the first selection method. Although a negative influence of salinity stress on regeneration has been observed by Nabors et al. (1980), the opposite was observed by Galiba and Yamada (1988). In our studies, regenerated cucumber plants had difficulties adapting to normal growth conditions and the regenerant of one line was unable to seed set (self sterility and sterile pollen). This was similar to cucumber plants regenerated after selection on herbicides (Malepszy et a., 1991) and *Fusarium oxysporum* (El-Kazzaz, 1990; Malepszy and El-Kazzaz, 1990). In the latter, regenerant plants were male fertile.

The reaction of regenerated plants to salinity was analyzed by comparing photosynthetic parameters and growth responses. The degree of salinity applied in the first series of experiments was chosen to be 75 mM, according to the results of Drew et al. (1990) who showed inhibition of cucumber photosynthesis at 50 mM. Our results indicate that photosynthetic and transpiration rates could not serve as reliable criteria for salinity stress discrimination. This may be due to differential cultivar sensitivity in their experiments as well as ours. Salinity is known to reduce the growth of salt sensitive species. We observed that salt had little effect on the growth of F2 plants.

Plants produced in our studies tolerated salinity differentially: 1) callus of Rl plants was resistant; 2) photosynthetic C02 fixation and stomatal conductance of R3 plants were not affected by salinity; and 3) some Fl and F2 plants were sensitive and tolerant. The mode of inheritance was not determined, but Fl phenotypic observation suggests dominant gene action for salt tolerance, similar to tobacco (Nabors et al., 1980), In contrast, tolerance in rice is recessive and conditioned 2 genes (Narayanan and Rangasamy, 1989). In another study (Kononowicz et al., 1990), all tobacco plants regenerated from salt tolerant selection experiments were hexaploid, suggesting that hexaploid cells were more likely to regenerate than cells of other ploidy levels. The results of our work indicate that NaCl-tolerant cucumber can be selected using *in vitro* culture and that the developed procedure can be applied in a plant improvement program.

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Micropropagation of the Cucumber Hybrids 'Brunex' and 'Bambina'

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The female parthenocarpic cucumber hybrids which are cultivated in greenhouses have considerable economic importance during the winter period in the European market. The plants are very productive and their fruits have desirable quality traits. Handley and Chambliss (1979) cultured axillary buds of the gynoecious cucumber hybrid 'Carolina'. The aim of the present work was to test different media and growth regulator combinations for micropropagating the hybrids 'Brunex' and 'Bambina' at low cost. These hybrids are important to Greek farmers.

Seeds of 'Brunex' (Bruinsma) and 'Bambina' (De Ruiter Seeds) cucumber hybrids were soaked in a Triton^R x 100 (Merck) 0.01% v/v solution for 20 min. Subsequently, they were surface sterilized in a Milton (Proctor & Gamble LTD) solution (containing 10 g/l NaOCl and 165 g/l NaCl) for 15 min. The seeds were then rinsed and placed under aseptic conditions in petri dishes containing 0.8% w/v agar-agar (Sigma). Dishes were placed in darkness at 25C for 5 days to allow germination. After germination the seedlings were transferred under aseptic conditions to vessels containing Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) solidified with 0.8% w/v agar-agar, and placed in a growth chamber to allow shoot growth until the stage of 6 leaves. All experiments were conducted in a growth chamber maintained at 25C provided with a 16-hours per day photoperiod by cool-white fluorescent light at 1500 lux.

All media used were solidified with 0.8% w/v agar-agar (Sigma). The pH was then adjusted to 5.7, and they were autoclaved at 121C for 20 min. Four experiments were initiated to estimate op-timum growth regulator levels and to quantify propagation rates. Eight replications per treatment were used in each experiment.

Experiment 1: To evaluate the need for auxin in combination with different cytokinin levels for culture, segments of shoots with two buds were transferred in test tubes each containing MS medium supplied with 0.0, 0.1, and 1.0 mg/l 6-benzylaminopurine (BA) and 0.0 and 0.1 mg/l 1-naphthalenacetic acid (NAA), and placed in a growth chamber.

Experiment 2: To refine the optimum level of cytokinin required for growth, shoots were established horizontally in vessels containing MS medium and incubated in a growth chamber. Two-week-old newly formed axillary shoots were established in test tubes containing MS medium supplied with 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 mg/l BA, and placed in the growth chamber.

Experiment 3: To estimate the optimum level of gibberellin required for growth, shoots were established horizontally in vessels containing MS medium and incubated in the growth chamber. One-week-old newly formed axillary shoots were established in test tubes containing MS medium supplied with 0.5 (in the case of 'Brunex') or 1.0 (in the case of 'Bambina') mg/l BA and 0.5, 1.0, 2.0, 5.0 and 10.0 mg/l gibberellic acid (GA_3) (in both cases).

Experiment 4: To quantify the propagation rate under optimal conditions, shoots with six lateral buds were cut in two segments and placed horizontally in vessels containing MS medium supplied with 0.5 mg/l BA and 10.0 mg/l GA_3 (in the case of

'Brunex') or with 1.0 mg/l BA and 5.0 mg/l GA_3 (in the case of 'Bambina'). Two weeks later, the formed callus was removed from the cultured explants and separated to six segments per each initial shoot, then transferred to new media of the same composition.

Four weeks after the establishment of each experiment, the number of new shoots were counted. Treatment differences in the first three experiments were determined at $P = 0.05$ as assessed by L.S.D. tests.

Results

Experiment 1: The means of the shoots produced per explant (shoot segment with 2 buds) of the cucumber hybrids 'Brunex' and 'Bambina' four weeks after cultivation on MS media supplemented with various levels of NAA and BA are presented in Table 1. When NAA was absent from the media more shoots developed than on media containing NAA. On auxin free media the presence of BA increased the number of shoots. In the case of 'Brunex' more shoots (3.0 per explant) were produced at the medium supplied with 1.0 mg/l BA. This value differs statistically from all other values except one. In the case of the hybrid 'Bambina', more shoots (3.6 per explant) were produced on media supplied with 0.1 and 1.0 mg/l BA. These values differ statistically from all the others. Moreover, they were also higher than the highest value obtained with the 'Brunex' hybrid.

Experiment 2: Since the results from the first experiment indicated that better growth is obtained with media lacking auxin, we used media without auxin in conjunction with more levels of BA in the second experiment. This was done to identify the optimum level of this growth regulator in each hybrid. The means of shoots per explant [newly formed axillary shoot (2-weeks-old)] of 'Brunex' and 'Bambina' four weeks after cultivation on MS media supplemented with various concentrations of BA are presented in the Table 2. It is obvious that the level of BA in the media affects shoot production and that the optimum level of BA for 'Brunex' is 0.5 mg/l (5.2 shoots produced per ex-

plant). This value differs statistically from all the other values, except the one achieved with medium supplied with 1.0 mg/l BA. In 'Bambina', BA level in the media also affects the number of shoots produced. The optimum BA level for this cultivar is 1.0 mg/l (1 1.0 shoots produced per explant). This value differs statistically from all others tested. The number of shoots produced by the hybrid 'Bambina' are higher than 'Brunex' at all the cytokinin levels. Thus, the cultivar differences observed in the previous experiments were verified in the second experiment.

Experiment 3: Keeping BA at the optimum level (i.e., 0.5 mg/l for 'Brunex' and 1.0 mg/l for 'Bambina'), we examined whether or not we could increase the number of shoots produced by adding different amounts of GA_3 to the medium. The means of shoots produced per explant [newly formed axillary shoot (1-week-old)] 'Brunex' four weeks after cultivation on MS media supplemented with 0.5 mg/l BA and various concentrations of GA_3 are presented in Table 3. Likewise, data of 'Bambina' four weeks after cultivation on MS media supplemented with 1.0 mg/l BA and various GA_3 concentrations are also presented. The level of GA_3 affects the number of the shoots produced in both hybrids. Better results for 'Brunex' were obtained at high GA_3 levels (5.0 and 10.0 mg/l). These two values (6.4 and 6.6 shoots per explant, respectively) differ statistically from all other values. For 'Bambina' the best shoot production was between 5-10 mg/l GA_3, but the optimum level was 5.0 mg/l. The value of 15.0 shoots per explant differs statistically from all the other values, except the one achieved at the medium supplied with 10.0 mg/l GA_3. At all the GA_3 levels, the number of shoots produced in 'Bambina' were higher than those of 'Brunex' indicating differences in performance between the two genotypes. In both cases, the increase in the number of produced shoots is due to the elongation of some small shoots. It is hypothesized that without the GA_3 treatment these shoots of small size would have gone uncounted.

Table 1. Means of shoots per explant from 'Brunex' and 'Bambina' cucumber explants four weeks after cultivation on MS media supplemented with various levels of NAA and BA.

		BA (mg/1)		
		0.0	0.1	1.0
NAA	0.0	1.62	2.75	3.00
(mg/1)	0.1	1.75	2.12	1.75

Table 2. Means of shoots per explant [newly formed auxiliary shoot (2-weeks-old)] produced from explants of 'Brunex' and 'Bambina' (in parentheses) cucumber four weeks after cultivation on MS media supplemented with various concentrations of BA.

BA (mg/1)	0.1	0.5	1.0	2.0	5.0	10.00
Shoots produced	3.5 (4.6)	5.2 (6.7)	4.6 (11.0)	2.6 (5.5)	1.7 (2.0)	0.7 (1.0)

Table 3. Means of shoots per explant [newly formed auxiliary shoot (1-week-old)] from 'Brunex' and 'Bambina' (in parentheses) cucumber explants four weeks after cultivation on MS media supplemented with BA and various concentrations of GA_3.

GA_3 (mg/1)	0.5	1.0	2.0	5.0	10.0
Shoots produced	4.5^1 $(10.0)^2$	3.5 (8.0)	3.6 (6.9)	6.4 (15.0)	6.6 (14.0)

[1]'Brunex' supplemented with 0.5 mg/l BA.
[2]'Bambina' supplemented with 1.0 mg/l BA.

Experiment 4: The optimum level of BA and GA$_3$ for propagation was calculated. In 'Brunex' four weeks after culture in MS medium supplemented with 0.5 mg/l BA and 10.0 mg/l GA$_3$, a cluster of shoots were produced (from shoot segments with six buds). After their separation into isolated propagules, an average of 48 shoots were produced from each initial explant. Elongation and rooting of the new shoots was carried out on MS medium free of growth regulators within four weeks. Similarly, in 'Bambina' 52 shoots were produced from each initial explant. These shoots were developed into plantlets.

In conclusion, the data indicate that:

1. The presence of auxin (NAA) in the culture media decreased the shoot propagation rate.

2. The cytokinin (BA) level in the culture media affected the number of shoots produced. The optimum level was 0.5 mg/l and 1.0 mg/l for the hybrids 'Brunex' and 'Bambina', respectively.

3. The presence of gibberellin (GA$_3$) in high levels (5.0-10 mg/l) increased the number of shoots produced.

4. In the case of 'Brunex', the highest propagation rate was achieved when the medium was supplemented with 0.5 mg/l BA and 10 mg/l GA$_3$. From every shoot with six buds, 48 new axillary shoots were produced within four weeks.

5. In the case of 'Bambina', the highest propagation rate was achieved when the medium was supplemented with 1.0 mg/l BA and 5 mg/l GA$_3$. From every shoot with six buds, 52 new axillary shoots were produced within four weeks.

6. Elongation and rooting of the produced new shoots can be carried out on MS medium free of growth regulators within four weeks.

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A Seed Dryer for Cucumber Seeds

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A dryer was built using readily available parts for use in drying cucumber seeds from single-fruit harvest, as well as bulk increases from isolation blocks. The dryer would undoubtedly work on seeds of other crops as well, but we have no test experience with them in this dryer. The dryer described here is the product of several years of modification, this being the fifth version we have built.

The dryer consists of a large sheet metal box with 2 squirrel cage fans on top (Fig. 1). The fans blow air across heating elements and into a chamber in the back of the box. A baffle lets the air flow across each of the 12 shelves inside the main part of the box. Each of the shelves has a hardware cloth bottom, and can hold up to 2300 g (dry weight) of seeds. The bottom of the box has a screened opening for the hot air to exit after passing across the seed-drying shelves. A thermostat above the exit opening keeps the seeds from being overheated during the drying process.

The shelves are loaded with seeds that have been washed (after extracting from mature fruits). We have the best success in drying bulk lots of seeds from isolation blocks when we have less than a 14-mm-thick layer of seeds on the screen. Alternatively, shelves can be loaded with seeds from individual fruits (from hand-pollinations). The seeds from each fruit are placed in the bottom half of a plastic, 100-mm-diameter petri plate (the tops are not used) along with the pollination tag. Each shelf will hold 40 plates.

Drying time is faster if the intake air is cool and dry, so the process takes longer under North Carolina conditions in the summer. We set the thermostat at 30 to 32C to dry the seeds, and have them ready to put in packets or bags after 24 (winter) to 36 (summer) hours.

The dryer is inexpensive and easy to build (we use three in our program). Also, it dries seeds safely and rapidly, and keeps rodents and insects away. Dimensions for construction of the dryer are shown in Fig. 1.

The main parts, available commercially, include: one remote thermostat (O to 50C, 1.5 m capillary, 12 amperes, 240 volts), two fans (2.95 m^3/hr) with motors (150 watts, 1610 revolutions per minute, continuous duty), one motor starter (single pole, double throw, 30 amperes, 600 volts maximum), and six heaters (45 cm long, 650C, 500 watts, 240 volts).

A photograph (Fig. 2) of the dryer shows the general appearance when constructed. We estimate that it required 50 worker-hours and $400 in parts to construct the dryer as shown.

TOP VIEW
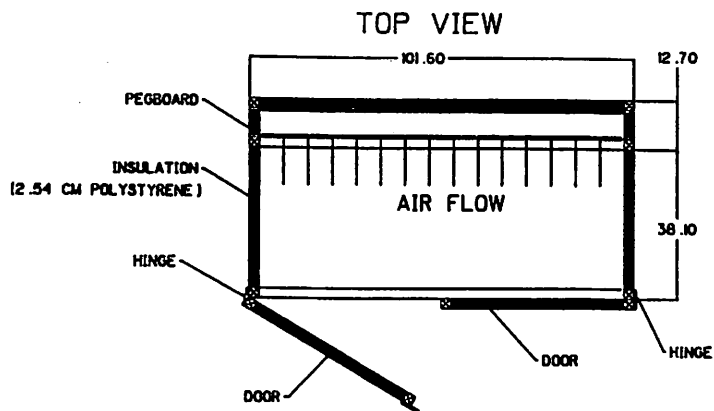
NOTES:

A: ALL DIMENSIONS ARE IN CM

B: THE TRAYS HAVE THE FOLLOWING
DIMENSIONS: HEIGHT = 8.89 CM
LENGTH = 38.1 CM
WIDTH = 95.25 CM

C: THE TRAYS HAVE .635 CM X .635 CM
HARDWARE CLOTH BOTTOMS: THE SIDES
ARE CONSTRUCTED OUT OF WOOD

D: THE BOTTOM OF THE DRYER HAS TWO OPENINGS
5.08 CM X 26.67 CM COVERED WITH .635 CM X
.635 CM HARDWARE CLOTH

FRONT VIEW

SIDE VIEW
(WITHOUT DOORS)
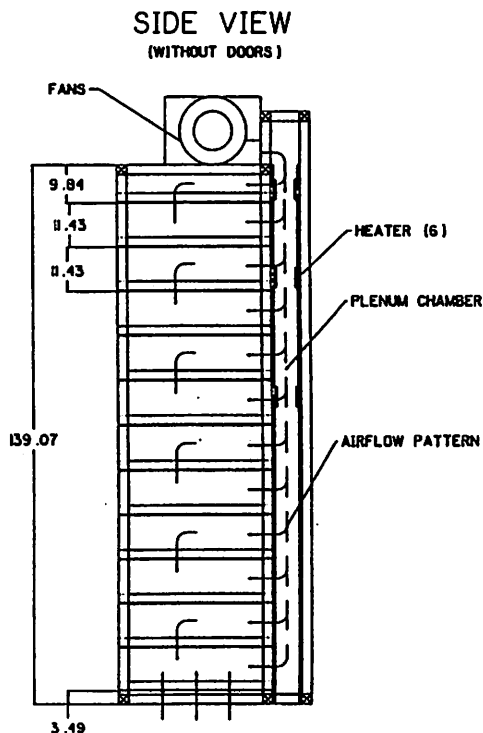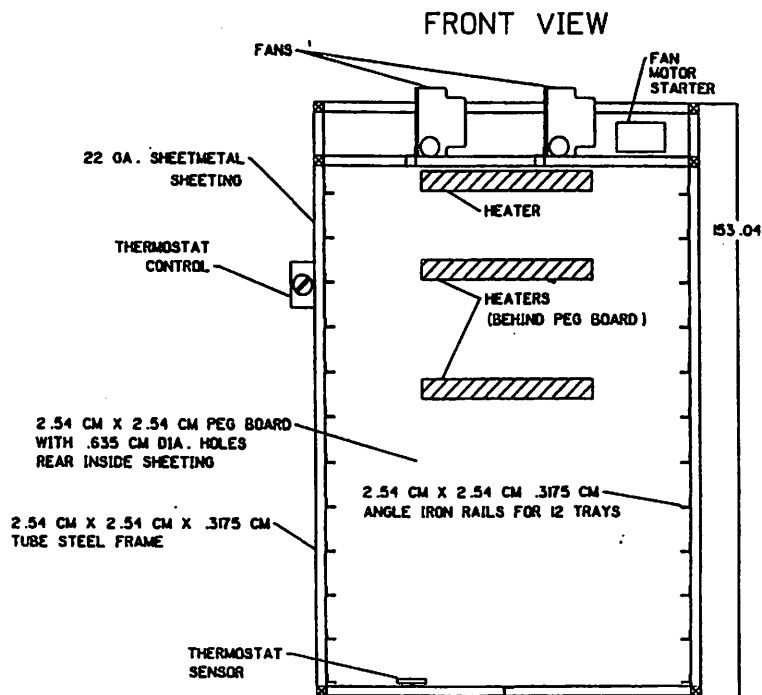
Figure 1. Schematic diagram of the cucumber seed dryer showing dimensions (in cm) for construction.
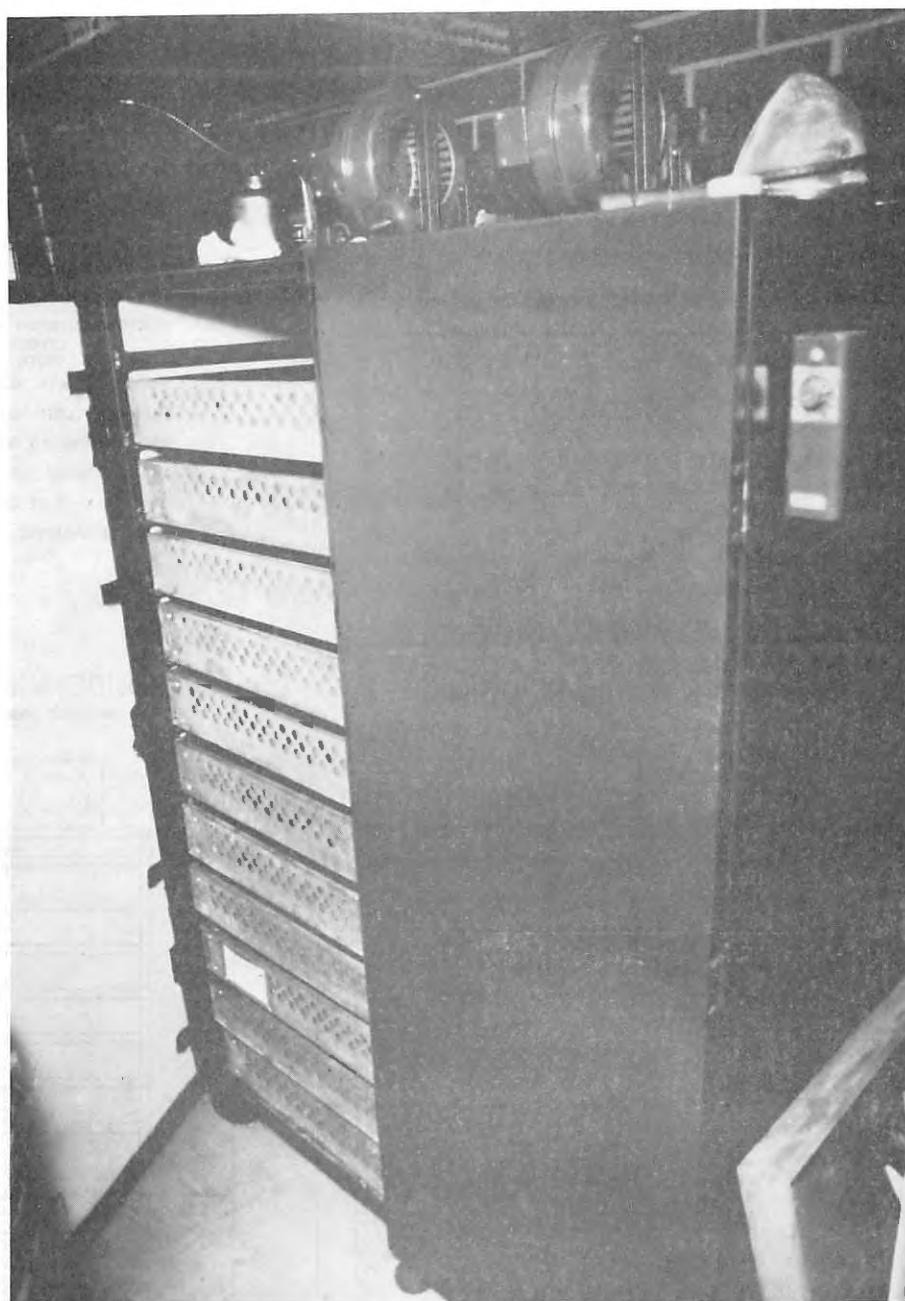
Figure 2. A cucumber seed dryer for use in handling bulk and single-fruit harvests. Photograph shows the sheet metal body, 2 squirrel cage fans on top, 2 access doors (1 closed), 12 seed shelves (1 removed) with 40 petri plate bottoms per shelf, and the remote thermostat on the right side (with the sensor over the exit screen on the bottom of the dryer).

Cucumis melo L. Accessions of the Genebank of the Polytechnical University of Valencia.

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The Genebank of the Polytechnical University of Valencia (Documentation Center of genus *Cucumis*) currently has available more than 500 accessions of melon (*Cucumis melo* L.). Although most of them have been collected in Spain (3,6,7,8), there are some materials from Latin America and Mediterranean Basin.

Most of these accessions, have been characterized and multiplied (2). These works, as well as the collection of new materials, have been supported by the International Board for Plant Genetic Resources (IBPGR) through the "Collecting vegetable germplasm in Spain" project, and by the National Institute of Agricultural Research (INIA) through the "Collection, Multiplication and Evaluation of the Genetic Resources for their Conservation in Genebanks" project. Furthermore, the Genebank was designed in 1984 as a Documentation Center of *Cucumis* genus.

The great diversity of types contained in this collection is outstanding particularly in relation with the adaptation to environmental conditions, due to a long time of stay in the same place. There is also a great diversity for agronomic characters (yield, diseases resistance, etc.) vegetative characters (vigour, leaf colour, etc.) and for fruit characters, both external (skin colour, netted, ribbed, etc.) and internal (flesh colour, Brix grades, etc.).

The accessions have been arranged by description in some groups, which have been established in accordance with commercial types on the Spanish market.

The types are the following:

□ 'Amarillo' - Yellow skin with a wide range of tonalities, usually without either secondary colour or design. An example of this type could be the 'Jaune Canaria'. Other shapes like globular, oblate, elliptical, ovate, etc. are present in the collection.

□ 'Piel de Sapo' - Green skin with dark green spots. Elliptical and oblate shapes are predominant, although oval fruits are also present.

□ 'Rochet' - Light green skin with yellow speckling.

□ 'Tendral' - Dark green, very wrinkled and thick skin, which make them specially resistant to transportation and gives them a long conservation period. Typical Spanish late melons are included in this type.

□ 'Blanco' - White or whitish skin. Netted can be present although with low intensity.

□ 'Cantalupo' - Globular or flattened shape in most cases small in size. Flesh is aromatic but few sweet and usually is orange coloured. Melons included in this type usually are deeply ribbed and both can be netted ('Cantalupo americano') or not ('Cantalupo charentais').

□ 'Hilo Carrete' - Light green skin with long dark green spots almost striped. This type is grown in South Spain.

□ 'Alficoz' - This type belongs to the var. *flexuosus*. White skin and snake-like shaped (the fruits can reach more than 1 m. long). It is basically used for salad.

□ 'Un-Named' - Accessions whose characteristics do not allow their inclusion in any of the former types have been included in this type. There is a great diversity for a lot of interesting characters, as shape, skin colour, wrinkled, ribbed, etc.

Moreover, there is an important group of accessions (nearly 70), which have not been included in the previous groups because they have shown a great variability for some of the characters within each accession.

Despite the established groupings, a great diversity for a wide number of characters remains within each type. At this moment a cataloguing task is underway, distributing each type in groups according to their potentially interesting commercial characters in order to produce a catalogue which will include all the available melon accessions of the Genebank of the Polytechnical University of Valencia. In the characterizations carried out, there are some accessions of almost all types, which due to their good agronomic behaviour and fruit features could be competitive with the present commercial hybrid varieties.

Table 1 shows the characters used in the grouping done, as well as the number of accessions belonging to each type, excepting those types containing a low number of accessions as 'Cantalupo' (6 accessions of 'Cantalupo americano' and 8 of 'Cantalupo charentais'), 'Hilo Carrete' (5 accessions) and 'Alficoz' (4 accessions).

In addition to this collecting, characterization and multiplication works, trials for testing to dieback (4) and yellowing (5) diseases and stress conditions have been carried out. Resistance to salinity has been shown by some accessions (1).

Conservation of this kind of materials is highly interesting as it represents a great source of variability which could be very useful for genetic breeders, both characters currently demanded by the market and others that would possibly be interesting in the future.

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Table 1.   Characters used and number of accessions for each type in the grouping performed.

'Amarillo' Type

Time of Maturity	Skin Netted	Skin Texture	Ribbing	Num. of Accessions
Early	Yes	Indifferent	Yes	4
			No	2
	No	Smooth	No	8
		Wrinkled	No	13
Intermediate	Yes	Smooth	No	12
		Wrinkled	Yes	2
			No	5
	No	Smooth	No	21 (Oblate)
				11 (Globular)
Late	No	Wrinkled	No	11
				89

'Piel de Sapo' Type

Skin Texture	Fruit Shape	Ribbing	Num. of Accessions
Wrinkled	Ovate	No	15
	Elliptical	No	21
	Oblate	No	10
Smooth or Finally Wrinkled	Ovate	Yes	3
		No	10
	Elliptical	Yes	1
		No	26
	Oblate	No	6
			92

'Rochet' Type

Skin Texture	Skin Netted	Fruit Shape	Num. of Accessions
Smooth	Yes	Ovate	8
	No	Ovate	17
		Elliptical	10
Wrinkled	Yes	Ovate	5
		Elliptical	18
	No	Ovate	6
		Elliptical	13
		Oblate	2
			79

Table 1.   Characters used and number of accessions for each type in the grouping performed.
(continued)

'Tendral' Type			
Skin Colour	Skin Texture	Fruit Shape	Num. of Accessions
Green	Fairly Wrinkled	Ovate	4
		Elliptical	4
	Very Wrinkled	Ovate	6
		Globular	1
		Elliptical	10
Very Dark Green	Fairly Wrinkled	Ovate	11
		Elliptical	2
	Very Wrinkled	Ovate	5
		Globular	3
		Elliptical	8
			54

'Blanco' Type		
Skin Netted	Skin Texture	Num. of Accessions
Yes	Smooth	4
	Wrinkled	3
No	Smooth	8
	Wrinkled	8
		23

'Un-Named' Type			
Skin Texture	Fruit Colour	Fruit Shape	Num. of Accessions
Smooth	Pale green	Elliptical	12
		Globular	2
	Yellow with Green Strips	Ovate	3
	Green	Ovate-Globular	12
		Pyriform	4
		Elliptical-Cylindrical	3
Wrinkled	Yellowish	Elliptical	14
		Cylindrical	2
	Green	Elliptical	17
		Ovate-Globular	5
	Very Dark Green	Ovate-Elliptical	4
		Cylindrical	1
			79

Chromosome Number and Karyotype of Melons (*Cucumis melo* L.)

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The number of chromosomes in 36 melon cultigens was identified and the karyotypes of two typical melon cltivars were analysed by the improved cytological method. The results were shown as follows: 1) all of the materials identified, except a tetraploid strain with 48 chromosomes (4n=48) in somatic cells, are normal diploid (2n=24); 2) the karyotypes of an America muskmelon (*Cucumis melo* var. *reticulatus*) and "Hetain" (*Cucumis melo* ssp. *melo* Pang) could be described as: 2n=2x=24=20m+2sm+2sm (SAT) and 2n=2x=24=18m+4M+2sm, respectively. Both karyotypes showed better symmetry which belong to 2A and 1A type, respectively.

In China, melon (*Cucumis melo*, L.) is an important horticultural crop and the germplasm resources are very rich and have a long growing history. In the world, there are more than 3000 cultivars and forms which belong to 8 variety groups. However, because of the heavy plasm and small chromosomes in melon cells, it is quite difficult to stain the chromosome and to identify the morphological characteristics of the chromosomes. So far, the reports on melon chromosomes have been extremely rare. In this paper, some characteristics of melon chromosomes were studied which would be of significance in melon breeding, classification, evolution, etc.

Newly developed root tips or shoot apices from each of melon cultigens were used. Chromosomes were identified by the improved cytological method of Zhang Zili (1977). Thirty metaphase somatic cells from each of the materials were selected and the number of chromosomes in each of cells was determined. The karyotypes of two

typical cultivars, American muskmelon (*Cucumis melo* var. *reticulatus*) and "Hetian," (*C. melo* ssp. *melo* Pang.) were analysed according to the chromosome classification and nomenclature method of Leven, 1964.

Number of chromosomes and ploidy in each of 36 melon materials. The results are shown in Table 1. Among all of the 36 melon cultigens, 32 (numbers 1-32), including cultivars, hemi-wild species and wild species from various regions of the world, are normal diploid with a common number of chromosomes (2n=2x=24) in spite of remarkble differences in the origin and habits; 3 (numbers 33-35) are mixoploid, i.e. many diploid somatic cells are mixed with few tetraploid cells in same material; and only 1 (number 36) is an artificial tetraploid melon strain (4n=4x=48).

Chromosome shape and karyotypes. Two typical cultigens, America *Reticulatus* and Hetian, were used as the materials for karyotype analysis. The length and arm ratio of chromosome in America Reticulatus are shown in Table 2. The absolute length of chromosomes at metaphase ranged from 1.65 to 1.18 m with the relative length ranging from 10.02 to 7.13 %m. Ratio of the longest chromosome to the shortest one is 1.4. The mean length of 12 chromosomes is 1.37 m. Chromosomes 1, 2, 4, 5, 7, 8, 9, 10, 11, and 12 belong to metacentric chromosome (m) with their arm ratios ranging from 1.08 to 1.52. Chromosome 3 belongs to submetacentric chromosome (sm) with its arm ratio being 2.17. Chromosome 6 with a larger satellite on the short arm belongs to submetacentric SAT-chromosome with its arm ratio being 1.84. Considering the morphological char-

Table 1. Number of chromosomes in somatic cell.

Order Number	Name	Origin	Variety	Number of Chromosome
1	Hongxin melon	Liaoning, China	*makuwa*	24
2		Japan	*makuwa*	24
3	Yi wofeng	Shaanxi, China	*makuwa*	24
4	Dajing *reticulatus*	Japan	*reticulatus*	24
5		Japan	*reticulatus*	24
6	Baidi	Japan	*inodorus*	24
7	Bailangua	Lanzhou, China	*inodorus*	24
8	81-49	Hebei, China	*reticulatus*	24
9	Spain	Spain	*cantalupensis*	24
10	Mapao melon	Shaanxi, China	wild melon	24
11	KH-242	South Asia	*reticulatus*	24
12	Lan pang	U.S.A.	*inodorus*	24
13	Dudaim	Hebei, China	*dudaim*	24
14	America *reticulatus*	U.S.A.	*reticulatus*	24
15	Haidongqing	Shanghai, China	*makuwa*	24
16	Balixiang	Changchun, China	*makuwa*	24
17	Huanan 108	Hunan, China	*makuwa*	24
18	Huangli	Hebei, China	*makuwa*	24
19	Longtian-1	Heilongjiang, China	*makuwa*	24
20	Yilianghu	Japan	*reticulatus*	24
21	84-29	Hebei, China	*cantalupensis*	24
22	81-48	Hebei, China	*cantalupensis*	24
23	Huangzuixian	Xinjiang, China	*cantalupensis*	24
24	Baicaigua	Hebei, China	*conomon*	24
25	Snaky melon	Middle East	*flexuosus*	24
26	Lantiangua	Lanzhou, China	*makuwa*	24
27	Wild Hami	Xinjiang, China	*Pubescens*	24
28	Lantian-5	Lanzhou, China	F_1	24
29	Gold melon	Shanghai, China	*makuwa*	24
30	Hetian	Xinjiang, China	ssp. *melo* Pang.	24
31	Kaer	Xinjiang, China	ssp. *melo* Pang.	24
32	Baipicui	Xinjiang, China	ssp. *melo* Pang.	24
33	Egypt melon	Egypt	*reticulatus*	24,48
34	Halesbest	U.S.A.	*cantalupensis*	24,48
35	Aheqi	Xinjiang, China	*cantalupensis*	24,48
36	Tetraploid	Artificial	*cantalupensis*	48

Table 2. Karyotype date of America muskmelon (*Cucumis melo* var. *reticulatus*) and Hetian.

Order number of Chromosome	Absolute length (μm)	Relative length (%)	Arm ratio (long/short)	Type
American muskmelon				
1	0.93 + 0.72 = 1.65	5.65 + 4.37 = 10.02	1.29	m
2	0.86 + 0.73 = 1.59	5.22 + 4.44 = 9.66	1.18	m
3	1.09 + 0.5 = 1.59	6.59 + 3.04 = 9.63	2.17	sm
4	0.78 + 0.72 = 1.50	4.47 + 4.37 = 9.11	1.08	m
5	0.77 + 0.71 = 1.48	4.68 + 4.31 = 8.99	1.09	m
6	0.86 + 0.47 = 1.33	5.22 + 2.83 = 8.05	1.84	sm
7	0.76 + 0.54 = 1.30	4.60 + 3.28 = 7.88	1.4	m
8	0.75 + 0.54 = 1.29	4.53 + 3.28 = 7.81	1.38	m
9	0.73 + 0.53 = 1.26	4.59 + 3.13 = 7.72	1.47	m
10	0.77 + 0.49 = 1.26	4.53 + 3.03 = 7.56	1.50	m
11	0.75 + 0.49 = 1.24	4.53 + 2.98 = 7.51	1.52	m
12	0.61 + 0.54 = 1.18	3.85 + 3.28 = 7.13	1.17	m
Hetian				
1	0.91 + 0.64 = 1.55	6.56 + 4.61 = 11.17	1.43	m
2	0.84 + 0.47 = 1.31	6.05 + 3.39 = 9.44	1.78	sm
3	1.78 + 0.49 = 1.27	5.62 + 3.53 = 9.15	1.59	m
4	0.75 + 0.48 = 1.23	5.4 + 3.45 = 8.85	1.57	m
5	0.73 + 0.47 = 1.20	5.26 + 4.39 = 8.65	1.55	m
6	0.69 + 0.47 = 1.16	4.97 + 2.39 = 8.36	1.47	m
7	0.71 + 0.45 = 1.16	5.12 + 3.24 = 8.36	1.58	m
8	0.66 + 0.45 = 1.11	4.76 + 3.24 = 8.00	1.47	m
9	0.55 + 0.51 = 1.02	3.67 + 3.67 = 7.34	1.0	M
10	0.49 + 0.49 = 0.98	3.53 + 3.53 = 7.06	1.0	M
11	0.51 + 0.47 = 0.98	3.67 + 3.39 = 7.06	1.08	m
12	0.49 + 0.42 = 0.91	3.53 + 3.03 = 6.56	1.17	m
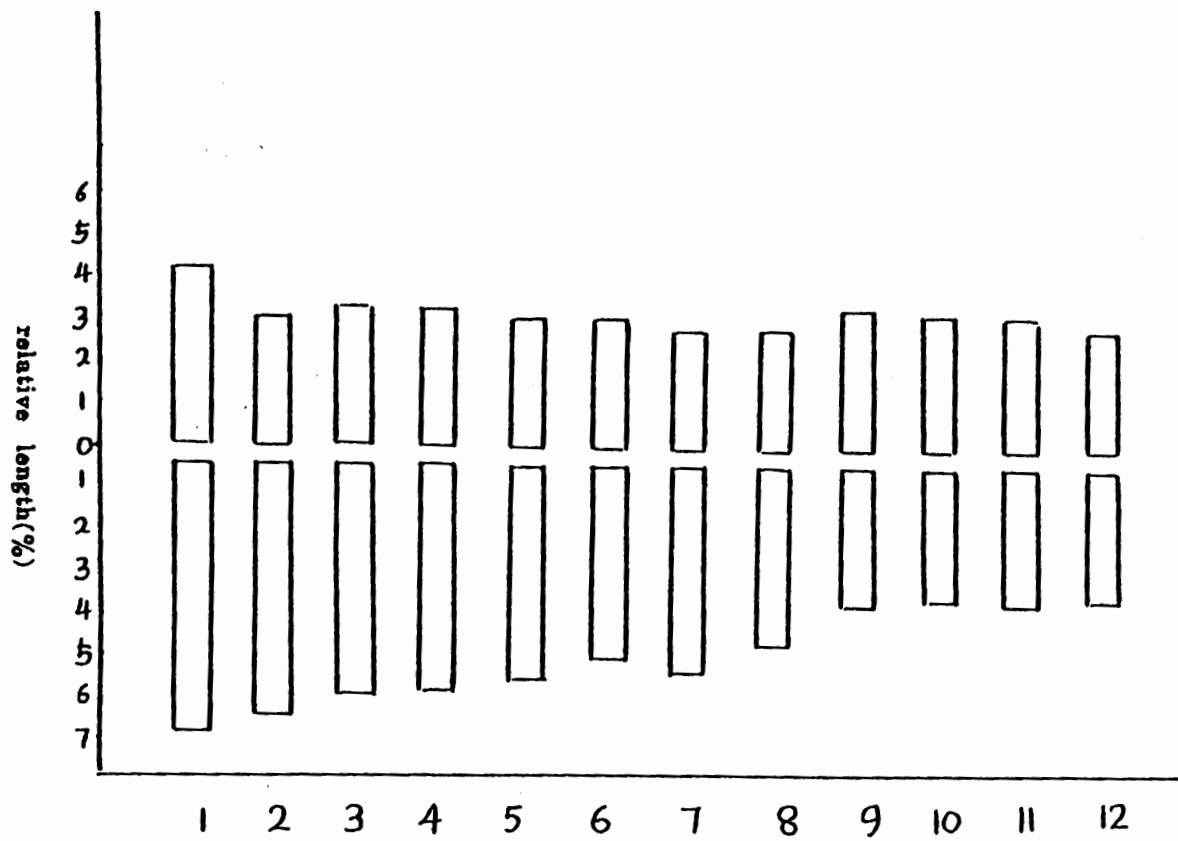
Fig 2   Karyotype in 'Hetian' variety (mold graphigue)
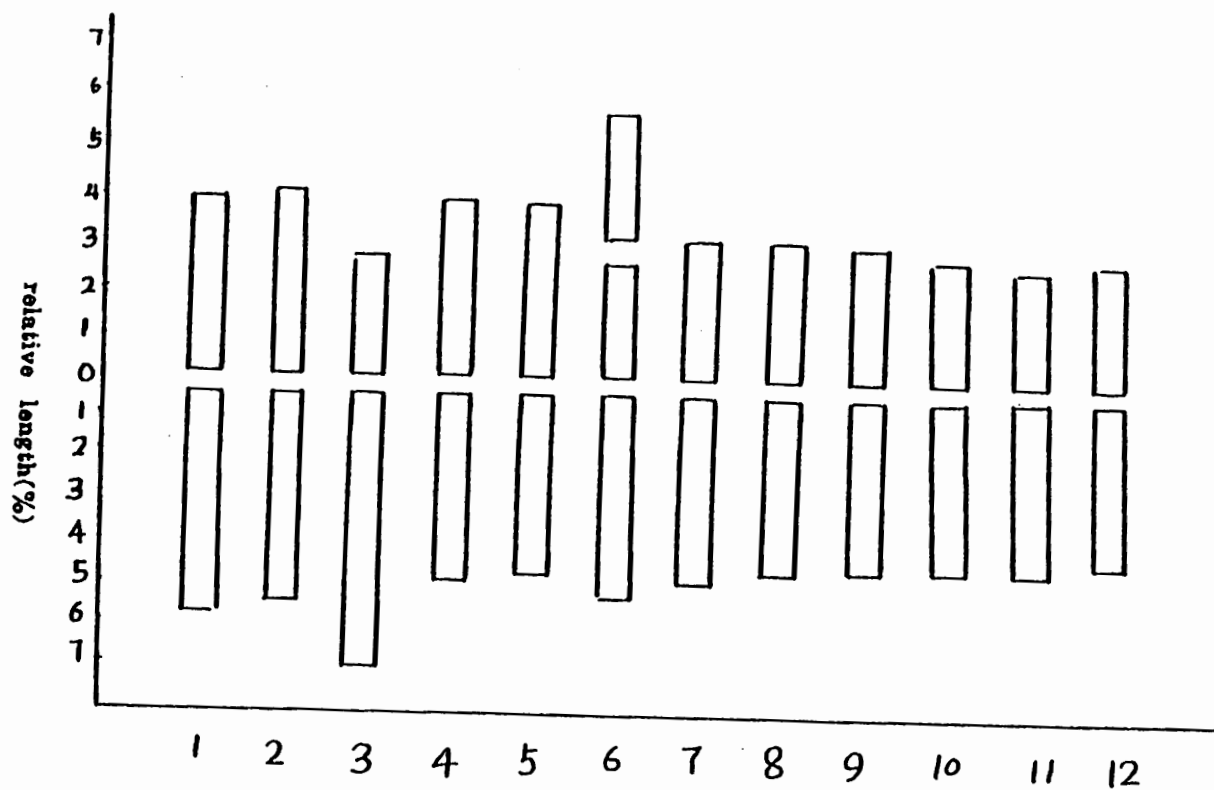
Fig 1 Karyotype in America muskmelon (mold graphique)

acteristics and the arm ratios, all of the 12 pairs of chromosomes could be distinguished clearly at metaphase as shown in Fig. 1. The chromosomes were arranged in descending order of the length and the karyotype formula can be expressed as: 2n=2x=24=20m+2sm+2sm (SAT), which belong to 2A type.

Hetian is a local melon cultivar from Xinjiang, China, belonging to middle Asia ecological group. Its karyotype data are shown in Table 2. Absolute length of the chromosomes ranged from 1.55 to 0.91 m, and relative length from 11.17 to 6.56. The ratio of the longest chromosome to the shortest one was 1.70. None of STA-chromosomes was recognized. Nine pairs of chromosomes (chromosome numbers 1, 3, 4, 5, 6, 7, 8, 11, and 12) are metacentric with their arm ratios ranging from 1.08 to 1.59. Only one pair of chromosome (chromosome number 2) is submetacentric with arm ratio being 1.78. Two pairs of chromosome (chromosome number 9 and 10) are right metacentric chromosomes (M) with their arm ratios being 1. According to the length, arm ratios, and other morphological characteristics, all 12 pairs of chromosomes could be distinguished and arranged in a descending order of the length as shown in Fig. 2. Its karyotype belongs to 1A type and the karyotype formula could be expressed as: 2n=2x=24=18m+4M+2sm.

Melon karyotype and its position in Cucurbitaceae. The results in this study showed that the melon karyotypes belong to 1A or 2A types. A better symmetry of chromosomes was found as compared with that in cucumber (1A), gourd (1A), sponge gourd (1A), balsampear (2A, 1B), watermelon (2A), white gourd (2A), Chinese squash (2A), Indian squash (2A), America squash (2B), chayote (2B), and snake gourd (2A) (Rongqian Li, 1989). According to Stebbins' (1971) theory concerning plant karyotype evolution, we could deduce that melon belongs to species of a lower evolutive degree in Cucurbitaceae on the basis of the karyotypes mentioned, while the cucumber, gourd and sponge gourd etc., belong to the very old crop type in evolution.

Morphological characteristics of the chromosomes in melon. Chromosomes in melons have the following main features: 1) the range of chromosome length is narrow; 2) chromosomes are mainly metacentric or submetacentric with a better symmetry; 3) the size of chromosomes is small, shorter than 2 m in length and melons belong to the crops with the smallest chromosomes in *Cucurbitaceae* plants; 4) secondary constriction is not recognized.

Karyotype variation in melons. Although the number and shape of the chromosomes are the same or similar in different melon species, some differences exist, such as chromosome size and karyotype, etc. These differences revealed a certain variation in chromosome structure in melon plants. Similar phenomena were discovered in garlic (Verna, 1978), Senvy, tomato, cucumber, pepo and white gourd (Rongqian Li, 1989). So far, no satisfactory explanation from genetic aspect has been provided and further investigations are needed.

In the experiment, a few tetraploid cells in a couple of diploid melon materials were observed. We consider that the origin of the tetraploid cell might be: 1) the number of chromosomes in few cells was doubled during the treatment with chemicals; 2) the tetraploid cells were induced to be doubled spontaneously.

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Races of *Fusarium oxysporum* f. sp. *melonis* Causing Wilt of Melons in Central Sudan

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Fusarium wilt of melons is a disease of worldwide occurrence. Four races of the causal organism, *Fusarium oxysporum* Schlecht f. sp. *melonis* Snyyder & Hansen, are known to occur. These are RO, R1, R2, and R1-2. Race 1-2 is further subdivided into isolates that cause wilt and others that cause necrotic yellowing. This nomenclature, proposed by Risser et al. (1976), is the one adopted in the present study.

In Sudan, Fusarium wilt is observed to pose a grave threat to melons in the major melon producing areas in central Sudan, especially when the fall crop, which coincides with the rainy season (July - October), is grown in the central heavy clay plains. Early infections under these conditions could lead to total crop loss. The winter crop of the 'Gallia' type F_1's which is intended for export to Western Europe and the Middle East, also faces a similar threat from the disease, and crop losses of 40% or more are common. In contrast, melon land races, that still predominate in the White Nile growing areas of central Sudan, are less affected by the disease and have shown variable degrees of resistance.

The fungus attacks muskmelon at various stages of its growth and typical symptoms of the disease are often observed to occur at flowering and during early fruit-setting. In the mature infected plants, wilt is observed to progress rather slowly, with later symptoms appearing as tip-burning of leaf edges, foliar chlorosis, stunting of plants, and greatly reduced fruit sizes. When affected stems are sliced, yellow, orange or brownish staining is observed in the water-conducting tissues. Under moist conditions, a white or pink fungal growth may be visible on dead stems.

On further examination of diseased plants of 'Gallia', 'Alma', 'Ananas', and 'Regal' hybrids collected from melon fields in central Sudan and examined at INRA-Avignon, at Montfavet Cedex-France, the pathogen isolated was positively identified as *F. oxysporum* f. sp. *melonis*. To identify the prevalent races of the fungal pathogen in central Sudan, two isolates of the fungus recovered from the diseased plants were inoculated into five differential hosts of *C. melo*, viz., 'Charentais T', 'Isoblon', 'Isovac', 'Margot', and 'Isabelle'. Two weeks later, the inoculated differential hosts were graded for resistance and susceptibility and the results are shown in Table 1. These results clearly indicate the presence of Race 0 and Race 1 in the Sudan.

In another test, the popular muskmelon hybrids and cultivars grown in Sudan were exposed to inoculum from each of the four known races of the fungus. The results shown in Table 2 indicate that 'Gallia' and 'Alma' are susceptible to all races, 'Ananas' is susceptible to races 1 and 1-2, while 'Regal' is resistant to all races except race 1-2. These results are clearly in line with our own field observations under Sudan conditions where 'Gallia' and 'Alma' are seen to suffer most, followed by 'Ananas' and then 'Regal', which was the least affected.

When field-tested during last summer and fall seasons at the University of Gezira research fields,

Table 1. Reaction of differential cultivars to known races and to Sudanese isolates.

Cultivar	Resistance genes	Reaction to known races				Reaction to Sudanese races	
		0	1	2	1-2	Isolate 1	Isolate 2
Charentais T	Non	S	S	S	S	S	S
Isoblon	Fom-1	R	S	R	S	S	R
Isovac	Fom-2	R	R	S	S	R	R
Margot	Fom-1 + Fom-2	R	R	R	S	R	R
Isabelle	Fom-1 + Fom-2 + Polygenic recessives	R	R	R	R	R	R

Table 2. Reaction of major cultivars grown in sudan to the four wilt races.

Cultivar	Race 0	Race 1	Race 2	Race 1-2
Alma F1	S	S	S	S
Ananas	R	S	R	S
Gallia F1	S	S	S	S
Reagal	R	R	R	S

S = Susceptible reaction

R = Resistant reaction

varieties 'Isabelle', 'Margot' and 'Isovac' have proved highly resistant to the disease. Consequently, 'Isabelle' is now used as a donor parent in a backcross program to transfer wilt resistance to 'Ananas' and some other breeding lines intended for hybrid seed production. The wilt problem in the F_1 hybrids grown for the export markets will probably remain with us for some time, until resistant 'Gallia' or 'Gallia'-type melons are developed in Sudan or otherwise supplied by foreign seed companies.

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Preliminary Screening of Indigenous Cultivars and a Few Known Marker Lines of *Cucumis melo* for Fusarium Wilt and CGMMV Resistance

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Fusarium wilt (3,5,6) among the fungal diseases and CGMMV (4) among the viral diseases have been found to be responsible for the total eradication of the crop in Delhi and surrounding areas. In an attempt to identify various accessions of *C. melo* responsive to nonconventional means of hybridization, a number of known marker lines (Tables 1 and 2) were utilized in the present study to outline their percentage germinability, field sensitivity to *Fusarium oxysporum* f. sp. *melonis* and CGMMV. The percentage germination of seeds under controlled conditions was found to be 40.0 percent or above, except in marker *dl* (dissected leaf), where percentage germination of seeds was only 4.0 1.53 percent. The germinated material transplanted to well manured and fertilized soil in the field, at the 4- to 5-true-leaf stage, was screened against *Fusarium oxysporum* f. sp. *melonis* and CGMMV. Except 'Pusa Madhuras' (PM), M4, and 'Perlita FR' (*Fom-3*), all other marker lines succumbed to Fusarium wilt and could not grow beyond 7- to 8-true-leaf stage, whereas PM, M4, and Perlita FR (*Fom-3*) could bear fruits and seeds. Scoring of various horticultural fruit characters and response to prestandardized regeneration medium of various marker lines is under study.

Materials and Methods. In the present study, 4 seeds per bag in replicates of six were sown under controlled nethouse conditions in the second week of February, using various indigenous cvs. and marker lines (Table 1) obtained from Station D'-Amelioration, Des Plantes Maraicheres, Maurice-84140, Montfavet, France, in 1992 and again in 1993. Percent germination of each marker line was scored at the 2-true-leaf stage. Seedlings were allowed to establish in bags up to 4- to 5-true-leaf stage under controlled conditions, after which

transplanting was done in a well manured prepared soil in the field.

Seedlings were allowed to establish and grow under field conditions with proper care. Sensitivity of seedlings to Fusarium wilt was scored at the 7- to 8-true-leaf stage and sensitivity to CGMMV was scored from the 7- to 8-true-leaf stage to flowering stage. The insensitive/resistant lines could bear seeded fruits.

This study was a preliminary step towards identifying the known marker lines suitable for growing, under Delhi conditions, in order to incorporate these marker lines in conventional hybridization or somatic hybridization studies.

Results. Various indigenous cvs. (e.g., 'Pusa Madhuras' [PM]; M4, and 'Kakri') were found to be insensitive to Fusarium wilt and CGMMV. These indigenous cvs. also exhibited percentage germination to be 75 percent and above (Table 2). 'H'arela was 100% sensitive to Fusarium wilt at the 7- to 8-true-leaf stage and was not available for scoring for CGMMV resistance. 'Arka-Jeet' obtained from I.I.H.R., Bangalore, was 50% sensitive to Fusarium wilt, the rest of the 50% were sensitive to CGMMV. Out of the various marker lines obtained from Montfavet, France, the percentage germination was 40% or above, except 4.0 ± 1.53% in EC-327438 (*dl*). Almost all the marker lines obtained from France were found to be 100% sensitive to Fusarium wilt and could not grow beyond 7- to 8-true-leaf stage and hence were not available for fruit harvest, whereas EC-327435 (PMR-5) was insensitive to Fusarium wilt and CGMMV and could bear seeded fruits.

Table 1. Description of various accessions obtained from station d'Amelioration des plantes Maraicheres, Domaine St-Maurice-84140 Montfavet, France.

Accession No.	Variety	Description
EC-327434	PI 124112 (PM-4, PM-5)	Powdery mildew resistance 4 and 5
EC-327435	PMR-5 (PM-1, PM-2)	Powdery mildew resistance 1 and 2
EC-327436	Va 435 (nsv)	Necrotic spot virus resistance
EC-327437	Charentais (Fom-1)	*Fusarium oxysporum* f. sp. *melonis* resistance-1
EC-327438	Marker (dl)	Dissected leaf
EC-327439	gf	Green flesh fruits
EC-327440	Perlita FR, (Fom-3)	*Fusarium oxysporum* f. sp. *melonis* resistance -3

Table 2. Germinability and sensitivity to *Fusarium oxysporum* f. sp. *melonis* and CGMMV of indigenous cvs. and known marker lines.

S.No.	Accession	Percent germination	Sensitivity to Fusarium wilt	Sensitivity to CGMMV	Fruits with seeds harvested
1.	PM^z	100.00 ± 0.00	–^{y}	–	Yes
2.	M4	83.33 ± 07.86	–	–	Yes
3.	Kakri	75.00 ± 25.00	–	–	Yes
4.	Harela	50.00 ± 8.00	++^{x}	NAS^w	No
5.	Arka-Jeet	66.66 ± 38.18	+^{v}	+	No
6.	EC-327434	100.00 ± 0.00	++	NAS	No
7.	EC-327435	83.33 ± 14.43	–	–	Yes
8.	EC-327436	41.66 ± 14.43	++	NAS	No
9.	EC-327437	83.33 ± 14.43	++	NAS	No
10.	EC-327438	4.00 ± 1.53	++	NAS	No
11.	EC-327439	41.66 ± 14.43	++	NAS	No
12.	EC-327440	100.00 ± 0.00	–	–	Yes

^{z}Control

^{y}Not sensitive

^{x}100% sensitive

^{w}Not available for scoring

^{v}50% sensitive

Discussion. This study has outlined indigenous cvs. PM, M4, 'Kakri', and the marker line EC-327435 (PMR-5) to be insensitive to Fusarium wilt and CGMMV and thus can be replicated for seeds. Further, they can be suitably utilized in conventional and nonconventional hybridization programs (e.g., somatic hybridization). A further study outlining the regeneration response of various marker lines is in progress. PM and M4 have already been reported to be highly regenerative due to the presence of dominant marker *G* (1).

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Appearance of a Possible New Melon Yellowing Disease in Spain

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A previously unknown melon yellowing disease associated with the presence of *Bemisia tabaci* (5) has been found in melon, *Cucumis melo* L., cultivated under plastic greenhouses in southeastern Spain. In this area, the vector of melon yellowing disease virus in melon was *Trialeurodes vaporariorum* (9), but since 1989 this has been progressively displaced by *Bemisia tabaci*. In some crops, the two vectors were found co-existing.

Experiments were started to study the causal agent-vector relationship and the nature of the pathogen to help the search for possible sources of resistance to this new melon yellowing disease.

In advanced stages, it is difficult to differentiate the symptoms from those of yellowing disease transmitted by *Trialeurodes vaporariorum* (5) but, in the initial stages, chlorotic leaf spots are usually more diffuse. Early symptoms appear around 20 to 22 days after inoculation and are well established at 30 days.

The causal agent was not transmitted mechanically by the two extraction techniques employed in this work: using either phosphate buffer 0.1M pH 7, or the same buffer plus DIECA, a compound usually needed for difficult-to-transmit viruses.

The efficiency of *B. tabaci* as a vector of the pathogen was determined with a series of seven plant groups inoculated with 1, 5, 10, 20, 30, 40, and 60, *B. tabaci* individuals that had previously been allowed to feed for 48 h on plants showing symptoms. In three replicates of the experiment we observed that, although one individual of *B. tabaci*

transmitted the disease, 100% transmission required 60 individuals.

The persistence of the infective particle inside the vector was determined by inoculating batches of plants each day with 50 individuals of *B. tabaci* that had previously been allowed to feed for 48 h on plants showing clear symptoms; three replicates were used for this experiment. The results revealed that transmission occurred until the seventh day after inoculation and this indicated that the causal agent-vector relationship was of the semi-persistent, non-circulative type described by Duffus (4).

To study the minimum period needed to acquire the pathogen and transmit it to healthy plants, six groups of 50 *B. tabaci* individuals were allowed to feed on plants showing symptoms for one of the following periods: 2, 6, 18, 24, 48, and 54 h. Afterwards, the groups were transferred to healthy plants and allowed to feed for 72 h. Initial results showed that two hours of feeding on plants showing symptoms were sufficient for the individuals of *B. tabaci* to acquire the pathogen and to transmit it to healthy plants in 50% of cases. Twenty-four hour feeding times gave slightly less than 100% transmission.

Electron microscopy of differentially-centrifuged concentrates of sap extracted from plants that showed symptoms revealed rod-shaped and flexuous virus particles around 790 nm long that were not present in sap from healthy control plants.

The symptoms of this disease and the morphology of the causal virus appear very similar to those attributed to YSD (7) and LIYV (3) and transmitted

by *Bemisia tabaci*, and to CuYV (10), to MYV (8), to BPYV (2), and to another yellowing disease of melon (6), transmitted by *Trialeurodes vaporariorum*, although in our case, the virus particles are appreciably shorter.

To seed sources of resistance, 45 accessions of *C. melo* and related species from widely separated areas in Spain and from other parts of the world were evaluated under natural infection conditions. The transmission tests showed that the yellowing was transmitted by *Bemisia tabaci*, but not by *Trialeurodes vaporariorum*. The *C. dipsaceus, C. metuliferus*, and *C. longipes* species and six accessions of *C. melo* showed no symptoms.

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Resistance of *Cucumis melo* var. *agrestis* against Melon-Yellowing Disease

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Several accessions of *Cucumis melo* L. with different degrees of resistance to melon yellowing disease whose causal agent is a closterovirus transmitted by *Trialeurodes vaporariorum* Westwood (1) have been selected (4). One wild accession, *Cucumis melo* var. *agrestis* belongs to the same species as the cultivar and so can be exploited without difficulty in a breeding program designed to find varieties of melon with tolerance or resistance to yellowing disease.

Nevertheless, this accession has shown variable responses to the melon yellowing disease regarding the results when controlled or natural conditions of infection are used. So, under natural conditions, around the 16% of the plants became non-infected and the rest showed mild symptoms which appeared later than on susceptible genotypes (2,3). This behavior suggested that this genotype should have some kind of resistance against muskmelon yellowing disease. When controlled inoculations are carried out, the response of this genotype is quite irregular because in some experiments all the plants showed mild symptoms while in others some plants showed no symptoms (2). Probably, factors depending on the virus and/or its vector are involved.

Because of that, the behavior against the melon yellowing virus of *Cucumis melo* var. *agrestis* was compared under controlled-infection conditions with other accessions known to be either resistant: *C. dipsaceus* and *C. metuliferus* of susceptible: *C. melo* var. 'Piel de Sapo', cv. 'Bola de Oro', and PI-505601 (2,4). Each plant at two-leaf stage was inoculated by 40 whiteflies inside a clip-on cage attached to the first true leaf (5). Table 1 shows that, although *C. melo* var. *agrestis* developed the characteristic symptoms of the disease, these appeared ten days later than in the susceptible accessions.

C. melo var. *agrestis* showed itself susceptible to the virus responsible for yellowing disease. The fact that the symptoms emerged later could be due to the existence of a certain degree of resistance to the spread or multiplication of the virus particles, or to both these factors.

The fact that no infection occurred under natural conditions when the virus vector was not forced to feed on a particular genotype could suggest certain degree of resistance to the vector to be present in *C. melo* var. *agrestis*.

Both, tolerance to the virus causing yellowing disease and possible resistance to its vector *T. vaporariorum* found in this genotype could be useful in melon breeding.

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Table 1. Incidence of melon yellowing disease.

Genotypes	Days after inoculations			
	20	25	30	35
C. melo cv. 'Piel de Sapo'	8/10^z	10/10	10/10	10/10
C. melo cv. 'Bola de Oro'	4/10	10/10	10/10	10/10
C. melo PI-505601	2/10	9/10	9/10	9/10
C. melo var. *agrestis*	0/10	0/10	3/10	9/10
C. dipsaceus	0/10	0/10	0/10	0/10
C. metuliferus	0/10	0/10	0/10	0/10

a/b^z a: plants with symptoms; b: plants inoculated.

Field Resistance to Melon Dieback in *Cucumis melo* L.

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Melon dieback continues being a serious vine decline disease in melons grown in Spain. The latest reports of CGC (1,2,3) comment on the nature of this disease. In previous experiments, accessions Pat 81 (1) and Acc 6 (3) were those which showed the greatest resistance.

These accessions were evaluated again in field conditions during the spring and summer of 1993. The trials were carried out in two locations (Puzol and Torrepacheco) both on the Spanish Mediterranean coast. The plot in Puzol was the same as that used in the 1992 experiment. Accession VC-120, which is very susceptible to melon dieback, was used as a control. In Puzol, the number of replicates per accession was three and the number of plants per replicate was ten. About 500 plants of 'Rochet' type melon were also cultivated together with accession Pat 81, VC-120 and Acc 6.

The incidence of disease was nil in Torrepacheco. Symptoms of melon dieback were not even observed on the controls. This location is part of the "Campo de Cartagena" region, where the melon dieback attack was in fact very weak during 1993. Nevertheless, the disease was very severe in Puzol, where all controls and 'Rochet' plants died between 18th and 28th July. Some plants of Acc 6 also died during this time. No plants of Pat 81 were affected by the disease (Table 1).

The incidence of melon dieback on Acc 6 was superior to that expected although it did show a certain level of resistance. However, the behaviour of Pat 81 clearly differed in relation to the controls. Pat 81 is of assiatic origin. It has been crossed with Spanish cultivars which are susceptible to melon dieback and diverse segregant generations have thus been obtained. These materials could be useful in the genetic analysis of resistance to melon dieback and for the initiation of a breeding programme for resistance to melon dieback.

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Table 1. Melon dieback incidence in Puzol.

Accession	Incidence*	Replicates				
		R-1	R-2	R-3	R-4	R-5
Acc 6	z	10	10	10	10	10
Acc 6	y	3	2	3	1	1
Pat 81	z	6	10	10	10	10
Pat 81	y	0	0	0	0	0
VC-120	z	10	10	10	10	10
VC-120	y	10	10	10	10	10

* z = tested plants; y = affected plants.

Preharvest Foliar Calcium Treatments for Reduction of Postharvest *Myrothecium* Fruit Rot of Muskmelon

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Muskmelons (*Cucumis melo* L.) are perishable horticultural commodity with variable storage lifes depending on the botanical variety. For example, honeydew and casaba melons (*C. melo* var. *inodorus*) tend to be less perishable than netted melons (*C. melo* var. *recticulatus*) (8). Muskmelon decline in storage is usually manifested by flesh softening and breakdown which often lead to postharvest decay (6). Many postharvest fruit rots arise as quiescent or latent infection in the field or as a result of injuries during harvesting and handling operations which subsequently lead to infection from fungal spores. *Myrothecium roridum* Tode ex. Fries is one of the major preharvest and postharvest pathogens of muskmelon (1). *Myrothecium* fruit rot of muskmelon can occur in the field, in transit to the market, during marketing or in consumer refrigerators (3).

Many studies have shown that increasing calcium content of fruits may extend storage life by delaying fruit ripening and senescence (2), and by maintaining firmness (4). The slower decline in flesh firmness of ripening fruit have been attributed to ability of calcium ions (Ca^{++}) to combine with pectin to form calcium pectate in cell walls (10). Calcium enhances host resistance to fungal infection by strengthening or stabilizing the cell walls, thus preventing cell wall breakdown by pathogens (9). This study was initiated to determine the effect of preharvest foliar application of calcium on susceptibility of muskmelon to *Myrothecium* storage rot.

Two botanical varieties of muskmelon were used. One botanical variety is a honeydew melon 'Limelight' (Burpee Seed Co.), and the other is a netted melon 'Tam Uvalde' (Willhite Seed Co.). Both melon varieties were grown under identical conditions in Kingsville, Texas. At about 28 days postanthesis, fruits were sprayed over a six week period (i.e., at 2-week intervals) with calcium, Nutrical®, a complex of calcium (8%) and an organic chelating agent - trihydroxyglutarate (CSI Chemical Corp., Boudurant, IA), at the rates of 0, 0.5, 1.0 and 1.5 l ha^{-1} using a high-pressure hand gun sprayer. Developing fruits were sprayed until run-off. Treatments were assigned to 2-row plots in a completely randomized design and replicated 3 times. The honeydew melons were harvested at 84 days postanthesis and the netted melons harvested when abscission layer was evident. Five fruits were randomly selected from each of the treatments, weighed and assessed for soluble solids and flesh firmness. Flesh calcium content of fruits from each treatment was also determined by atomic absorption spectrophotometry.

Fruits were inoculated with 25 l spore suspensions of *M. roridum* (ATCC# 52485) at 4 sites around the fruit equatorial region using a modified multiple puncture inoculation technique as described by Reed and Stevenson (7). Inoculated fruits were covered with a large perforated polyethylene sheet to prevent dehydration and incubated under ambient storage conditions (25 2°C and 85% RH). Fruit decay volume was measured 14 days after inoculation by measuring surface area of decay and multiplying by the depth of decay. There were 5 fruits per calcium treatment for each melon in the botanical types. Inoculated fruits without Nutrical® application served as controls. All experiments were repeated at least twice and data were analyzed by analysis of variance procedures.

Results of fruit weight, firmness, soluble solids, flesh calcium content, and *Myrothecium*

Table 1.   Effect of foliar calcium treatments on fruit weight, firmness, soluble solids and flesh calcium content and susceptibility to *Myrothecium* fruit rot of two botanical varieties of muskmelon (honeydew melon, 'Limelight' and netted melon, 'Tam Uvalde').

Treatment (Nutrical®)	Weight (kg)	Firmness (kg)	Solids (%)	Ca^{++} content ($\mu g\ g^{-1}\ fw$)	Decay vol.[z] (cm^3)
Honeydew Melon 'Limelight'					
Control	1.53 a[y]	12.6 a	9.8 a	1.19 a	6.4 a
0.5 1 ha^{-1}	1.67 a	13.1 b	12.6 b	2.60 b	5.7 b
1.01 1 ha^{-1}	1.64 a	18.2 c	12.8 b	3.89 c	3.2 c
1.51 1 ha^{-1}	1.69 a	19.6 d	12.6 b	4.28 d	0.9 d
Netted Melon 'Tam Uvalde'					
Control	0.83 a	14.3 a	12.0 a	0.76 a	5.6 a
0.5 1 ha^{-1}	0.87 a	14.7 a	10.2 b	0.94 b	5.2 a
1.01 1 ha^{-1}	0.87 a	15.6 b	9.8 b	1.21 c	5.0 a
1.51 1 ha^{-1}	0.86 a	15.6 b	9.6 b	1.43 d	4.7 a

[z]Decay volume was determined by measuring surface area of decay and then multiplying by the depth of decay.

[y]Mean separation in the columns by Duncan's multiple range test (P = 0.05).

decay of inoculated fruits are shown in Table 1. Low calcium treatments resulted in lower flesh firmness and calcium content in both botanical varieties of muskmelon tested. By increasing flesh calcium concentration, decay severity by *M. roridum* was significantly reduced only in the honeydew melons, while the netted melons showed no difference. Calcium treatments did not affect the fruit weight of both melon types. While honeydew melons treated with calcium had higher soluble solids than untreated melons, netted melons treated with calcium had lower soluble solids when compared to the untreated controls. The differences in flesh calcium content of honeydew melons and netted melons in this study are consistent with previous findings of Ng and Carr (5).

The results of the present study indicate that foliar treatments of honeydew melons with Nutri-cal® or any other absorbable calcium salts may have the potential to reduce storage losses due to postharvest pathogens and may provide high quality melon for the consumer.

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Response Reaction of Melon Genotypes Heterozygous for the Prv[1] Gene to Mechanical Inoculation of PRSV-W

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In a previous study on the inheritance of Papaya Ring Spot Virus-Watermelon (PRSV-W) on melon (1) we have observed the appearing of necrotic local lesion on cotyledons of mechanically inoculated plants, which were heterozygous for the Prv[1] gene, that eventually became systemic, killing the entire plant. It was of interest to know whether this kind of response reaction to PRSV-W would be present in F_1 hybrids of melon heterozygous for the Prv[1] gene, with different genetic backgrounds.

Materials and Methods. The pedigree and the PRSV-W status of the inbreed lines used in the present study to produce the F_1 hybrids are presented in Table 1. Inoculum was prepared by grinding leaves of *C. pepo* cv. Caserta, showing strong symptoms of the virose, in 0.02 M phosphate buffer (pH 7.0) containing 0.01 M sodium sulfite (1:10 - w/v) and rubbing extracts on fully expanded cotyledons of 4- to 6-day old plants, previously dusted with 600-mesh Carborundum.

Results and Discussion. The response reaction of the melon genotypes to the mechanical inoculation of PRSV-W are presented in Table 2. Regardless of the genetic background, necrotic local lesion on the cotyledons and systemic necrotic response reaction were observed for all F_1 hybrids, except for the AF605LxAF647L F_1 hybrid, which is homozygous for the Prv[1] gene. On this F_1 hybrid and on the resistant inbreed lines the necrotic local lesions observed on the cotyledons were usually smaller in number and in size when compared to those observed on the heterozygous Prv[1] F_1 hybrids. These results suggest that it may not be commercially feasible to use the Prv[1] gene in the heterozygous condition for resistance to PRSV-W.

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Table 1.Pedigree and PRSV-W status of the melon inbreed lines used to produce the F1 hybrids to evaluate the response reaction to mechanical inoculation of PRSV-W.

Inbreed Line	Melon type	Origin	PRSV-W Status[1]
AF605L S9	Casaba	Brazil	R
AF647L S7	Casaba	Brazil	R
AF111L S3	Santa Claus	France	S
AF137L S3	Cantaloupe	USA	S
AF581L S4	Cantaloupe	Brazil	S
AF584L S5	Casaba	USA	S
AF652L S6	Casaba	USA	S
AF659L S6	Casaba	Israel	S

[1]R = resistant; S = susceptible.

Table 2. Response reaction of melon genotypes to mechanical inoculation of PRSV-W.

Genotype	18 D.A.I.[1]				26 D.A.I.				TOTAL
	NC[2]	NC+SN[3]	H[4]	M[5]	NC	NC+SN	H	M	
AF605L	1	0	11	0	1	0	11	0	12
AF647L	2	0	12	0	2	0	12	0	14
AF605LxAF647L	2	0	10	0	2	0	10	0	12
AF647LxAF111L	0	14	1	0	0	14	1	0	15
AF605LxAF137L	1	4	9	0	0	5	9	0	14
AF647LxAF137L	2	10	3	0	3	11	1	0	15
AF605LxAF426L	0	10	2	0	0	10	2	0	12
AF647LxAF426L	5	5	2	0	4	7	1	0	12
AF647LxAF581L	3	5	6	0	3	6	5	0	15
AF605LxAF584L	5	4	1	0	3	6	1	0	10
AF647LxAF584L	6	3	3	0	3	8	1	0	12
AF605LxAF652L	0	4	4	0	1	4	3	0	8
AF647LxAF652L	1	7	4	0	1	7	4	0	12
AF605LxAF659L	2	9	0	0	1	10	0	0	11
AF647LxAF659L	3	7	1	0	1	10	0	0	11
AF426L	0	0	0	12	0	0	0	12	12

[1]D.A.I. = days after inoculation.
[2]NC = necrotic local lesions on the cotyledons.
[3]SN = systemic necrotic lesions on the plants.
[4]H = healthy plants.
[5]M = systemic mosaic

Screening of Melons for Sweetpotato Whitefly Resistance: 1993

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Sweetpotato whitefly, *Bemisia tabaci* Genn., type B virtually destroyed the Fall 1991 melon crop in the lower desert valleys of Arizona and California (7). It does not appear to be an important vector of lettuce infectious yellows virus (LIYV) (3). This whitefly type was recently re-named the silverleaf whitefly (8,9,10), but not without controversy (1,2,11). This whitefly has recently been found in the San Joaquin Valley of California (4).

In 1991, 17 of 150 PIs from India appeared to have some level of resistance to *B. tabaci*, type B (5). In 1992, these 17 PIs were re-tested for *B. tabaci*, type B resistance along with 108 previously untested PIs from India plus 27 standard varieties, breeding lines, and F_1, F_2 and backcross families from crosses of susceptible parents with lines identified as potentially resistant to LIYV or *B. tabaci*, type B (6). None of the entries was superior for whitefly resistance.

In 1993, 276 melons from Afghanistan and Pakistan were evaluated for whitefly resistance in a naturally infested field test. Also included in the 1993 test were three cultivars (PMR 45, Top Mark, GF Honeydew), breeding line WMR 29, Snakemelon from the Middle East, and progenies 28479 an F_1 from the cross Top Mark FR x Snakemelon, 28478 a backcross from the series Top Mark FR (Snakemelon [Freeman Cucumber x Snakemelon]), and 28481 and 28482 which are backcrosses from the series PMR Honeydew (Snakemelon [Freeman Cucumber x Snakemelon]). Plots were planted on 80 inch centers and consisted of five two-plant hills spaced 30 inches apart. There were two replications. The test was evaluated on a plot basis 4 weeks (September 22) and 8 weeks (October 20) post-planting for number of live plants, plant size, plant condition,

yellowing, leaf necrosis (burn) and flowering. Plots were not treated with any pesticides or herbicides.

Of the 276 PIs planted, four failed to germinate in either one replication (three entries) or both replications (one entry). Four weeks post-planting, 63 entries were dead in one replication and an additional 17 PIs were dead in both replications. Eight weeks post-planting, all but 15 entries were dead. Of those 15, only one had live plants in both replications. This is in sharp contrast with the 1991 test when most of the entries were alive and 1992 when approximately 70% of the entries were alive after 8 weeks. Severity of the test is also indicated by differences in mean ratings of plant condition 4 weeks post-planting for Top Mark in 1992 and 1993, 7.0 and 2.0, respectively.

Table 1 summarizes the mean ratings of plant condition 4 weeks post-planting. At that time, nine entries showed promise as sources of whitefly resistance; they are the entries with means ranging 3.75 to 5.50. By 8 weeks post-planting, only one (28481) of those nine entries had any (one) live plants. Entry 28481 is a backcross progeny with the following pedigree: PMR HD (Snakemelon [Freeman Cucumber x Snakemelon]). Snakemelon appeared be a potential source of *B. tabaci*, type B resistance in 1991, but in this test it had a mean rating of 2.0 at 4 weeks and was dead at 8 weeks. Of the remaining eight best entries 4 weeks post-planting, PI 125918, PI 125890, PI 116915, and PI 125951 are of most interest for further study for the following reason. Each of these four entries had plant condition ratings of 2 or 3 in one replication, but was rated 6 or better in the other replication. Especially interesting are PI 125918, PI 125890 which were rated 9 in one

Table 1.   Mean plant condition on 22 September 1993 (four weeks post-planting) in response to whitefly feeding.

Mean^z	Entries									
5.5	125918	125890								
4.5	28481	116915								
4.0	125951	125966	125997	126165						
3.8	125861									
3.0 - 3.5	WMR 29	116917	125928	126017	126091	126154	127565	220786		
	28478	125860	125964	126050	126131	126174	211588	223768		
	28479	125879	125989	126057	126146	126199	220170	324525		
	28482	125886	126000	126076	126151	127564	220655			
2.0 - 2.5	GF Honeydew	125901	125952	125996	126056	126097	126164	127546	212291	300956
	PMR 45	125903	125953	126004	126059	126098	126166	127547	217525	323937
	Snakemelon	125905	125955	126006	126060	126101	126167	127548	217526	355051
	Top Mark	125906	125956	126008	126062	126105	126168	127550	217945	418766
	116916	125907	125957	126012	126064	126113	126170	127560	218070	420152
	125862	125910	125958	126013	126068	126116	126172	127567	218071	426628
	125865	125914	125961	126016	126074	126117	126178	127570	220515	532929
	125868	125919	125969	126018	126075	126126	126185	127572	220651	
	125870	125921	125970	126024	126078	126133	126195	127575	220654	
	125872	125922	125972	126027	126080	126140	126197	127576	220787	
	125876	125923	125974	126030	126083	126141	126198	127578	221439	
	125880	125925	125979	126032	126084	126142	127524	135315	222097	
	125885	125931	125981	126036	126086	126143	127528	204304	222098	
	125891	125932	125984	126040	126088	126145	127531	207478	242907	
	125892	125935	125986	126042	126089	126147	127534	211016	260651	
	125893	125943	125991	126044	126090	126150	127536	211726	261750	
	125895	125944	125992	126047	126093	126152	127538	212087	269367	
	125896	125946	125993	126052	126095	126160	127539	212089	269368	
	125897	125947	125994	126054	126096	126162	127545	212090	300955	
1.0 - 1.5	125863	125913	125938	125973	126037	126077	126110	126156	207477	
	125866	125920	125942	125976	126051	126079	126111	126159	223770	
	125881	125924	125948	125987	126053	126081	126112	126171	269474	
	125883	125927	125962	125990	126069	126082	126114	126180	269478	
	125900	125930	125963	126005	126070	126099	126125	127535	401646	
	125904	125933	125967	126020	126072	126106	126127	127551	418768	
	125911	125937	125971	126033	126073	126108	126134	135319	426627	

^{z}Condition was rated on a 1 (dead) to 9 (vigorous, flowers) scale.

replication. They appeared to have as many adults and immatures as other entries in the test, but the plants were normal in appearance, were flowering, and were the largest in the field. A greenhouse test of these nine entries is in progress.

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Resistance of *Cucumis melo* var. *agrestis* to *Trialeurodes vaporariorum*

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Cucumis melo var. *agrestis* Naud. appears to have some type of inherent resistance to greenhouse whitefly *Trialeurodes vaporariorum* Westwood. This work aimed to determine the possible existence of antixenosis or antibiosis by comparing the resistance of this accession to whitefly with *C. melo* and related species.

Two experiments have been carried out. Experiment 1 was designed to determine the reproductive capacities of *Trialeurodes vaporariorum* feeding on *C. melo* var. *agrestis* and each of two susceptible accessions of *C. melo* cv. 'Bola de Oro' and PI-505601, and in *C. metuliferus* that some authors describe as resistant (2). Five plants of each line were arranged under a fly-proof mesh box into which 800 whiteflies were introduced for 72 h. The files had a free choice of plants to feed on and reproduce themselves during this time. At 32 days, the number of *T. vaporariorum* empty pupal cases on each plant and each line were counted. Three replicates were made of this experiment. Variance analysis revealed significant differences between the lines, notably *C. metuliferus* on which whitefly reproduction was very high (Table 1). Possibly, this high value makes *C. melo* var. *agrestis* appear no different from the other lines, but the results show clearly that whitefly reproduction on it was quite low and this suggests that *T. vaporariorum* has a scale of preference and prefers other genotypes to reproduce on before *C. melo* var. *agrestis* when the others are present at the same time.

The second experiment determined the rate of reproduction of individuals of *Trialeurodes vaporariorum* and their development times (eggs-adult). This experiment also included *C. dipsaceus* as an accession known to be resistant to whitefly (1). Twenty plants of each accession at the five-leaves-completely-developed stage were randomly selected and, by the use of clip-on cages, a recently emerged female of *T. vaporariorum* was placed on each low leaf of each plant. Every 48 h, each of these individual whiteflies was transferred in its cage to a younger fully expanded leaf of the same plant. Twenty-six days later, the numbers of empty pupal cases on each leaf of each plant were counted daily (total reproduction of each individual). We also counted the number of days needed to complete the development from egg deposition to emergence of the adult. Analyses of variance detected significant effect of genotypes on both reproduction and development times.

The results show that *T. vaporariorum* reproduced well on *C. melo* cv. Bola de Oro, *C. metuliferus* and *C. dipsaceus* (Table 2), however, reproduction on *C. melo* var. *agrestis* and PI-505601 appeared difficult, although PI-505601 does not differ from the others. The accessions on which the whitefly development time decreased significantly were *C. metuliferus* and *C. melo* var. *agrestis* (Table 2).

The *C. metuliferus* accession used in these experiments was clearly susceptible to greenhouse whitefly. This is the same accession that was tested against the melon yellowing virus transmitted by this insect (3) and this suggests that *C. metuliferus* has resistance either to that virus or to its transmission.

C. dipsaceus did not really appear to be resistant to greenhouse whitefly even though its whitefly development time was longer (which apparently suggests the presence of antibiosis) because the net-rate of *T. vaporariorum* reproduction was the highest of all the genotypes tested and did not differ

from any of them, except that of *C. melo* var. *agrestis* (Table 2).

T. vaporariorum appeared to prefer to feed and reproduce on other genotypes before *C. melo* var. *agrestis*. The results suggest that in this accession there is an effective antibiosis mechanism against the greenhouse whitefly because its rate of reproduction on this accession is very low, however, on the other hand, the short whitefly development time contradicts this supposition; it appears that the whitefly shortens its development time to live on *Cucumis melo* var. *agrestis*, and perhaps, this could be an adaptive strategy of the insect to ensure future generations. The percentage of eggs that eventually resulted in adults with complete life cycles is not known and is the subject of on-going experiments. Perhaps, these will also determine if antibiosis is really the resistance mechanism in this accession

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Table 1.Rate of *T. vaporariorum* reproduction of four accessions of *C. melo* under free-choice conditions.

Genotypes	No. of Empty Pupal Cases
C. melo var. *agrestis*	55.9 a^z
C. melo PI-505601	101.3 a
C. melo cv. Bola de Oro	355.0 ab
C. metuliferus	514.2 b

a^{z}Means with the same letter are not significantly different, SNK test, p>0.95.

Table 2.Rates of reproduction and development times of *T. vaporariorum* in days.

Genotypes	No. of Empty Pupal Cases	Development Times
C. melo var. *agrestis*	15.73 a^z	30.97 b
C. melo PI-505601	26.00 ab	32.18 c
C. melo cv. Bola de Oro	69.80 b	33.26 cd
C. metuliferus	69.85 b	30.17 a
C. *dipsaceus*	72.67 b	33.60 d

a^{z}Means with the same letter are not significantly different, SNK test, p>0.95.

Callus Formation from Cotyledon and Hypocotyl of *Cucumis melo* L. and *Cucumis metuliferus*

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For many years, viral disease in muskmelon (*Cucumis melo*) (2n = 24) has been of great concern to seedsmen and growers (5). The development of resistant cultivars can eliminate the disease.

Various accessions of *Cucumis metuliferus* (African horned cucumber) (2n = 24) contain genes for some desirable characters that should be valuable additions to the gene pool of the muskmelon if they could be transferred (2,6 and literature cited therein).

Our efforts to achieve the interspecific hybrid between *C. melo* and *C. metuliferus* through conventional breeding procedure between a few reciprocal combinations (in summer 1992) were unsuccessful because of the existence of a prefertilization barrier (1).

Tissue culture is a promising tool for the recovery of important traits such as disease resistance. Selection *in vitro* would allow for rapid screening of large populations of cells if suitable selection agents are available and if cell culture techniques exist for the regeneration of plants from single cells or clumps of cells. We report here on the callus formation from explant of *C. melo* and *C. metuliferus* on various nutrient media.

Plant material consisted of cotyledon and hypocotyl of axenic cultures of *Cucumis melo* cultivar 'Charentais' and *Cucumis metuliferus* line 'Italia'. After seed coat removal they were sterilized in 0.75% solution of sodium hypochlorite for 30 and 20 min for *C. melo* and *C. metuliferus* seeds, respectively, followed by 3 rinses in sterile bidistilled water. This procedure was found to be suitable in preliminary study. The seeds were transferred to medium consisting of 1.6 g/liter Murashige and Skoog's (MS) (4) inorganic salts and vitamins, 10 g/liter sucrose and 1% agar

(Sigma). Cotyledons and hypocotyls from 8-day-old plants were cut to 3 mm wide strips and 8 mm length strips, respectively, and placed on the culture media.

The basal nutrient medium consisted of MS, 0.1 g/liter m-inositol, 1 mg/liter thiamine HCl, 40 g/liter sucrose, and various concentrations of BAP and NAA. The pH was adjusted to 5.75 prior to addition of 0.8% purified agar (Sigma). The media were autoclaved at 121°C for 20 min and about 20 ml were dispensed into petri dishes. The cultures were kept under 16 h of low light (1000 lux) at 25 ± 1°C.

Table 1 gives data on callus development after 25 days of culture. The best developments were observed on medium consisting of 1 mg/liter BAP with an addition of 1 or 2 mg/liter NAA for *C. melo* and with or without (only from hypocotyl) 2 mg/liter NAA for *C. metuliferus*. The results demonstrate the possibility of applying them: 1) to develop cell suspension culture from the callus culture and apply the suspension in fusion and transformation experiments; 2) to develop shoot regeneration from the callus culture; 3) the differences found between *C. melo* and *C. metuliferus* to the selection of hybrid cells after fusion.

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Table 1. Callus development of *Cucumis melo* ('Charentais') and *Cucumis metuliferus* ('Italia') after 25 days of culture with different BAP and NAA concentrations.

BAP (mg/liter)	NAA (mg/liter)	Callus Development[z]			
		C. melo		*C. metuliferus*	
		Cotyledon	Hypocotyl	Cotyledon	Hypocotyl
0.5	0[y]	1	2	0	b
1	0[x]	1	0	0	2
	1[x]	3	3	b	w
	2[x]	3	3	2	2

[z]Green callus rated from 0, none; 1, little; 2, moderate; 3, much.
b = brown callus; w = white callus.

[y]according to Jain and More (3).

[x]according to personal knowledge.

Attempts to Overcome Barrier of Interspecific Hybridization between *Cucumis melo* and *C. metuliferus*

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Our efforts to achieve the interspecific hybrid between *Cucumis melo* and *Cucumis metuliferus* through conventional breeding procedure between a few reciprocal combinations (in summer 1992) were unsuccessful because of the existence of a prefertilization barrier (1). The barrier was characterized by arrested pollen tube growth in the stigma or the upper style. In any event, viable embryos can be recovered 15 to 20 days after pollination, but not at fruit maturity, perhaps due to embryo abortion (8). To overcome the barrier, techniques like bud pollination (5), growth regulator application (5) and use of irradiated mentor pollen (5,6) - suggested in other crop plants and in *Cucumis* - combining with embryo culture, were employed to find out their potential to overcome the barrier of hybridization between the two species.

Plant material: The plants were grown in the field under insect-proof net and flowers were hand-pollinated during May-June 1993. We used 10 lines (Monoecious PI 124111F and 'Tam-Uvalde'; Andromonoecious 'Venrantais', 'Charentais-T', 'Mainstream', 'Gulfcost', 'Ananas-Yokneam' (Hazera Seed Co., Haifa, Israel), 'Hemed' (Hazera Seed Co., Haifa, Israel); and Genoecious 'Gylan' E6/10 and 'Gylan' 5/5/5) as a female parent. Six accessions of *C. metuliferus* (PI 292 190 So/1, 2, 3 and 4, 'Italia' and one from Tzofar, Israel) were used as a male parent. Using different cultivars of the same species in interspecific cross results in variable fruit set and embryo development (4,10).

Irradiated mentor pollen: Anthers with pollen were collected in the morning from fresh male *C. melo* flowers and irradiated with 100, 150 and 200 Krad gamma rays. The flowers were kept overnight at 5°C. The next morning, anthers with the mentor pollen (MP) were mixed with fresh anthers of *C. metuliferus* in a volume ratio of about 2:1 (7)

for use in crosses. We applied the MP first and the foreign pollen directly thereafter or in the reverse order. Standard pollination procedure for *Cucumis* were used with special precautions to avoid contamination by pollen from foreign sources.

Benzyladenine: 1% of the cytokinin N^6-benzyladenine (BA) in lanolin was applied to the base of ovaries immediately after pollination. This concentration of BA was suitable in promoting fruit set in hand-pollinated *C. melo* (9).

Bud pollination: Hand-pollination of the flowers was attempted at its stage: 24, 48 or 72 hr prior to anthesis.

Embryo culture: Fruits that developed were checked for ovules with embryos 20-25 days after pollination. After seed coat removal, they were washed during 24 hr and then placed on 5 culture media, with or without previous sterilization, depending on the culture media, of which the composition is given in Table 1. Seeds placed on media containing sucrose were sterilized in 0.75% solution of sodium hypochlorite for 0 (control), 5, 10 or 15 min, followed by 3 rinses in sterile bidistilled water. The pH of the media was adjusted to 5.75 prior to addition of 0.75% purified Bacto agar (Difco). The media were autoclaved at 121°C for 20 min at 15 psi. The cultures were kept under 16 h of low fluorescent light (1000-1500 lux) at 25 ±1°C.

Seeds that started to show signs of contamination were removed from the culture medium, immersed for 10 sec in 70% ethanol followed by 3 rinses in sterile bidistilled water, and then placed back on medium 4 (Table 1). Seedlings with fully expanded cotyledons were transplanted into pots with a sterilized mixture of peat substrate and sand, then irradiated with 1/10 MS as needed. Plantlets

at the initiation of the 2nd leaf developed from those seedlings were transferred into a glasshouse, where temperature ranged from 21°C (night) to 30°C (day). Plants at the stage of 3 leaves were planted out into soil in the field with a thick net.

Using the techniques of BA and bud pollination was ineffective in overcoming the hybridization barrier between the two species. Out of 50 and 45 pollination in various combinations, using the BA and bud pollination, respectively, no fruit set was observed.

Fruit and seed set of 10 lines of *C. melo* after pollination with self-irradiated pollen mixed with fresh pollen of *C. metuliferus* are presented in Table 2. Twenty and 21 normal fruits were developed from about 60 pollinations after irradiating the mentor pollen with 100 and 150 Krad, respectively. Fruit set was intensely reduced by the higher radiation dose (200 Krad). From about 35 pollinated flowers, only 2 normal fruits were developed after irradiation of the mentor pollen with this dose. Those results could easily be explained with our results about the effect of the various doses on pollen viability *in vitro* (2).

Mentor pollen technique effectively induced fruit set on all of the *C. melo* genotypes examined, but the success of obtaining fruits containing embryos was dependent on genotype (Table 2). In those fruits, the number of embyros was generally low.

The embryos which were sterilized and initially placed on media containing sucrose appeared to be highly contaminated, and after their removal to medium 4 (1/3 MS), most of them did not germinate. Some of the embryos which were cultured on media without sucrose were also infected, but most of them germinated even after being removed twice with a short immersion in ethanol. Embryos that were initially placed on medium consisting of 1/3 MS were observed to provide normal development for most of the embryos. On the contrary, embryos initially placed on medium consisting of only H_2O did not grow and, therefore, were transferred to medium 4 after a few days.

Nine embryos developed into plantlets and survived transplanting to soil. All 9 plants that developed from these embryos resembled the *C. melo* maternal parent. Fruits that developed after self-pollination of these plants were also similar to the maternal parent fruits and, therefore, the 9 plants were considered to be noninterspecific hybrid. Fruits of *C. melo* which developed after self-pollination with irradiated pollen with at least 100 Krad gamma-rays never contained mature seeds (2). Since all fruits which contained embryos in this study were obtained on the andromonoecious genotypes and never on the monoecious or genoecious lines (Table 2), we assume that those embryos resulted from viable maternal pollen, although all the anthers were removed from the hermafrodite flowers 24 h before flowering.

We reached the following conclusions:

1. A significant factor in the embryo culture was the age of isolated embryos. The age of 20-25 days, counting from the date of pollination, was suitable to form plants.

2. The seeds isolated at the above-mentioned age appeared to be highly contaminated, yet not sufficiently mature to survive after sterilization with sodium hypoclorite. Therefore, a method of disinfection must be worked out that will provide sterility and surviving embryos. One possibility is using a mixture of nonionic detergents, as described by Rakoczy-Trojanowska and Malepszy (12).

3. Initially cultured embryos at the above-mentioned age on media container 1/3 MS are suitable to allow for normal plantlet development.

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Table 1. General composition of the media used.

Designation	Composition of Medium
1	The content of mineral components and vitamins according to Murashige and Skoog (MS) (11), 1 g/l casein hydrolysate, 0.1 g/l m-inositol, 1 mg/l thiamine-HCl, 1 mg/l pyridoxin HCl, 1 mg/l nicotinic acid, 35 g/l sucrose, 0.02 mg/l IAA and 0.5 mg/l kinetin [according to Beharav and Cohen (3)].
2	Same as 1, with 2 modifications: I. Only 1/3 MS; II. Only 10 g/l sucrose.
3	1/3 MS and 10 g/l sucrose.
4	1/3 MS.
5	Only H_2O.

Table 2.   Effect of *Cucumis melo* genotype treated with various Gamma-irradiation doses of mentor pollen mixed with fresh pollen of *C. metuliferus* on fruit and seed sets.

Genotype	Irradiation dose (Krad) of mentor pollen	No. of fruit set	No. of fruits with embryos	No. of embryos in the fruits containing embryos
		MONOECIOUS		
PI 124111F	150	3	0	
	200	1	0	
'Tam-Uvalde'	150	4	0	
		GENOECIOUS		
'Gylan' E6/10	100	4	0	
	150	1	0	
'Gylan' 5/5/5	100	7	0	
	150	7	0	
		ANDROMONOECIOUS		
'Mainstream'	100	1	0	
'Gulfcost'	100	1	1	1
	150	2	1	8
'Ananas-Yokneam'	100	2	1	1
'Hemed'	100	2	1	2
'Vedrantais'	100	2	2	1,9
	150	2	1	5
	100	1	0	
	150	2	1	2
'Charentais-T'	200	1	*	

* This fruit harvested early due to closing of the field.

Effect of Gamma-Radiation on Vitality and Fertilization Ability of *Cucumis melo* and *C. metuliferus* Pollen

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The mentor pollen technique can be applied to overcome crossing barriers in certain interspecific hybridization, where pollen tube growth is arrested before the ovary (4). Oost and Den Nijs (3) reported on mentor pollen as a tool in interspecific hybridization in *Cucumis*. Our observations (1) show that the nature of cross incompatibility between *C. melo* and *C. metuliferus* is prefertilization barrier. The use of such double pollinations with foreign pollen and irradiated maternal mentor pollen in crosses between the two species is being investigated to introduce valuable resistance genes from *C. metuliferus* into the *C. melo*. Knowledge of the behavior of irradiated pollen may be useful for the application of the mentor pollen technique. The influence of Gamma-radiation on *in vitro* germination, pollen tube growth of *C. melo* and *C. metuliferus* and on *in vivo* fertilization of *C. melo*, were evaluated in the present study.

Determination of pollen tube growth after Gamma-radiation: Freshly harvested male flowers were irradiated with a caesium source. The dose rate was 0.473 Krad per min. The flowers were placed in a closed glass vial within 250 ml erlenmial icetin. Doses of 100, 150 and 200 Krad (100 Krad = 1 KGray) were given. Pollen from irradiated and non-irradiated flowers was germinated *in vitro* on a substrate composed of 20% sucrose, 20 ppm boric acid and 1% agar in the dark at 25 ±1°C, a modification of the procedure which was described by Van Den Boom and Den Nijs (6). Germination percentage and mean pollen tube length from 2 cultivars of *C. melo* ('Vedrantais' and 'Charentais') and 3 accessions of *C. metuliferus* (PI 292 190 So/1, 2 and 5) were determined under a light microscope after incubation for 120 min. A pollen grain was considered to have germinated when its tube length at least equalled the grain diameter. The mean

genotype effect was regarded as 'block' in the analysis of variance for pollen tube length, while the various tubes were regarded as samples.

Determination of *C. melo* pollen vitality *in vivo* after gamma-radiation: Anthers with pollen from the monoecious line PI 124111F were collected in the morning from fresh male flowers and irradiated with 100, 150 and 200 Krad gamma-rays. The flowers were kept overnight at 5°C. Pollination was carried out the next morning. Fruit and seed set of the genoecious line 'Gylan' E6/10 following pollination with non-irradiated or irradiated pollen were determined.

Effect of gamma-radiation on pollen vitality *in vitro*: The effect of different doses of gamma-radiation on *in vitro* germination and tube growth of pollen of *C. melo* and *C. metuliferus* is presented in Table 1. As the genotypes of each of the two species gave similar results, the data were combined for each species.

Radiation with 100 Krad did not have an effect whereas a dose of 150 Krad had only a limited, though significant, effect on the germination of the *C. melo* pollen. A dose of 200 Krad decreased the germination percentage by 35% (compared with the control). In contrast, the mean pollen tube length was already reduced in accordance with the dose radiation, by a radiation dose of 100 Krad. The germination of *C. metuliferus* appeared to be much less sensitive to gamma-rays. A 100 Krad radiation dose had no effect on the germination percentage, while a dose of 200 Krad decreased the germination percentage by only 14%. The mean tube length was not influenced by the radiation, even by a dose of 200 Krad. This is in agreement with Van Den Boom and Den Nijs (6).

Table 1. Effect of gamma-radiation on the *in vitro* germination and pollen tube length of *Cucumis melo* and *C. metuliferus*.

	C. melo		C. metuliferus	
Dose in Krad	Germination percentage[z]	Pollen tube length (μm)[y]	Germination percentage[z]	Pollen tube length (μm)[y]
0	96 a[x]	293 a[w]	84 a	403 a
100	95 a	259 ab	80 ab	416 a
150	89 b	227 bc		
200	62 c	192 c	72 b	413 a

[z]Percentage from 345 and 178 pollen grains of 2 cultivars of *C. melo* and 3 lines of *C. metuliferus*, respectively.

[y]Mean from 89 and 69 pollen tubes of 2 cultivars of *C. melo* and 3 lines of *C. metuliferus*, respectively.

[x]Percentage separation in columns by Chi square test, 5% level.

[w]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 2. Fruit and seed set following pollination of *Cucumis melo* line 'Gylan' E6/10 with non-irradiated or irradiated pollen of line PI 124111F.

Dose in Krad	Germination pollinations	Number of fruits
0	11	10
100	3	3[z]
150	3	2[z]
200	3	1[y]

[z]All seeds were flat and empty (samples of 50 seeds per fruit).

[y]Stopped developing after a few days.

Effect of gamma-radiation on the behavior of *C. melo* pollen *in vivo*: Fruit set of the genoecious line 'Gylan' E6/10 was reduced by increased radiation dose of pollen from the monoecious line PI 124111F (Table 2). After pollination with 200 Krad-irradiated pollen, only 1 out of 3 pollinated flowers set fruit, which stopped developing after a few days. This is in accordance with the high decrease of pollen vitality *in vitro* of this dose (Table 1). Fruit weight was also decreased in accordance with the radiation dose (unpublished data).

All seeds obtained after pollination with irradiated pollen were flat and devoid of embryo ['pseudo-fertilization' (2)].

The competition for ovules between irradiated and non-irradiated pollen is a major problem in the mentor pollen technique (5). When simultaneously applied, the irradiated maternal pollen probably grows faster down the style than the pollen of the foreign species, and thus has the opportunity to occupy many ovules. Therefore, it is necessary to irradiate the mentor pollen at a dose that is high enough to eliminate this competition but low enough to stimulate fruit set (6). Our results about *in vitro* germination and pollen tube growth of *C. melo* and *C. metuliferus* and *in vivo* fertilization of *C. melo* indicate that an irradiation dose of 100-150 Krad appeared to be suitable for the *C. melo* pollen when used as the maternal mentor pollen, while a dose of 200 Krad could be suitable for the *C. metuliferus* pollen when this species is used as the maternal mentor pollen.

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The Crossability of *Cucumis melo* and *C. metuliferus*, an Investigation of *In Vivo* Pollen Tube Growth

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For many years viral disease in muskmelon (*Cucumis melo*) (2n = 24) has been of great concern to seedmen and growers (8). The development of resistant cultivars can eliminate the disease. Various accessions of *Cucumis metuliferus* (African horned cucumber) (2n = 24) contain genes for some desirable characters that should be valuable additions to the gene pool of the muskmelon if they could be transferred (4,9 and literature cited therein).

Numerous attempts to cross *C. metuliferus* with *C. melo* have failed. Occasional fruit set and ovary enlargement occurred when *C. metuliferus* was used as the female parent, but the fruits were small, misshapen, and lacked viable seeds (2,4). Norton and Granberry (7) reported a successful cross with *C. melo* as the female parent, which gave viable F_1 and F_2 seeds, but attempts to duplicate the original cross were unsuccessful due to embryo abortion.

The nature of cross incompatibility between *C. metuliferus* and *C. melo* is controversial. Kho *et al.* (6) concluded from studies on *in vivo* pollen tube growth that it is inhibited in the stigma or style, while Fassuliotis (4) found a partial compatibility between the species that was indicated by a very small percentage of *C. metuliferus* ovules being fertilized by *C. melo*.

This paper presents observations on pollen tube growth in *C. melo* and *C. metuliferus* flowers after pollination within each of the species, and after cross-pollinations of the reciprocal crosses of the two species. The identification of the barriers to hybridization of those species led to attempts to develop procedures that would overcome the incompatibility.

C. melo ('Gylan' Genoecious E6/10) and *C. metuliferus* ('Italia') were grown in the field and female flowers were hand-pollinated on June 20th with male flowers of *C. melo* line PI 124111F or *C. metuliferus* ('Italia'). Temperatures ranged from $18°CN-39°CD$. Standard pollination procedures for *Cucumis* were used with special precautions to avoid contamination by pollen from foreign sources.

The fluorescence microscopic procedure, as described by Kho and Baer (5) and modified by Tomer and Gottreich (10), was used to observe pollen tube growth.

Flowers were collected 0.5, 5.5, 24 and 48 hr after pollination and immediately fixed in AA (water 4, absolute alcohol 3, acetic acid 1). The flowers were washed in water and sectioned free-hand longitudinally and transversely. Each section was softened in 8 N NaOH for 1 hr, washed with water and stained for 5 min with 0.1% aniline blue w.s. dissolved in $0.1 M K_3PO_4$. The stained tissues were then placed on a slide in glycerine and gently squashed by applying pressure on the cover slip. Observations were made at 390 (excitation) - 420 (emission) nm by use of FT425 and LP450 filters. Photomigraphs were taken on Kodak film.

The stigma of each of the two species was receptive to its own and to the foreign pollen. The pollen of both species germinated within 30 min after application to the stigma (Fig. 1a, b).

Pollen germination after cross-pollination of the reciprocal crosses between species was comparable to that of a self or cross-pollination within species, but the pollen tube grow more slowly. It already showed after 30 min (Fig. 2a, b) and was very recognizable after 5.5 hr (Fig. 2c). Pollen tubes of *C. melo* PI 124111F reached the style of

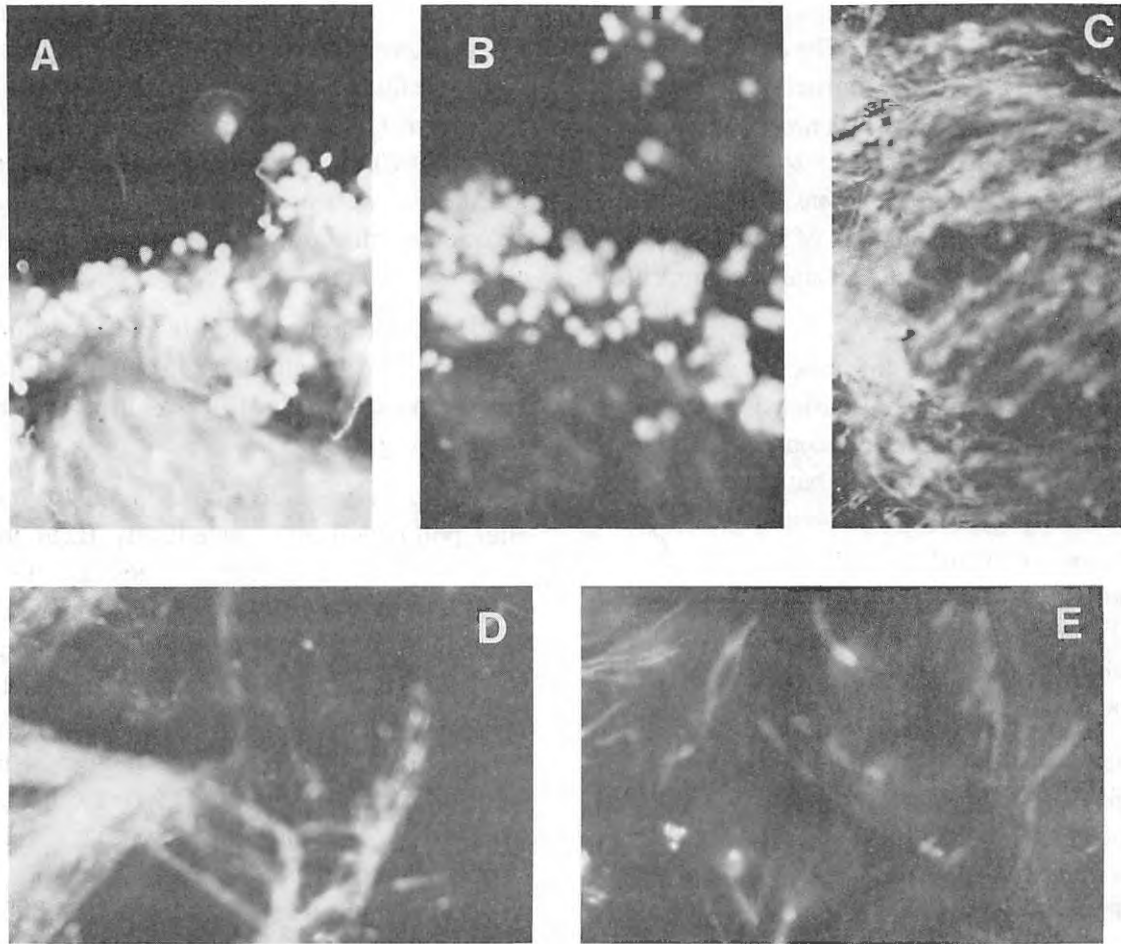Figure 1. Pollen tube growth of selfed *C. metuliferus* 0.5 hr after pollination (a), and of cross-pollination within *C. melo* 0.5 hr (b), 5.5 hr (c), 24 hr (d) and 48 hr (e) after pollination (x 250).
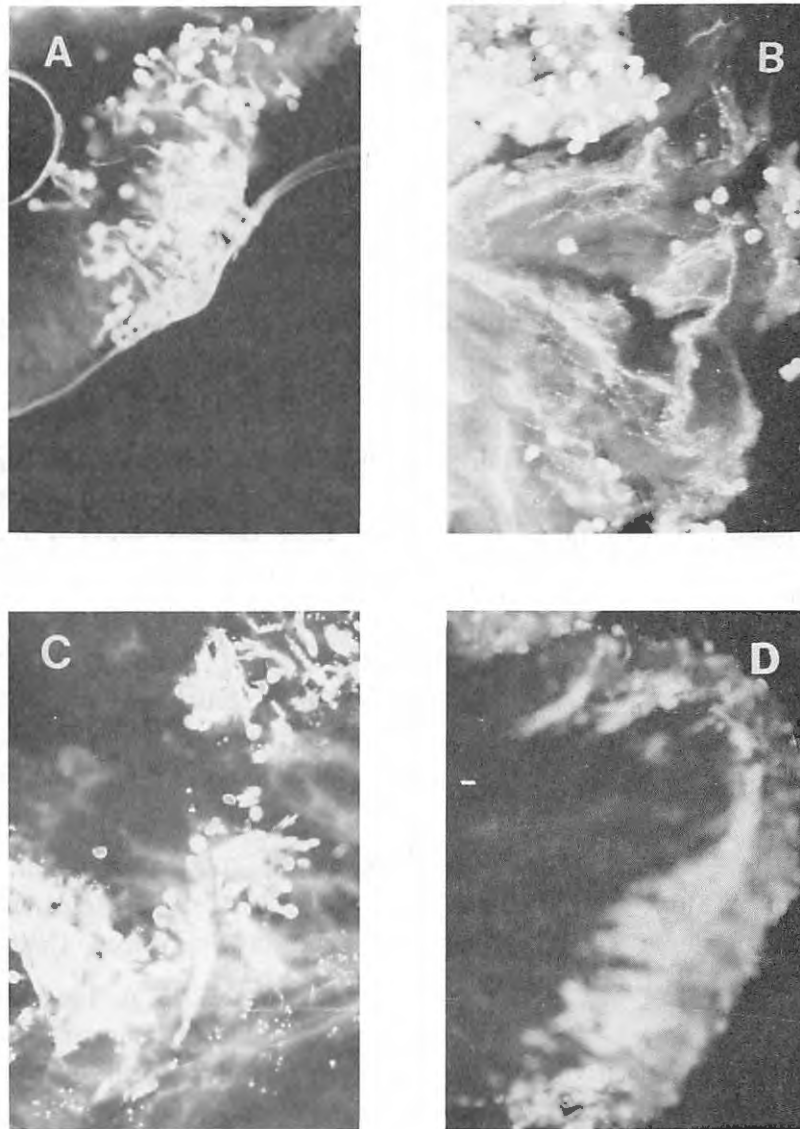
Figure 2. Growth of *C. melo* pollen tubes on *C. metuliferus* flower 0.5 hr after pollination (a), and of *C. metuliferus* pollen tubes on *C. melo* flower 0.5 hr (b), 5.5 hr (c), 24 hr (d) and 48 hr (e) after pollination (x 250).

'Gylan' E6/10 within 5.5 hr (Fig. 1c). By the 24th hr after pollination, they had entered the ovarian cavity (Fig. 1d) and fertilization took place within 48 hr. After 48 hr, the epical end of the tube contained a highly fluorescent elongated callose plug within the ovules (Fig. 1e).

When *C. melo* was treated with *C. metuliferus* pollen, the pollen tubes were mostly arrested in the stigma or in the upper style (Fig. 2d). The pictures of arrested pollen tube growth (Fig. 2c, d) are similar to what is usually found as a result of incompatibility, namely, thick(er) tubes, fully filled with callose (5,6). Thickened ends, bifurcated tubes and other deformations are sometimes found, in no particular combination. In contrast, the normally-growing tubes are slender, with dots of callose spread along the length of tubes (Fig. 1c, d).

We anticipate that using special pollination techniques such as irradiated mentor pollen (1,3), benzyladerine (1) and bud pollination, will be necessary to overcome the prefertilization barrier between the two species.

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A New Morphological Marker in Watermelon, *Juvenile Albino (ja)*

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In 1992, a new morphological variant, which we name "juvenile albino" (*ja*) was observed in two populations - selfed 'Dixielee' and an F_2 population of G17AB (*ms ms*) x 'Dixielee'. To our knowledge, this variant has not been documented previously (1). The cotyledon is yellowish when the seedlings emerge from the soil and gradually becomes greenish-yellow. However, the hypocotyl of the variant remains pure white for a longer time. The shoot, especially the shoot tip, tendrils, male and female flower buds on the main shoot, and new young leaves are all white. Albino leaves gradually become green, and the margin of the leaf remains albino for a much longer time than the interior of the leaf. Surprisingly, the young leaves and flower buds on the secondary branch are not as white as those on the main shoot (Fig. 1). Growth is much slower than normal in the seedling stage, but increases as the plant matures.

Normal pollen and fruit set occur on all branches. The gene affects leaf, tendrils, shoot and flower, as well as rind color. Some portions of the fruit rind are unevenly changed to yellow (Fig. 2). Flesh color is normal. Soluble solids content varied in a few fruit from 9.2-12.8%.

The segregation of normal: juvenile albino in the two populations, selfed 'Dixielee' and F_2 (GI7AB *ms ms* x 'Dixielee') was 23:8 and 34:11, respectively. Some of the *ja* variants in the F_2 population were selfed. The F_1 population from the cross between the breeding line JX-91 and juvenile albino were all normal. Preliminary results suggest that the juvenile albino may be controlled by a single recessive gene. Families are being developed for analysis of larger populations. Improvement of existing lines and transfer of the gene to other lines is underway. The value of the gene in hybrid seed production will be assessed.

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Fig. 1. Vine characters of mutant *ja*. Note main (m) and secondary (s) shoot.

Fig. 2. Mutant *ja* and normal fruit.

Determination of Watermelon Ploidy Level using Flow Cytometry

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Several approaches have been used to identify ploidy level in watermelon, *Citrullus lanatus*. Generally, ploidy levels are suggested by plant morphology, leaf shape/size, pollen size, guard cell size, chloroplast number in guard cells, fruit shape/size and the size of blossom end scar. The ploidy level is determined directly by chromosome number (Angel, 1969; Kihara, 1951; Lower et al., 1969). Chromosome numbers are considered proof of ploidy level. However, because of the difficulties associated with chromosome staining and metaphase arrest, chromosome counts are time consuming and experience dependent. Tetraploid breeding for triploid parents is one of the major breeding objectives in watermelon, and therefore, ploidy level determinations are frequently made. Unfortunately, watermelon chromosome observations are difficult because of the small size and special difficulties associated with chromosome staining and metaphase arrest. An efficient and reliable ploidy determination technique other than chromosome counting is specially useful to expedite watermelon tetraploid breeding.

Flow cytometry is a powerful technique for estimating plant nuclear DNA content because this technique permits sensitive measurement of fluorescence intensity of large numbers of stained nuclei within seconds (Arumuganathan and Earle, 1991a). Flow cytometry also permits greater statistical accuracy than either conventional Feulgen microdensitometry or reassociation kinetics (Leutwiler et al., 1984). Nuclear DNA content of more than 100 important plant species have been measured by flow cytometry (Arumuganathan and Earle, 1991a,1991b). Chromosome number in-creases or decreases will result in the increase or decrease of nuclear DNA content. Therefore, flow cytometry can be used for determining ploidy level (De Laat et al., 1987; De Rocher et al., 1990). This experiment was conducted to confirm putative tetraploids regenerated from several diploid watermelon genotypes using tissue culture.

Materials. Putative tetraploid regenerants 4xG17AB (G4x), 4xYF91 (YF4x) and 4xAuP (AuP4x), and their original diploids 617AB (G2x), YF91-1 (YF2x) and AuP7 (AuP2x), respectively, were used in this experiment. Leaf materials for nuclear isolation were taken from greenhouse-grown plants in October 1993.

Methods. Nuclear isolation procedures were from Arumuganathan and Earle (1991a), and Michaelson et al. (1991) with minor modification. About 25 milligrams of newly expanded true leaf tissue were sliced as thin as possible in $MgSO_4$ buffer. The nuclei isolated were stained with propidium iodide (PI) in a solution containing DNase-free RNase. Samples were analyzed on an Epics 751 flow cytometer (Coulter Corporation) equipped with a data acquisition system. Excitation of PI was provided by the 488 nm line (400 mW) of an argon laser (model I-90, Coherent) and the red fluorescence emitted by PI was collected through a 635 nm band pass filter. Chicken red blood cells (CRBC), with nuclear DNA content of 2.397 pg as calibrated by using male human white blood cells (7.0 pg/nucleus) (Tiersch and Chandler, 1989), were used as an internal standard. Five thousand cells per sample were analyzed and the data were represented as histograms. The formula, plant nuclear DNA amount=(position of plant
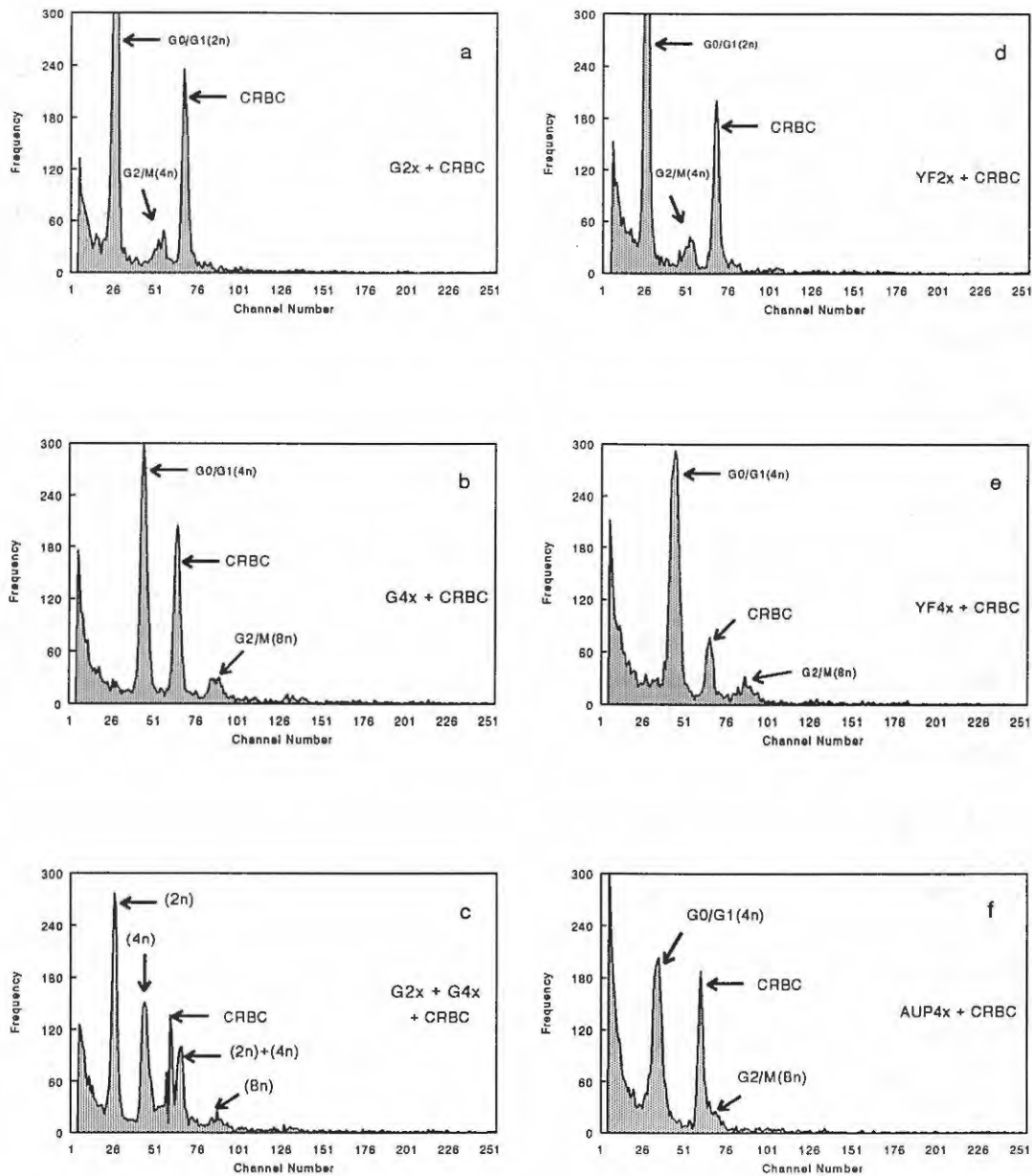
Figure 1. Histograms of number of nuclei per channel as a function of relative fluorescence intensity resulting from flow cytometric analysis of nuclei stained with propidium iodide.

nuclear peak/position of CRBC nuclear peak) x 2.397 pg, was used to calculate DNA content of different genotypes with different ploidy levels.

Results. High quality nuclear preparation was obtained from watermelon leaf tissue. Newly expanded true leaves provided a better source for nuclear isolation than cotyledons. All nuclear preparations from the true leaves gave sharp peaks (Figure 1). Nuclear preparations from cotyledons gave "fat" peaks as a result of a large amount of starch particles in the preparation (histograms are not shown). The yields of nuclei from all of the leaf materials were good enough for flow cytometry to distinguish different categories of the nuclei. However, more nuclei were isolated from the same amount of diploid leaf tissue than from the putative tetraploid leaf tissue.

Nuclei of all six preparations produced two peaks of fluorescence intensity besides the peak produced by CRBC (Figure 1a,b,d,e,f), one corresponding to G0 - G1 phase cells (2C and 4C complement of DNA for diploid and tetraploid, respectively) and the other to G2 + M phase cells (4C and 8C complement of DNA for diploid and tetraploid, respectively). The G2 + M nuclei produced twice the amount of fluorescence intensity of the G0 + G1 nuclei because they contained twice as much DNA. Mixed nuclei of G2x and G4x produced four peaks of fluorescence intensity, for 2C, 4C, 2C+4C and 8C complement of DNA, besides the peak produced by CRBC (Figure 1c). The positions of fluorescence peaks of the putative tetraploids were almost in the expected position. Therefore, the tetraploidy of these putative tetraploid regenerants were confirmed by the histogram of flow cytometric analysis.

The peak position of G0 - G1 nuclei of six preparations was used to calculate nuclear DNA content. The calculated nuclear DNA content of three genotypes at diploid and tetraploid levels are shown in Table 1. Nuclear DNA contents were 0.878 ± 0.111 for diploids, and 1.593 ± 0.103 for tetraploids. Nuclear DNA content of tetraploids appeared 6% smaller than the expected amount

from their original diploids. However, the chi-square statistic indicated that the measured nuclear DNA content of the tetraploids has a probability of 0.95-0.975 to be the expected content. The nuclear DNA content determined in our experiment was very close to the reported numbers 0.88 and 0.90 pg (Arumuganathan and Earle, 1991b). Watermelon genome size is small compared to wheat (33.09 pg), leek (50.27 pg), onion (31.69 pg) and tomato (1.88-2.07 pg), and is only about three times of that of *Arabidopsis* (Arumuganathan and Earle, 1991b). Therefore, a high density watermelon genetic map would not require very many markers.

In summary, the published plant nuclear isolation protocols (Arumuganathan and Earle, 1991a; Michaelson et al. 1991) worked well for watermelon nuclei isolation from a young true leaf. Flow cytometry is a very rapid and efficient technique for determining watermelon ploidy and, therefore, is helpful for watermelon polyploid breeding. The histogram obtained from flow cytometric analysis can be directly used for determining tetraploidy if the original diploid nuclei are included in the sample preparation.

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Table 1. Nuclear DNA content (pg) of diploid and tetraploid watermelons as determined by flow cytometry.

Genotypes	Diploids	Tetraploids
617AB	0.980	1.662
YF91	0.938	1.634
AuP	0.723	1.415
Mean S.E.	0.878 ± 0.111	1.593 ± 0.103

Screening for Resistance to Anthracnose (Race 2), Gummy Stem Blight, and Root Knot Nematode in Watermelon Germplasm

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Over the past six years Auburn University has increased watermelon germplasm for the USDA-ARS. This germplasm has been routinely screened for resistance to gummy stem blight, root knot nematodes and race 2 anthracnose. To date 138 watermelon accessions have been screened for resistance to these diseases (Table 1).

Each accession evaluated for gummy stem blight resistance was arranged in a completely randomized block design with three replications and eight seed planted for each accession within a replication. Plants were grown in 28 x 53 cm flats in Metromix 300 (Cambridge, MA).

Gummy stem blight (*Didymella bryoniae* (Auersw.) Rehm.) inoculum was prepared from culture number MJH 602-5 obtained from Dr. M. J. Havey, University of Wisconsin. The fungal cultures were maintained on PDA and grown for inoculation purposes on sterile green beans. Spore suspensions in sterile water were adjusted to 100,000 spores per ml. using a hemocytometer (3). This spore suspension was sprayed to runoff on two week old seedlings which were then incubated at 100% relative humidity and 25°C for 48 hours. After incubation seedlings were placed in a greenhouse (20-35°C) for two weeks at which time the plants were evaluated for disease resistance on a 1-9 scale with 1 indicating resistance and 9, susceptibility.

Resistance to anthracnose (*Colletotrichum orbiculare* (Pass.) Ell. & Halst.) race 2 was handled in a similar fashion to gummy stem blight. The culture used was CP3 from Dr. J. C. Correll of the University of Arkansas. The spore suspension was adjusted to 50,000 spores per ml (5).

Root knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) races 3 and 4 were used by continual cropping of susceptible tomato and cotton plants (4) in a root knot nematode infested soil. These plants were greenhouse grown in rotation in a sandy-loam soil obtained from the E. V. Smith Plant Breeding Unit, Shorter, AL. Nematode eggs were obtained from these tomato or cotton roots by placing roots in a closed container containing a 10% clorox solution which was shaken for four minutes. This solution containing the nematode eggs was passed through an 80, 200, and 500 mesh screen with the eggs collected on the 500 mesh screen and adjusted, using a hemocytometer, in a sterile water solution to 2,500-3,000 eggs per ml (1). One ml. of this solution was placed in a 7.5 cm pot containing a nematode free sandy-loam soil. Two seed of each accession was planted in each pot so inoculated. The experimental design was a completely randomized block design of three replications with three pots for each accession within a replication. Plants were lifted and the roots carefully washed and evaluated one month after planting on a 1-9 scale with 1 indicating resistance and 9, susceptibility.

As seed increases for accessions were completed, they were evaluated for resistance. Thus, these accessions were not evaluated all at the same time for the three diseases, therefore caution should be exercised in interpreting these results, due to the subjective nature of rating scales and changes in test conditions. The top five accessions for gummy stem blight (GSB) resistance were PIs 500335, 505590, 512373, 164247, and 500334 with ratings of 1.8, 1.9, 1.9, 2.1 and 2.2 respectively. The top 5 PIs for root-knot nematode resistance are 512369,

Table 1. Plant introduction means, standard errors and numbers of entries for resistance to gummy stem blight (GSB), nematodes, and anthracnose (race 2).

Plant Introduction	GSB			Nematodes			Anthracnose		
	Rating[z]	±SE	No. of entries	Rating[z]	±SE	No. of entries	Rating[z]	±SE	No. of entries
164247	2.1	0.3	3	2.8	0.4	3	8.4	0.4	3
167126	3.5	0.6	3	6.2	1.3	3	7.2	1.2	3
487458	5.9	0.7	3	6.7	1.2	3	3.0	0.6	3
487459	2.7	0.6	3	5.8	0.7	3	3.5	2.3	3
487476	5.4	0.3	3	5.3	1	3	3.2	0.5	3
494815	6.1	0.3	2	3.3	0.2	3	2.8	0.3	3
494816	4.4	0.7	3	5.1	1.1	3	2.3	0.1	3
494817	2.3	0.5	3	4.7	0.9	3	2.3	0.5	3
494818	7.7	1.3	2	3.7	-	1	-	-	-
494819	4.5	1	3	4.4	1.3	3	3.7	1.7	3
494820	3.4	0.7	3	5.6	1.7	3	4.6	2.2	3
494821	5.3	0.2	3	5.3	1.5	3	5.3	1.9	3
500306	3.9	0.4	3	5.8	1.5	3	4.1	1.8	3
500309	2.4	0.8	3	4.8	0.2	3	2.7	0.6	3
500310	3.9	0.3	3	6.4	1.3	3	4.0	1.0	3
500312	3.5	0.8	3	5.4	0.9	3	3.8	0.6	3
500315	4.8	1.3	6	4.6	0.8	3	1.9	0.3	3
500317	4.5	0.5	3	6.1	0.6	3	2.6	0.1	3
500327	3.3	0.7	3	4.3	1.1	3	2.1	0.1	3
500329	2.7	0.9	3	3.4	0.1	3	1.8	0.2	3
500331	3.1	0.9	3	3.7	0.7	3	3.9	0.3	2
500332	2.9	0.3	3	6	1.5	3	3.5	0.9	3
500334	2.2	0.2	6	7	0.7	3	2.0	0.1	3
500335	1.8	0.3	3	4	-	1	3.2	0.7	3
500337	-	-	-	-	-	-	2.2	-	1
500345	-	-	-	8	0.6	3	2.4	0.3	6
500353	4.3	1.1	3	6	1.3	3	2.9	0.7	3
502315	5.4	0.9	6	7.1	0.6	6	3.3	1.0	6
502316	-	-	-	4.3	-	1	-	-	-
502317	4.8	0.6	2	7	0.9	4	3.1	1.2	3
502318	6	1.1	3	7	-	1	1.4	0.1	3
502319	8.5	0.5	3	7.3	1.3	2	2.2	0.2	3
504519	5	1.3	2	5	1.5	3	1.9	0.6	3
505584	4.3	1.4	3	6.6	0.8	3	2.4	0.5	3
505585	4.1	0.6	4	6.1	1.2	3	2.9	0.6	3
505586	2.5	0.5	3	8.5	0.5	2	4.2	0.9	3
505587	4.3	0.5	2	5.2	0.8	3	2.3	0.4	3
505588	5.1	0.9	3	6.4	1.5	3	3.8	1.3	3
505589	3.3	0.9	3	5	0.7	2	2.3	0.2	3
505590	1.9	0.3	3	6.5	0.5	2	2.0	0.1	3
505591	4.1	1	3	6	1.2	3	1.9	0.3	3
505592	2.9	1	4	5.3	1.4	3	1.7	0.3	3
505593	2.9	0.5	4	4.9	0.9	3	1.9	0.4	3
505594	3.6	0.8	3	6.4	1.4	3	2.4	0.4	3
505595	3.6	0.7	2	4.6	0.2	3	3.0	0.6	3
505935	4.4	0.7	6	5	0.6	6	8.4	0.3	3
506439	4.8	0.7	3	4.3	1.3	2	2.7	0.4	3

507858	5.1	1	3	5.8	0.9	3	3.3	0.5	3
507859	8.6	0.4	3	7.4	0.4	3	2.7	0.7	3
507860	3.8	1.3	2	6.7	0.9	3	6.1	1.5	3
507861	5.2	1.1	3	6.3	1	3	2.0	0.1	3
507863	6	0.2	3	8.5	0.5	2	4.3	1.5	3
507864	6.8	1.1	3	7.8	0.7	3	2.1	0.8	3
507865	9	0	3	7.7	0.7	3	8.9	0.1	3
507866	7.7	0.7	3	8.1	0.9	3	7.6	1.3	3
507867	4.9	1.4	3	7.9	0.3	3	3.1	0.9	3
507868	7.8	0.4	3	8.8	0.2	3	3.5	1.1	3
507869	8.6	0.2	3	8.7	0.1	3	5.8	1.3	3
508441	5.8	1.7	3	8.1	0.2	3	4.4	1.0	3
508442	7.7	1.1	3	7.9	0.9	3	2.6	0.6	3
508443	8.4	0.5	3	7.3	0.9	3	3.7	2.7	2
508444	8.5	0.5	3	8.8	0.1	3	2.6	0.5	3
508445	6.4	0.9	3	7.6	1.1	3	5.2	1.8	3
508446	7.4	0.7	3	7.8	0.3	3	4.2	1.3	3
512331	6.8	1.1	3	7.3	0.7	3	3.8	2.6	3
512332	8	0.5	3	8.6	0.2	3	3.6	1.9	3
512339	5.7	0.9	6	7.8	0.6	6	6.3	1.5	5
512340	3.2	0.5	3	5.5	3.5	2	7.9	0.1	3
512341	8.3	0.8	2	7	0.7	3	5.4	1.1	3
512342	8.4	0.4	3	8.2	0.3	3	8.3	0.4	3
512343	7.5	0.8	3	8.8	0.2	3	4.5	1.5	3
512344	7.1	1.3	3	7	2	3	5.7	1.4	3
512345	8.2	0.8	3	7.2	0.9	3	2.5	0.5	3
512346	3	0.1	3	8	-	1	9.0	0.0	3
512347	8.9	0.1	3	5	1.5	3	7.2	1.0	3
512348	8.9	0	3	6.6	1	3	4.6	0.6	3
512349	8.6	0.2	3	8.1	0.3	3	5.5	1.6	3
512350	5	1.6	5	4.3	1.4	4	7.7	1.0	5
512351	5.1	1.1	5	5.7	0.9	6	5.9	1.4	6
512352	4.3	0.7	6	6.5	0.8	6	6.4	1.4	6
512353	3.9	1.6	2	5.6	0.7	3	2.4	0.6	3
512354	6.3	0.8	3	7	1	3	8.6	0.3	3
512355	6.2	0.9	6	6.2	1.1	6	6.5	1.6	5
512356	5.5	1.2	6	5.5	1	6	5.8	1.3	6
512358	5.6	2.2	3	8.6	0.3	3	2.9	1.3	3
512359	4.3	0.9	6	7.5	0.7	6	5.9	1.3	6
512360	5	1.2	6	5	0.5	5	5.1	1.1	6
512361	4.4	1.6	4	6.5	0.7	5	8.9	0.1	5
512362	5.2	1.6	5	7.6	1	3	7.7	0.2	3
512363	3.1	0.2	3	6	1.2	3	8.8	0.2	3
512364	3.7	0.7	3	6.3	1.8	2	8.7	0.2	3
512365	8.8	0.2	3	7.7	1	3	3.6	1.5	3
512366	3.5	0.2	3	4.9	1.6	3	9.0	0.0	3
512367	3.5	0.2	3	5.5	2.5	2	9.0	0.0	3
512368	3.9	0.3	3	7.1	0.5	3	7.8	0.0	2
512369	4.5	0.1	3	2	-	1	7.0	0.9	3
512370	3.2	0.2	3	5.8	1.5	3	7.7	0.7	3
512373	1.9	0.1	3	7.5	0.3	3	8.9	0.1	3
512374	3.3	0.4	3	5.8	0.8	2	8.3	0.3	3
512375	2.7	0.2	3	7.4	0.6	3	8.5	0.3	3
512376	3.1	0.3	3	6.4	0.7	3	8.1	0.5	3

512377	2.5	0.5	3	7	-	1	8.6	0.3	3
512378	3.6	0.7	3	5.5	1.9	3	7.7	0.7	3
512379	3.5	0.4	3	6.8	0.8	2	7.8	0.2	3
512381	3.4	0.6	3	6.9	0.7	3	8.0	0.3	3
512382	3.1	0.2	3	8	-	1	8.9	0.1	2
512383	3.1	0.7	3	7.7	0.2	3	7.9	0.6	3
512384	3.2	0.2	3	6.3	0.7	3	8.7	0.3	3
512385	5.6	0.8	3	5.7	1.3	3	4.5	1.1	3
512386	4.2	0.8	3	5.9	0.8	3	8.6	0.4	3
512387	2.8	0.2	3	9	-	1	8.4	0.3	3
512388	4.3	1.1	3	7.8	0.2	3	8.1	0.5	3
512389	2.9	0.2	3	6.3	0.8	3	8.6	0.2	3
512390	3.1	0.6	3	5.1	1.2	3	8.2	0.5	3
512391	2.4	0.1	3	7.8	0.7	3	8.7	0.2	3
512392	2.6	0.3	3	7	1	2	9.0	0.0	3
512393	3.6	0.4	6	5.9	0.9	6	8.1	0.5	3
512394	2.9	0.2	3	7.2	1	3	8.5	0.5	3
512395	3.2	0.3	3	6.6	1.1	3	8.3	0.4	3
512396	3.3	0.5	3	8.3	0.3	2	8.8	0.1	3
512397	3.5	0.4	3	7.8	0.8	2	8.3	0.5	3
512398	3.3	0.4	3	4.6	1.2	3	8.2	0.4	3
512399	2.9	0.4	3	7.5	0.3	3	8.5	0.3	3
512400	2.6	0.2	3	7.3	0.7	3	7.6	0.9	3
512401	3.6	0.7	3	6.8	0.8	3	8.8	0.2	3
512402	3.3	0.8	3	6.3	0.8	2	8.0	0.5	3
512403	3.5	0.4	3	5.6	0.3	3	8.8	0.1	3
512404	2.8	0.8	3	6.9	0.5	3	9.0	0.0	3
512405	3.5	0.3	3	7.2	0.4	3	8.6	0.3	3
512406	3.5	1.2	3	7	1	3	8.5	0.4	2
512407	3.1	0.1	3	7.3	0.5	3	6.5	0.3	3
532811	3.7	0.6	3	3.3	1.8	2	7.0	1.1	3
534593	4.3	0.6	4	4.9	0.9	6	8.7	0.3	3
537461	2.3	0.1	3	6.5	0.5	3	8.8	0.2	3
537465	3.1	0.4	5	5.6	0.4	3	8.3	0.0	3
537467	2.4	0.4	3	7.5	1	2	6.8	0.8	3
537468	3	0.7	3	4.7	1.4	3	7.9	0.8	3
537470	3.8	0.5	3	7.7	1.3	3	8.7	0.3	3

Ratings: 1-9 with 1-resistant and 9-susceptible.

164247, 494815, 532811, and 500329 with ratings of 2, 2.8, 3.3, 3.3, and 3.4 respectively. Finally, the top 5 performers with respect to race 2 anthracnose are PIs 502318, 505592, 500329, 505593, and 500315 with ratings of 1.4, 1.7, 1.8, 1.9, and 1.9 respectively.

Resistance to GSB and anthracnose race 2 have been found in several PIs and have been successfully incorporated into watermelon varieties (2). The PIs listed here may be additional sources of resistance, however additional testing is required to confirm this. It should be noted that there is no widely accepted source of resistance to root-knot nematode in watermelon. Our testing method used a lower concentration of nematodes (2,500-3000 eggs/ml.) compared to the standard method of 8,000 eggs/ml. The relatively low ratings of theses accessions may be due to the lower egg counts however these results warrant further investigation and selection.

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Shoot Regeneration From Immature Cotyledons of Watermelon

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Genetic transformation and somaclonal variation can be efficient alternatives to traditional breeding techniques for varietal improvement. However, an efficient regeneration system is essential for *in vitro* techniques to be used in cucurbit crops (4, 7).

Adventitious shoot regeneration from cotyledons and hypocotyls of watermelon seedlings was first reported by Srivastava et al. (1989) who determined that cotyledons were more regenerative than hypocotyls. The effects of growth regulators, genotypes, explant age and explant location in the determination of adventitious shoot organogenesis were previously documented in diploids (2, 3) and more recently in triploids and tetraploids (2). High frequency shoot regeneration was observed from 5-day-old cotyledons of diploid cultivars cultured on Murashige and Skoog (MS) medium with 22.19 μM BA and 2.85 μM IAA (3) or 5-10 μM BA (2). Two growth regulator combinations, 10 μM BA combined with 1 μM NAA (10) and 10 μM BA alone (1) gave high frequency shoot organogenesis.

Adventitious shoot organogenesis studies in watermelon have been conducted using cotyledons from mature seeds. Cotyledons from immature seeds can be another useful explant source for adventitious shoot regeneration (1). The cotyledons of immature seeds taken from a disinfected fruit tend to be bacteria-free, obviating the need for sterilization of explant tissue. Immature cotyledons may be more responsive to *in vitro* regulation than mature cotyledons because of their active physiological condition. The watermelon cotyledon is large enough to be easily sectioned. Knowledge of the location of responsive regions for regeneration can be very useful for explant preparations in somaclonal variation and transformation studies. This research explores the effect of the inclusion of NAA, age, genotype, location and size of explants on shoot regeneration from immature watermelon cotyledons.

Methods. Immature fruits from self-pollinated flowers were harvested from greenhouse grown plants during the spring-summer and fall-winter of 1991. The fruit were surface-disinfested with 95% ethanol, flamed under a laminar flow hood and sliced open. Fruit development in the greenhouse took about 35 days for the spring crop and 40 days to mature for the fall crop. Immature seeds were removed from the fruit and the developing cotyledons were excised. Either entire (minus a small segment of tissue near the apical end of the embryo to avoid the confounded factor of axillary shoot formation) cotyledons or section of cotyledons (Fig. 1) were used as explants. The cotyledon tissue was placed on the medium in abaxial orientation. MS (8) medium was prepared and supplemented according to Wilkins (1985) with 100 mg/l myo-inositol, 2 mg/l glycine, 0.2 mg/l thiamine HCl, 0.5 mg/l pyroxidine, 0.5 mg/l nicotinic acid, 7 g/l agar (Agar-Agar, Gum Agar; Catalog no. A 1296, SIGMA Chemical Co., St. Louis, MO), 30 g/l sucrose, and 10 μM BA with or without 1 μM NAA. Medium pH was adjusted to 5.7 before autoclaving. Because of the limitation of available immature embryos, eight to 16 explants per vessel were cultured on 50 ml medium in Magenta GA 7 vessels (Sigma) under cool white fluorescent lamps that provided a photosynthetic flux density of 35 μmol m^{-2}sec^{-1} for a 16 hr period at 25 ± 2°C.

Shoot regeneration was compared from immature cotyledons cultured on medium with 10 μM

BA + 1 μM NAA (1,10). One hundred twenty and 104 entire cotyledons dissected from seeds of G17AB 17 days after pollination in June were cultured on 10 μM BA and 10 μM BA + 1 μM NAA medium, respectively. The percentage of cotyledons that callused before producing shoot buds (i.e., the shoots before elongation) was recorded. After 34 days, the number of cotyledons that regenerated one or more shoot buds was recorded.

Cotyledons from immature seeds collected 14, 18 and 20 days after pollination in June were evaluated for shoot regeneration competence. The genotype G17AB was used as above with 10 μM BA. At least four replications of 8 whole cotyledonary explants from each age were cultured on MS medium. The number of cotyledons that regenerated one or more shoots were recorded 35 days after culture initiation.

To determine the effect of genotype on shoot regeneration, immature fruit from 8 inbred diploid genotypes were harvested 20 days after pollination in November. Lines JXP-91, G17AB, B1-91, DRE, and 91.7.4 were from our breeding program. Commercial cultigens were 'Yellow Sweet', 'AU-Producer', and 'Mickylee'. Entire cotyledons (32-127) from each genotype were cultured on medium with 10 μM BA for 35 days and scored for the presence of shoot bud regeneration.

Genotype JXP-91, not different from genotype G17AB in previous testing, was used to determine the effect of location of the cotyledonary explant on shoot regeneration. Immature cotyledons were dissected (see Fig. 1) 28 days after pollination because embryo development was slow at low temperature (in December). Explant A was a large block of one cotyledon comprising all but a small segment of tissue near the apical end of the embryo and a small segment of tissue at the distal tip of the cotyledon. The other cotyledon from the same immature embryo was cut the same way as A but then cross-sectioned into two equal portions: B (proximal portion) and C (distal portion). To obtain D, E and F, a longitudinal cut was made to

bisect the proximal block into two equal portions. One portion was bisected again to produce explants E and F. E was the center portion of the proximal portion of one cotyledon. G was taken from the same location as B, but the rest of the cotyledon was discarded. Forty-eight explants from each location were cultured on medium with 10 μM BA. The number of explants with shoot buds was recorded after 35 days in vitro. To promote further shoot development, buds regenerated from six explants of each type were transferred to medium with 5 μM BA for 14 days.
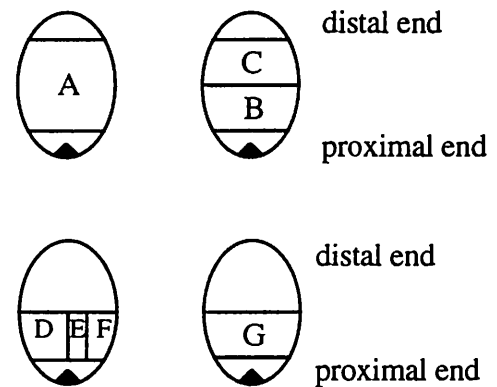
Figure 1. Regions and relative sizes of cotyledon pairs sectioned.

To obtain plantlets, shoot buds formed from cotyledons on medium with 10 μM BA were transferred to medium with 5 uM IBA for 12 days. These plantlets were planted into Todd Planter Flats (Catalog No. 14-2574, A. H. Hummert Seed Co., St. Louis, MO) containing pre-moistened commercial soilless potting mix (Fafard Superfine, Fafard, Inc., Anderson, SC) and placed under mist in the greenhouse. Shoots without roots were rooted with Hormodin #1 (Merck & Co., Rahway, NJ).

The shoot regeneration frequency data (percentage) were subjected to an arcsin √y transformation and the number of shoots per explant data were subjected to a square root transformation before the analysis of variance. An LSD was performed to determine significant differences when the analysis of variance was significant.

Results. Immature cotyledons of G17AB cultured on media with 10 μM BA produced more shoot buds than those incubated on similar medium containing 1 μM NAA (60% and 38%, respectively). Although the apparent increase of callus on NAA was not statistically significant (77% versus 64%), auxin (NAA and IAA) increased callus formation and inhibited watermelon shoot regeneration from mature cotyledons in studies by Srivastava et al. (1989) and Compton and Gray (1993). Because of the negative effect of auxin on watermelon shoot regeneration, medium with BA alone was used for future experiments.

The effect of explant age on shoot regeneration from cotyledons excised 14-20 days after pollination was not significant in the range tested. The age of the immature cotyledon explants was not as critical as the age of cotyledons from mature seeds for optimal shoot regeneration (2,3). The differential response may be related to the developmental difference of explant tissue from immature and mature cotyledons. The fate of cell differentiation and development in the immature cotyledon may be less determined than in the mature cotyledon, and therefore the cells in the immature cotyledon might be regulated *in vitro* over a longer period. It would be enlightening to compare shoot regeneration from the immature versus the mature cotyledon. Cotyledons from 20-day-old (summer) or 28-day-old (winter) embryos were chosen for future experiments because dissection of cotyledons from younger embryos was difficult.

More explants from genotypes JXP-91, G17AB and B1-91 regenerated more shoots than the other genotypes tested (Table 1). Shoot bud initiation was observed one week later for 'Mickylee' than the other genotypes. Genotypic differences for adventitious shoot regeneration in watermelon were previously observed using mature cotyledons (3, 2). We noted that genotypes that were difficult to regenerate either produced fewer shoots (DRE and 91.7.4) or callused profusely ('Mickylee'). In fact, more recent observations not reported here indicate that genotype not only

affects shoot regeneration but also rooting and acclimatization.

Cotyledon sections, explant B, D, G and E, proximal to the cotyledonary node regenerated shoots more often and produced more shoots than cotyledon sections, explant C, from distal regions (Table 2). The excised cotyledon, explant A, produced 14 shoots per explant. When twin cotyledons were bisected, distal section, explant C, produced only 3 shoots per explant whereas the proximal section, explant B, produced 19 shoots per explant. In a parallel experiment, proximal section G (similar to B) yielded 21 shoots per explant, not different from explant B. When twin cotyledon sections were dissected into sections D, E and F, 22, 18 and 8 shoots per explant were produced, respectively, from the same quantity of tissue. Dividing explant G into D, E and F with two cuts resulted in 2.3 times as many regenerants as produced by explant G. Bisection of explant D, into E and F, yielded 1.6 times as many shoots as D alone. Within proximal portions, explants containing the midrib of cotyledons regenerated more frequently than explants from marginal portions. When explant G was bisected into D, E and F, the interior portion E always produced shoots whereas only 69% of portion F explants regenerated shoots.

The cells capable of shoot organogenesis in immature watermelon cotyledons were distributed mainly in the proximal region, especially the portion along the cotyledon midrib where the vascular system is located. Shoot buds were more uniform from proximal and interior portions than distal and exterior portions. Our results support the similar observations reported in cucumber (5), melon (6) and mature watermelon cotyledons (2).

Shoots and roots developed 12 days after buds were transferred to medium with 5 uM IBA. Depending on the genotype, 7% to 63% of the shoots produced roots. Shoots (94%), with or without roots, developed into transplants in 5 weeks in the greenhouse. More than 1000 plants regenerated from the 8 genotypes (Table 1) survived transplanting in the field. Plants from tissue

Table 1. Shoot regeneration from immature watermelon cotyledons[1] of different genotypes.

Genotype	Total explants	%Explants with shoot buds[2]
JXP-91	127	84 a[3]
G17AB	124	75 a
B1-91	102	61 ab
Yellow Sweet	32	50 bc
Au-Producer	32	34 bcd
DRE	57	26 cd
91.7.4	96	20 d
Mickylee	112	19 d

[1]All cotyledons were taken from immature seeds 20 days after pollination.
[2]Data were collected 35 days after culture initiation.
[3]Denote differences at the 0.01 level of significance using the LSD test.

Table 2. Shoot regeneration from sections of immature watermelon cotyledon tissue[1].

Region[2]	Total Explants	% Explants with shoot buds	Shoot buds per Explant[4]
E	48	100.0 a[3]	17.8 a
G	48	93.8 ab	21.3 a
D	48	87.5 ab	21.7 a
B	48	87.5 bc	19.2 a
A	48	77.1 bc	14.0 a
F	48	68.8 cd	7.5 ab
C	48	52.1 d	3.2 b

[1]Cotyledons were from JXP-91 seeds 28 days after pollination.
[2]Refer to Figure 1.
[3]Denote significant differences at 0.01 confidence level using the LSD test.
[4]Based on six explants with shoots.

culture produced fruit comparable in size to fruit from plants from seed. The plants appeared uniform and true-to-type. However, variants in ploidy level, suggested by pollen size and leaf characters, and chlorophyll deficiency were observed. The occurrence of tetraploids was rare. Tetraploid regenerants were observed from G17AB (2/200), JXP-91 (5/241), B1-91 (1/387) and 'Mickylee' (2/133). Two regenerants from B1-91 were chimeric, and chlorophyll deficiency was observed on the majority of leaves. No fruits were obtained from the vine with chlorophyll deficiency because of delayed female flower occurrence on those vines and lack of synchronous male flowers. Albino shoots were regenerated from G17AB in 1992 and the plantlets did not survive in the greenhouse. The only somaclonal variants obtained that could be maintained in this research were tetraploids. Some of the self- pollinated regenerants from G17AB, JXP-91, B1-91 and 'Au-Producer' were evaluated in the field in 1993. There was no variation observed in their progeny populations.

This research illustrated that the immature cotyledon explant was the source of high frequency shoot regeneration in watermelon, and the immature cotyledon from a disinfected fruit facilitated clean explant tissue. The effects of genotype and explant location should be considered for high frequency shoot regeneration. Our experiments on effect of cotyledon explant location on shoot regeneration provided useful information for precise selection of explant tissue, and these results can be applied to either genetic transformation experiments or tetraploid regeneration. Based on this study, an *in vitro* system for efficient tetraploid watermelon regeneration has been developed in our laboratory.

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RAPD Molecular Markers in Watermelon

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Watermelon is grown worldwide, yet genetic evaluation is far behind other important crops. Fewer genes were described in watermelon than other important cucurbit crops such as cucumber and melon (Henderson, 1991; Robinson, et al., 1976). More genetic information is needed for efficient genetic improvement of this crop.

Genetic markers closely linked with genes of interest are useful for efficient selection of the traits controlled by the genes in the breeding population. Navot and Zamir (1986) have developed a partial linkage map using isozyme and seed protein markers. Seven linkage groups were identified based on populations derived from interspecific crosses. Five of these groups consist of two or three marker loci (Navot and Zamir, 1986; Navot et al., 1990).

A major limitation of using isozyme/protein markers is that limited polymorphic loci can be detected in watermelon, especially in the species *C. lanatus* (Biles et al., 1989; Navot and Zamir, 1987, Zamir et al., 1984; Zhang and Wang, 1989). A new marker system, which offers a large number of polymorphic loci, is needed to develop a genetic map with high density or map the genomic regions around the genes of interest. DNA markers have introduced a new dimension to the development of genetic maps and the mapping of agronomically and physiologically important characters since they have the potential to reveal an almost unlimited number of polymorphisms. DNA markers commonly used are restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD). RFLP assay detects DNA polymorphism through endonuclease digestion, coupled with DNA blot hybridization, and are in general time-consuming and labor- intensive. RAPD assay is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence. These primers detect polymorphisms in the absence of specific nucleotide sequence information and the polymorphisms function as genetic markers. The major advantage of this assay is that there is no requirement for DNA sequence information. The protocol is relatively quick and easy to perform and automation is feasible. The RAPD assay uses fluorescence in lieu of radioactivity and only nanogram quantities of DNA are required. The RAPD markers have been used for many genetic studies of plant crops, and are suitable for marker assisted selection in applied breeding (Martin et al., 1991; Michelmore et al., 1991; Paran et al., 1991; Willams et al., 1991).

The objective of this study was to investigate RAPD molecular polymorphisms, and identify polymorphic RAPD markers for developing a molecular linkage map and mapping Fusarium wilt resistance gene(s) in watermelon.

Materials and Methods. We used three cultigens and one primitive watermelon for most of the primers tested: 1) 617AB (*ms ms*), a male-sterile line with round fruit, red flesh and stripe rind; 2) 'Dixielee', a late variety with round fruit, red flesh and stripe rind; 3) PI 296341, a primitive watermelon with resistances to race 0, 1 and 2 of the Fusarium wilt pathogen, and small round fruit with white flesh; and 4) 'New Hampshire Midget' (NHM), an early variety with small round fruit, red flesh, light green fruit color and susceptibility to all races of Fusarium wilt pathogens. Eight more

genotypes and the F_1 hybrid NHM x PI 296341 were used for testing some primers.

DNA Isolation and PCR Amplification. The DNA isolation procedures and PCR amplification conditions were the same as described by us (Zhang et al., 1992). Amplification was performed in a Perkins-Elmer/Cetus Model 480 DNA thermal cycler. Fifty-three 10-mer primers from the Operon RAPD Primer Kit C, E and M (Operon, Alameda, CA) were used in this study.

Cluster Analysis. A cluster analysis of four genotypes was performed using UPGMA (unweighted pairgroup method using arithmetic average) method on SAS program. Eighty-nine polymorphic RAPD loci observed from the four genotypes were used for the average cluster analysis.

Results. Three (5.6%) primers of the 53 primers tested were unable to prime amplification for the four watermelon genotypes under the conditions used in this experiment. By changing the reaction condition, amplification can be obtained from those primers that were unable to prime amplification in this experiment. Fourteen (26.4%) primers were only able to prime amplification for some genotypes. A total of 159 readable bands were obtained from the 36 primers that were able to prime DNA amplification for all the accessions tested, yielding 4.4 bands per primer. Among the total of 159 bands, 89 (56.0%) of the bands were polymorphic among the four accessions, 82 (51.6%) of the bands were polymorphic between NHM and PI 296341, but only 16 (10.1%) of the bands were polymorphic among the three cultigens. About 2.5 polymorphic bands were revealed per primer among the accessions tested. Therefore, RAPD markers offer a high level of polymorphism in watermelon when a primitive type was included. Fig. 1 shows the RAPD bands generated from cultigens 617AB *(ms ms)*, 'Dixielee' and NHM, and primitive watermelon PI 296341 using primers OPM01, OPM02, OPM03, OPM04 and OPM05. Fig. 2 shows polymorphic RAPD loci generated

from 13 watermelon genotypes using primer OPC08.

RAPD markers were further tested on F_1 hybrid NHM x PI 296341 and its parents (NHM and PI 296341) to confirm polymorphism and inheritance of the RAPD marker. Similar to the RAPD markers detected in other crops (Williams et al., 1990; Michelmore et al., 1991), RAPD markers were inherited in a dominant fashion in watermelon (Fig. 2).

We did a cluster analysis using the RAPD data obtained from the three cultigens 61 7AB(*ms ms*), 'Dixielee' and NHM, and one primitive type PI 296341 to test if the RAPD data are correlated to the variation in agronomic/morphologic character. As shown in Fig. 3, 617AB(*ms ms*) and 'Dixielee', which are morphologically and agronomically similar, were joined as one cluster at an average distance of 0.239619, NHM was joined to the first cluster at an average distance of 0.262833, and PI 296341, which is distant from all three cultigens, was joined to the second cluster at an average distance of 1.744839. We confirmed the great genetic distance between cultigens and primitive watermelon at the DNA level. However, the three cultigens were very similar at the DNA level although they were developed at different times and locations. Breeding has considerably narrowed the genetic background of this crop.

In summary, the use of RAPD markers is an effective marker system for watermelon genome mapping because of the high polymorphism in the species. The existence of a relatively small genome (Arumuganathan and Earle, 1991; and our unpublished data) and a large number of polymorphic RAPD markers provides a potential for the development of a high density map efficiently. To be successful, however, a primitive type should be used as one of the parents to generate enough segregating marker loci in F_2 or any population used for cosegregation analysis.
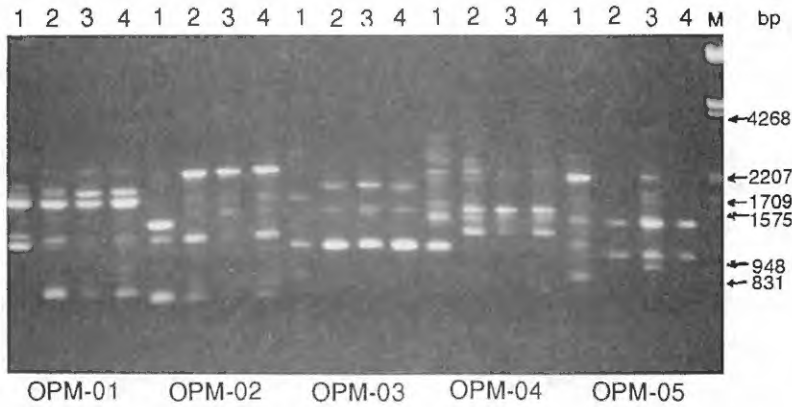
Figure 1 RAPD polymorphism in watermelon obtained with primers OPM- 01, OPM-02, OPM-03, OPM-04, OPM-05. 1) PI 296341, 2) NHM, 3) G17AB(msms), 4) Dixielee.

Figure 2 RAPD polymorphism in watermelon obtained with primer OPC-08. 1) G17AB(*msms*), 2) Dixielee, 3) PI 296341, 4) NHM, 5) Tomato Seed Watermelon(*tsts*), 6) Edible Seed Watermelon, 7) SC-7, 8) bl-91, 9) NHMxPI 296341, 10) Au-Producer, 11) Sweet Princess, 12) Yellow Sweet, 13) G17AB(*Msms*).
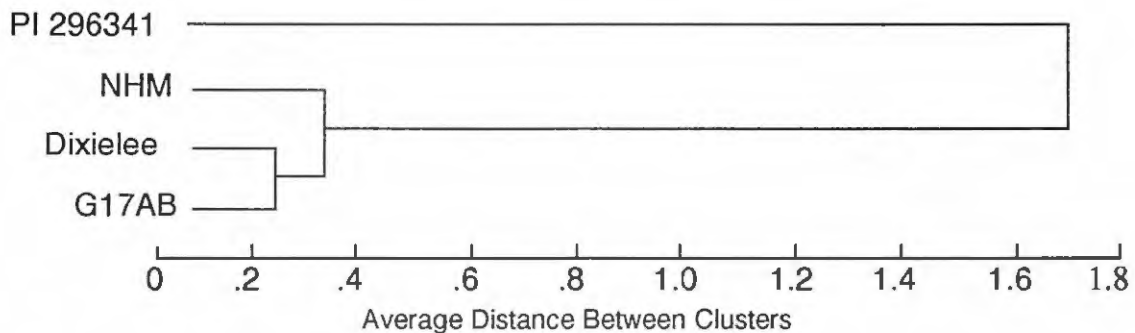
Figure 3. Average linkage cluster analysis of four watermelon genotypes based on 89 polymorphic RAPD markers.

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On the Selective Activator of Gene $\underline{B2}$ in *C. moschata*

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Gene $\underline{B2}$ originated as mutation in *C. maxima* (1,2). This gene conditions precocious depletion of chlorophyll in fruits of all known genetic backgrounds. But in some backgrounds, $\underline{B2}$ is also expressed in one or more other aerial organs. Results of breeding experiments suggested the existence in *C. maxima* of nuclear elements that selectively activate or selectively suppress the expression of $\underline{B2}$ in aerial organs other than fruit.

This report provides evidence for the existence of a selective activator or selective activators of $\underline{B2}$ in *C. moschata*, a species in which $\underline{B2}$ has not been reported to occur naturally. The evidence is based on results obtained from two interspecific crosses of *C. maxima* x *C. moschata* involving different cultivars. In each case, the cultivar of *C. moschata* was used as a pollen parent in the initial crossing as well as the recurrent pollen parent in repeated backcrosses.

The first cross involved PI 165558, $\underline{B2}$ $\underline{B2}$, of *C. maxima* and 'Waltham Butternut', $\underline{B2+}$ $\underline{B2+}$, of *C. moschata*. Both the initial cross and all the backcrosses were made by A.M. Rhodes (personal communication). The present writer merely extracted a $\underline{B2}$ $\underline{B2}$ inbred of *C. moschata* from one of Rhodes' sixth backcross progenies. This new $\underline{B2}$ $\underline{B2}$ inbred is known as the IL-B breeding line (2). In his transfer of $\underline{B2}$ from *C. maxima* to *C. moschata*, Rhodes employed a special "bridge" the nature of which will be described elsewhere.

PI 165558 is an introduction from India. In this accession, $\underline{B2}$ is expressed in both fruits and stems. However, unlike the uniformly golden pigmentation and high phenotypic stability of $\underline{B2}$ expression in fruits, the golden pigmentation in stems is subject to phenotypic plasticity, i.e., under varying field conditions the stems often exhibit a mosaic phenotype, being predominantly golden but with some green patches or stripes (3).

In IL-B, the expression of $\underline{B2}$ is consistently stable in both fruits and stems. When compared to PI 165558 stems, the IL-B stems are particularly striking in their uniformly golden pigmentation and high phenotypic stability under varying field conditions. What then is the cause for the high phenotypic stability of $\underline{B2}$ expression in IL-B stems?

The second interspecific cross was made in part as an attempt to find an answer to the above question. This cross involved 'Pink Banana', $\underline{B2}$ $\underline{B2}$, of *C. maxima* and 'Chirimen', $\underline{B2+}$ $\underline{B2+}$, of *C. moschata*. 'Pink Banana' was represented by an advanced inbred, PB#1. In this inbred, the fruits are precociously pigmented and pink in color, but the stems are persistently green. 'Chirimen' (4) was represented by an inbred obtained from Theodore H. Superak. When used as pollen parent, 'Chirimen' is known to be cross-compatible with *C. maxima*. The F_1 is vigorous, self-sterile but moderately cross-fertile to 'Chirimen' as pollen parent. The F_1 plants bear precociously pigmented golden fruits. The stems of these F_1 plants are predominantly but not persistently green, i.e., under some field conditions a portion of one of the stems may undergo chlorophyll depletion and appear golden. Field rooting of rare golden shoots and their subsequent growth in a warm greenhouse led to F_1 plants that produced precociously pigmented fruits and persistently green stems.

A sample of 159 BC_1 plants was observed for a period of three months. Over 90% of this sample manifested different types of developmental abnormalities, including leaf distortion and varying degrees of stunting. However, with the passage of time, 82 plants recovered sufficiently to allow decisive classification of both fruits and stems. Of these 82 plants, 62 had precociously-pigmented fruits and stems, and 20 had precociously pig-

mented fruits and persistently green stems. In addition, there were 29 plants that appeared to have persistently green fruits and green stems, but the classification of some of them was not entirely certain. Of the remaining 48 plants, 13 died prematurely and 35 were too stunted and abnormal to permit adequate classification.

Two additional backcrosses and some selfing resulted in the isolation of two distinct breeding lines: one bearing precociously-pigmented golden fruits and uniformly golden stems, and the other bearing precociously-pigmented golden fruits and persistently green stems. Although the level of fertility of these lines remains to be determined, it is clear that 'Chirimen' carries a genetic material (one or more nuclear elements) capable of strongly activating $B2$ in stems. Furthermore, one cannot exclude the possibility that the strong selective activator of $B2$ in IL-B was derived from 'Waltham Butternut' rather than from PI 165558. Is the strong selective activator of $B2$ widely spread in *C. moschata*? What function, if any, does it have in a $B2+$ $B2+$ species?

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Inheritance of Hard Rind in *C. maxima* x *C. moschata* Crosses

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Along with the development of cv. Alice (1), a bush type of *C. maxima*, we have selected a sister line with fruit carrying the hard rind trait. Because hard rind lines show good field resistance to fruit rotting, there is some interest in using it as the female parent in F_1 seed production. Hard rind in *C. pepo* and *C. andeana* x *C. maxima* is determined by a single dominant gene (3). Herrington et al. (2) presented data from a cross between *C. maxima* x *C. ecuadorensis* suggesting that hard rind was recessive to soft rind in this cross and that the *C. maxima* cv. Queensland Blue has a dominant gene (*Hi*) which inhibits the expression of hard rind. It was of interest to know whether this inheritance pattern would hold true for other *C. maxima* accessions and the inheritance of this trait in crosses between *C. maxima* x *C. moschata*.

Materials and Methods. The pedigree and main characteristics of the breeding lines and cultivars used in the present study are presented in Table 1. Inbred lines were selfed for at least three generations and cultivars were observed for at least two generations before use, and were uniform for the trait under investigation. F_1 seeds were produced by crossing the *C. maxima* hard rind inbred line AF839L as the female parent with all other lines and cultivars. A segregating F_2 population was obtained for the cross (AF839L x AF724). Two hard rind and four soft rind F_3 lines were selected from this cross to further investigate the inheritance of the trait. Parents, F_1 and F_2 plants were grown under field conditions during the summer season of 1991, and the F_3 lines during the Autumn season of 1992. Rind classification was made after steaming pieces of the mature fruits for 10 minutes. Soft rinds disintegrate after this treatment, whereas hard rinds remain hard.

Results and Discussion. Hard rind in F_1 plants of crosses between *C. maxima* x *C. moschata* was dominant to soft rind (Table 2). In all crosses between *C. maxima* x *C. maxima* lines or cultivars, hard rind in F_1 plants was recessive to soft rind (Table 2). Segregation for the trait in the F_2 and in the F_3 (selected lines) generations did not differ significantly from the expected 3:1 and 1:0 ratios, based on chi-square values, for a single gene model of inheritance (Table 2). These data support that of Herrington et al. (2), and suggest that the *Hi* gene (Hard rind inhibitor) may be a common feature among the *C. maxima* cultivars. If this holds true for other *C. maxima* cultivars, it may be feasible to use hard rind lines as the female parents in F_1 seed production in *C. maxima* x *C. maxima* crosses for a better control of fruit rot under tropical conditions.

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Table 1.   Pedigree and main characteristics of the breeding lines and cultivars used in the study of the inheritance of hard rind in *C. maxima* x *C. maxima* and *C. maxima C. moschata* crosses.

Line or Cultivar Number	Pedigree	Origin	Rind Type	Plant Type
		C. maxima		
AF89	cv. Exposicao	Brazil	soft	large indeterminate
AF139	cv. Sumare	Brazil	soft	large indeterminate
AF600L	(T4 x Delicious)S6	Brazil	soft	medium indeterminate
AF721	cv. Golden Nugget	Australia	soft	bush
AF723	cv. Jarrahdale	Australia	soft	large indeterminate
AF724	cv. Baby Blue	Australia	soft	short indeterminate
AF839L	(Corôa x Zapallo de Tronco)S6	Brazil	hard	bush
		C. moschata		
AF543L	Kurokawa S6	Japan	soft	medium indeterminate
AF822L	Aizuwase S3	Japan	soft	medium indeterminate

Table 2. Segregation pattern for rind texture in *C. maxima* × *C. maxima* and *C. maxima* × *C. moschata*.

Lines or Crosses	Number of Plants			Expected Ratio of Soft:Hard	X^2	P
	Total	Soft Rind	Hard Rind			
AF89 (*C. maxima*)	12	12	0			
AF139 (*C. maxima*)	11	11	0			
AF6001 (*C. maxima*)	12	12	0			
AF721 (*C. maxima*)	12	12	0			
AF723 (*C. maxima*)	10	10	0			
AF724 (*C. maxima*)	9	9	0			
AF89 (*C. maxima*)	12	0	12			
AF543L (*C. moschata*)	12	12	0			
AF822L (*C. moschata*)	11	11	0			
C. maxima × *C. moschata*						
AF839L × AF543L F_1	25	0	25			
AF839L × AF822L F_1	22	0	22			
C. maxima × *C. maxima*						
AF839L × AF89 F_1	21	21	0			
AF839L × AF139 F_1	18	18	0			
AF839L × AF600L F_1	23	23	0			
AF839L × AF721 F_1	25	25	0			
AF839L × AF723 F_1	19	10	0			
AF839L × AF724 F_1	23	23	0			
AF839L × AF822L F_2	353	260	83	3:1	0.12	0.50-0.75
HR-1[1] AF839L × AF724 F_3	51	0	51	0:1		
HR-2 AF839 × AF724 F_3	45	0	45	0:1		
SH-1[2] AF839L × AF724 F_3	60	60	0	1:0		
SH-2 AF839L × AF724 F_3	67	54	13	3:1	1.12	0.25-0.50
SH-3 AF839L × AF724 F_3	62	51	11	3:1	1.74	0.10-0.25
SH-4 AF839L × AF724 F_3	64	44	20	3:1	1.33	0.10-0.25

[1] HR = Hard rind
[2] SH = Soft rind

[Handwritten margin note: This seems to be an error (Should be AF839)]

Heritability of Seed Size in Hull-less Seeded Strains of *Cucurbita pepo* L.

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A breeding project to develop high seed yielding strains of hull- less (naked) seeded pumpkins has been ongoing for the past twelve years at the University of New Hampshire.

Seed size is an important trait in a hull-less seeded pumpkin, both from the standpoint of seed yields and for consumer acceptance. Because earlier investigations indicated that the highest seed yields could be obtained in small-fruited strains, we were interested in the relationship of fruit size to seed size. A recently developed hybrid out of our program, 'Snackjack', has reasonably large hull-less seed (av. 13 g/100) and relatively small fruit (0.5 to 1.0 kg). Two large-fruited, hull-less seeded plant introduction lines (PI 264241 and PI 285611) have very large seeds (23 to 26 g/100 seed). Initial attempts to transfer large seed size from PI264241 into small- fruited genotypes met with failure. In the present study crosses were made between PI 285611 and a small-fruited, hull-less seeded strain (NH29-13-5, F_6) with moderately large seed (av. 14 g/100) to study heritability of fruit and seed traits and the relationship between fruit and seed size.

PI 285611 was crossed to NH29-13-5 in the greenhouse, spring 1992. F_1 plants were grown in the field during summer 1994, and three selfed fruits were obtained to provide F_2 seed for the genetic study. During the summer of 1993, a population of 450 F_2 plants were grown in a single plot at the Woodman Research Farm in Durham, NH. In an adjacent plot 10 plants of PI 285611 and 25 of NH29-13-5 were grown; the additional plants of NH29-13-5 were needed for other breeding purposes. We attempted to obtain as many selfs as possible on F_2 plants so that most selections could be carried to the F_3 generation. For the heritability study we were able to obtain seed samples from fruits of 250 F_2 plants; environmental variances

were computed from data on 5 fruits and seeds of PI 285611 and 9 fruits and seeds of NH29-13-5. Data were taken on fruit weight and diameter. Additional seed samples were obtained from small-fruited, large-seeded selections that were not included in the heritability study because they were not randomly selected. Seeds were dried thoroughly on screens at ca. 30°C in a forced air dryer, and the following measurements were taken on 5 randomly sampled seeds per seed lot per fruit: seed weight/100, seed thickness, seed width and seed length, using a digital caliper. The data presented here represent only the first 100 fruits sampled to date.

Heritability of fruit weight was relatively low (61.7%). This was not unexpected given the fact that even within a plant there is considerable variation in fruit size, depending upon the fruit load and vigor of the plant. Heritabilities for seed weight, length, and thickness were 76.4, 78.7, and 85.2%, respectively. A lower heritability for seed width (68.1%) can probably be ascribed to the effect of different degrees of expression of the hull-less trait on seedcoat development along the seed margins.

Seed thickness and fruit weight were not correlated ($R^2 = 0.05$); both parents had fairly thick seeds. Seed weight and fruit weight ($R^2 = 0.38$) and seed length and fruit weight ($R^2 = 0.46$) were both positively correlated, but to a moderate degree. Therefore, it was possible to obtain small-fruited selections with fairly large seed. However, only one selection out of 450 F_2 plants had both small (1.2 kg) fruit and a seed weight (25.4 g/100) similar to PI 285611. All seed size traits showed transgressive segregation (Table 1); whereas, no small-fruited genotypes were recovered in the F_2 generation approaching the size of NH29-13-5, the small-fruited parent. This was unexpected because

Table 1. Fruit and seed size attributes for PI285611 and NH29-13-5 pumpkin cultigens and an F_2 population derived from them.

Seed and fruit variables[1]	Mean	Variance	Range High	Range Low
Fruit weight (kg)				
PI285611 (5 fr)	5.17	1.86	7.50	4.00
NH29-13-5 (9 fr)	0.61	0.01	0.75	0.45
F_2	3.44	2.43	8.39	1.13
Seed Weight (g/100)				
PI285611	23.62	3.73	25.94	20.18
NH29-13-5	14.27	2.57	16.74	11.56
F_2	17.88	13.37	27.76	10.38
Seed length (mm)				
PI285611	18.56	0.32	19.35	17.76
NH29-13-5	14.93	0.26	15.76	14.32
F_2	16.05	1.38	18.98	13.52
Seed width (mm)				
PI285611	10.14	0.33	10.59	9.10
NH29-13-5	8.96	0.15	9.59	8.28
F_2	9.14	0.77	12.84	7.57
Seed thickness (mm)				
PI285611	2.32	0.02	2.45	2.06
NH29-13-5	2.28	0.01	2.38	2.14
F_2	2.44	0.09	3.03	1.81

[1]Values for seed parameters represent average measurements of 5 seeds randomly sampled from seed lots obtained from individual fruits.

fruits and seeds of F_1 plants grown in 1992 were about as large as PI 285611. This indicates the existence of heterotic effects for fruit and seed size in the above cross. It would be interesting to know if heterosis for seed size would be displayed in crosses of two small-fruited strains, one with large and the other with small seeds.

The prospects for selecting small-fruited strains of pumpkin with very large seeds appear good. Heritability of seed size appears to be high and is possibly underestimated in this study because of the small sample size of parents and likely greater variance displayed for the environmental component. It should be noted, however, that we attempted to eliminate fruit likely to have poor seed fill by not including fruit from plants that looked diseased or from fruit not deemed to be fully developed. A major question to be answered is whether small fruit with large seed will show lower total seed yields because of greater early seed abortion. Larger seeds are larger nutrient sinks; therefore, carbohydrates and other resources translocated to a small developing fruit may be limiting for allocation to the large number of fertilized ovules (300 to 600) within a fruit.

One final phenomenon worth mentioning is that seed size is almost totally determined by maternal factors, and most probably by genetically determined differences in size of the seed coat. Histochemical and biochemical studies of normal and hull-less seed coats during embryogenesis indicate that the seed coat is a transient storage organ for reserve materials that appear to be later utilized by the developing embryo (1, 2). In addition, size of the seed coat may mechanically limit expansion of the embryo within it. Whatever the mechanism, seed size differences are not evident in F_2 seed taken from F_1 plants, but rather the differences occur in F_3 seed harvested from F_2 plants.

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Response Reaction of *C. moschata* Breeding Lines to Mechanical Inoculations of CMV, PRSV-W, and WMV-II.

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In a previous report (1) we presented the pedigree of some *C. moschata* breeding lines which showed field resistance to PRSV-W. As these lines were selected under natural field infection conditions, using plants of *C. pepo* cv. Caserta as control and spread rows, the mechanism of this field resistance is unknown. It was of interest to know the response reaction of these lines under mechanical inoculations to CMV. PRSV-W and WMV-II, which are the main viruses reported to occur on Cucurbitacease in Brazil.

Materials and Methods. Virus identification was previously made based on differential host reactions and serology. The inoculum of each virus was made by grinding leaves of *C. pepo* cv. Caserta showing strong symptoms of the respective virus in 0.02 M phosphate buffer (pH 7.0) containing 0.01 M sodium sulfite (1:10, w/v) and rubbing extracts on fully expanded cotyledons of 4-6 day old plants, previously dusted with 600-mesh carborundum. Twenty plants of each breeding line were inoculated for each virus.

Results and Discussion. The response reaction of the inbred lines to each virus is presented in Table 1. All lines showed susceptible response reactions to mechanical inoculation of PRSV-W and WMV-II. Inbred lines AF1075L and AF1094L, however, showed resistance to mechanical inoculation of CMV. Plants of both lines presented some mosaic symptoms on the first true leaves that completely disappeared as the plants developed. Back inoculations, using leaves with no CMV symptoms taken individually from all these plants, were made on *C. pepo* cv. Caserta for further investigation. No CMV symptoms were observed on the *C. pepo* plants, suggesting that the CMV virus is not multiplying in plants of the *C. moschata* inbred lines AF1075L and AF1094L.

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Table 1.Response reaction of *C. moschata* breeding lines to mechanical inoculations of CMV, PRSV-W and WMV-II.

| | Response Reaction[1] | | | | | |
| | CMV | | PRSV-W | | WMV-II | |
Inbred Lines	R	S	R	S	R	S
Piramoita	0	20	0	20	0	20
AF1075L	20	0	0	20	0	20
AF1094L	15	0	1	19	0	20

[1]R = Resistant, S = Susceptible

Genetic Analysis of Bitter Principals in *Momordica charantia* Linn.

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Bitterness of bitter gourd (*Momordica charantia* Linn.) is due to presence of Momordicosides, which are glycosides of tetracyclic triterpinoids with a cucurbitane skeleton (1). The cucurbitane triterpenes have received attention because of their anticancer and other biological activities. Bitter form was reported to be dominant over mild bitter fruits (4), but detailed genetic studies on triterpinoid content of fruits are lacking.

The present study was conducted in the Tamil Nadu Agricultural University, Coimbatore, during 1991-92. Twelve morphologically and geographically diverse bitter gourd parents were analyzed for total triterpinoid content and the two highest (P7 and P12) and two lowest (P3 and P5) bitter parents were selected. The selected parents were MC 41 (P7), 'White Long' (P12), MC 13-Coimbatore Local (P3) and 'Arka Harit' (P5). Bitter principals of their hybrids and the parents recorded were analyzed in a 4 x 4 full diallel fashion using graphical and numerical methods (2,3). Mean performance of the parents and hybrids for triterpinoid content are furnished in Table 1. Validity of the basic assumptions for a diallel experiment was found true in this experiment because t^2 (1.16), deviation of b from zero (2.53) ,and that from unity (-0.51) were not statistically significant. The regression value, b, was 1.25 ± 0.50.

The Vr-Wr graph for total triterpinoid content (Fig. 1) indicated overdominance gene action because the regression line intercepted the Y axis below the point of origin. Array points of three parents were located below the unit slope indicating predominance of additive gene action. Less bitter

parents, P3 and P5, had maximum dominant alleles due to their position near the origin. The scattered position of the parental array points indicated the wide genetical diversity.

Numerical analysis indicated that variances due to D (additiveness) and H1 (dominance) components were statistically significant, their values being 4.06 ± 1.04 (at 1 % level) and 7.99 ± 3.09 (at 5% level). The magnitude of dominance was higher than additiveness. Variances due to F (1.81 ± 2.67), H2 (-1.99 ± 2.78), h^2 (-0.15 ± 1.89) and E (0.22 ± 0.46) were not significant. Ratios of genetic parameters indicated overdominance ($\sqrt{H1/D} = 1.40$), unequal proportion of dominant genes over the recessive genes (KD/KR = 1.38), influence of one block of genes on the character ($h^2/H2 = 0.08$) and a high heritability (h^2 n.s. = 0.97).

Graphic and numerical analyses indicated presence of overdominance, dominance and additive gene action. Hence a breeding program combining selection and hybridization (i.e., reciprocal recurrent selection) would be appropriate for the triterpinoid content of fruits.
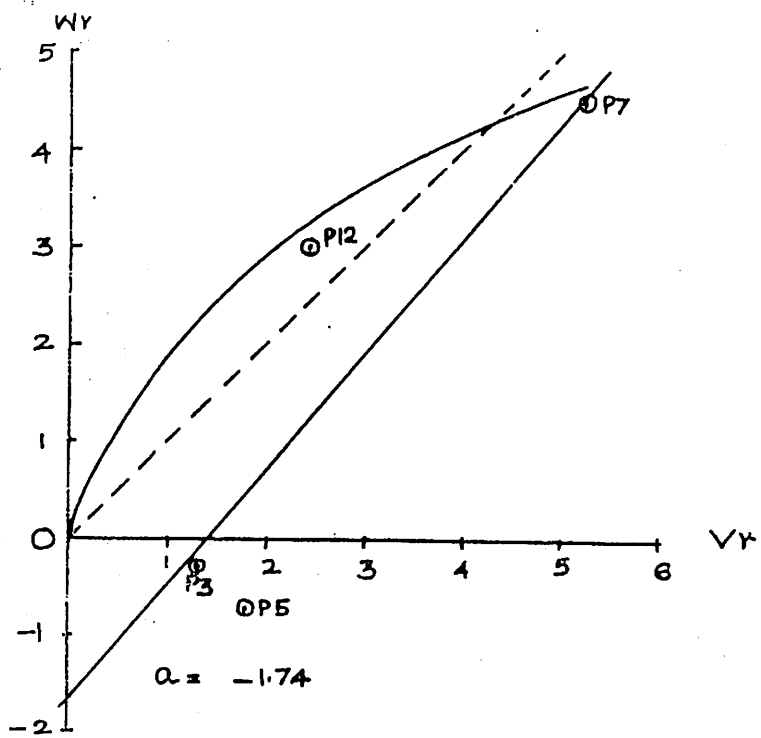
Figure 1. Vr-Wr graph for total triterpinoid content of fruits.

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Table 1. Mean performance of the parents and hybrids for triterpinoid content of fruits (mg/g of triterpinoids on dry weight basis.

Female/Male	P3	P5	P7	P12
P3	0.9050	0.999	2.147	1.133
P5	6.333	1.262	0.929	2.314
P7	1.457	1.186	4.905	9.381
P12	3.038	4.195	2.081	4.376

SE = 0.461; CD (P=0.05) = 1.332

Heterosis and Combining Ability for Bitter Principals in *Momordica charantia* Linn.

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All parts of the bitter gourd plant are bitter in taste except the ripe aril covering the seed. The bitter principals are designated as Momordicosides, which are glycosides of triterpinoids with a cucurbitane skeleton (3). Cucurbitane triterpenes have received attention because of their cytotoxic, anticancer and other biological activities. Information on heterosis and combining ability for the specific bitter principals in bitter gourd are lacking.

Triterpinoid content of fruits of twelve morphologically and geographically diverse bitter gourd parents were analyzed (2) as a part of a 12 x 12 diallel experiment. Fruits of 12 hybrid combinations involving four parents (P3 and P5 with the lowest content and P7 and P12 with the highest content) were also analyzed for the triterpinoid content, and heterosis and combining ability were estimated. Mean triterpinoid content of the 12 parents and the 12 hybrids, and heterosis (di = relative heterosis based on mid parent value; dii = heterosis based on the higher parent value; and diii = standard heterosis based on the value of standard parent (i.e., P8)) observed are furnished in Table 1.

Triterpinoid content of the parents differed significantly. The highest amounts were observed in P7 and P12, and the lowest in P3 and P5. The positive heterosis (di, dii and diii) was expressed by P5 x P3 and P7 x P12; and the lowest (negative) heterosis was seen in P5 x P7 and its reciprocal combination. In general, high x high and low x low parental combinations exhibited maximum positive heterosis and the low x high or high x low combinations exhibited maximum negative heterosis.

General and specific combining ability effects (GCA, SCA) were statistically significant and are presented in Table 2. A high proportion of GCA:SCA (1.413:1.00) was observed indicating preponderance of additive gene action. P7 and P12 had significant positive GCA effects, and the maximum triterpinoid content (per se values as well as heterosis) of P7 x P12 indicated additive x additive epistatic interaction. Additive, dominance, and additive x dominance gene actions in controlling the total crude bitter contents of bitter gourd fruits have been reported earlier (1). Since both additive and non-additive gene actions are observed, the appropriate breeding method would be reciprocal recurrent selection.

Table 1. Mean triterpinoid content (mg/g on dry weight basis) of fruits and heterosis.

For 12 Parents			For 12 Hybrids		Heterosis		
Parent S1.No.	Name	Mean triterpinoids (mg/g)	Combination	Mean triterpinoids (mg.g)	di	dii	diii
P1	'Pusa Do Mausami'	2.333	P3 x P5	0.999	-7.74	-20.79	-56.72
P2	'MDu 1'	3.438	P3 x P7	2.147	-26.08	-56.22**	-7.04
P3	MC 13	0.905	P3 x P12	1.133	-57.08**	-74.10**	-50.94
P4	'Priya'	2.500	P5 x P3	6.333	484.56**	401.88**	174.18**
P5	'Arka Harit'	1.262	P5 x P7	0.929	-69.88**	-81.07**	-59.80**
P6	MC 36	2.024	P5 x P12	2.314	-17.90	-47.11**	0.19
P7	MC 41	4.905	P7 x P3	1.457	-49.83	-70.29**	-36.91
P8	'Co 1'	2.095	P7 x P5	1.186	-61.55**	-75.83**	-48.67
P9	MC 78	3.367	P7 x P12	9.381	102.16**	91.26**	306.12**
P10	'Coimbatore Long Green'	1.595	P12 x P3	3.038	15.05	-30.58*	31.52
P11	KAU Cluster MC 84	2.524	P12 x P5	4.195	48.82**	-4.13	81.62**
P12	White Long'	4.376	P12 x P7	4.376	-55.16**	-57.57**	-9.91
	SE (±)	0.239	SE (±)	0.461	* Significant at 5% level		
	C.D. (P=0.05)	0.701	C.D. (P=).05)	1.332	** Significant at 1% level		

Table 2. General and specific combining ability effects for triterpinoid content of fruits.

Female/Male	P3	P5	P7	P12
P3	-0.800**	2.158**	-0.771**	0.976**
P5	-2.667**	-0.605**	-1.712**	-2.021**
P7	-0.345	-0.129	0.459**	1.411**
P12	-0.952**	-0.941	3.650**	0.947**

	SE (±)	CD (P=0.05)	
GCA	0.141	0.41	
SCA	0.258	0.73	
reciprocal	0.326	0.94	

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Gene list for *Cucumis melo* L.

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Lists of the known genes for the melon have been published previously (15, 16, 17, 62, 74). In order to update and collect information on the melon, the following represents the current list of described genes for *Cucumis melo* L. In **bold characters** are the genes which are maintained by the curators or which are very common in collections (like *andromonoecious* or *white testa*). In light characters are genes which either have been apparently lost, are not yet maintained by curators, or have uncertain descriptions.

It is hoped that scientists will consult the following list as well as the rules of gene nomenclature for the Cucurbitaceae (see appendix) before choosing a gene name and symbol. Thus inadvertent duplication will be prevented.

New symbols have been attributed to genes due to previous use:

The symbol *dl* for *delayed lethal* (87) has been used for *dissected leaf* and we propose to use *dlet* for the new mutant.

The symbol *M* for *Maleness* (43) was first used for *monoecious* before *andromonoecious* (symbol *a*) was prefered to describe this character. As both characters (*Maleness* and *monoecious*) are refering to sex expression it seems better to have another name and symbol. In reference 41 the symbol *n* was used and in the previous list the name *gynoecious* (symbol *gy*) was preferred.

The character *Subtended floral leaf* has been described (30) with symbol *S*. As this symbol has been already used for presence of *sutures* on the fruit we propose that the symbol *Sfl* will be used for *Subtended floral leaf*.

The situation concerning powdery mildew resistance is highly confusing! Six genes were already in the previous list (from *Pm-1* to *Pm-6*) and new have been published. All the allelism tests have not been done. Powdery mildew can be provoked by two fungi *Sphaerotheca fuliginea* and *Erysiphe cichoracearum* and some genes are specific for *S. fuliginea* resistance (*Pm-1* for instance), others are specific for *E. cichoracearum* (*e.g. Pm-H* in 'Nantais oblong') while probably other genes have an effect on both fungi. A working group named 'Club Mildew' (54) intends to clarify the interaction between melon and powdery mildew strains. We propose to wait the results of this group to have a better view on the resistance genes to powdery mildew and to propose new names and symbols (may be *Sf-x* for resistance to *S. fuliginea*, *Ec-x* for resistance to *E. cichoracearum*, and *Pm-x* for resistance to both fungi?).

Gene symbol			Linkage	
Prefer.	Synonym	Character	group	Refer
a	*M*	*andromonoecious*. **Mostly staminate, fewer perfect flowers; on a^+_ plants, pistillate flowers have no stamens; epistatic to *g*.**	4	69, 75, 82
ab	-	*abrachiate*. Lacking lateral branches. Interacts with *a* and *g* (e.g. *abab aa* g^+_ plants produce only staminate flowers).		27
Ac	-	*Alternaria cucumerina* **resistance (in MR-1).**		79
Af	-	*Aulacophora foveicollis* resistance. Resistance to the red pumpkin beetle.		80

Gene symbol			Linkage	
Prefer.	Synonym	Character	group	Refer
Ag	-	*Aphis gossypii* **tolerance. Freedom of leaf curling following aphid infestation (in PI 414723).**		**4**
Ala	-	*Acute leaf apex.* Dominant over obtuse apex, linked with *Lobed* leaf. (*Ala* in Maine Rock, *Ala+* in PV Green).		31
Al-1	*Al$_1$*	*Abscission layer-1.* One of two dominant genes for abscission layer formation. See *Al-2.* (*Al-1 Al-2* in C68, *Al-1+ Al-2+* in Pearl).		77
Al-2	*Al$_2$*	*Abscission layer-2.* One of two dominant genes for abscission layer formation. See *Al-1.*		77
Ap-1^1	*APS-1^1*	*Acid phosphatase-1^1.* One of two codominant alleles, each regulating one band. The heterozygote has two bands. See *Ap-1^2.*		25
Ap-1^2	*APS-1^2*	*Acid phosphatase-1^2.* One of two codominant alleles, each regulating one band. The heterozygote has two bands. See *Ap-1^1.*		25
bd	-	*brittle dwarf.* Rosette growth with thick leaf. Male fertile, female sterile (in TAM-Perlita45).		12
Bi	-	*Bitter.* **Bitter seedling (common in honeydew or in Charentais type while most American cantaloupes are *Bi+*).**		**48**
Bif	-	*Bitter fruit.* Bitterness of tender fruit in wild melon. Relations with *Bi* are unknown.		61
cb	*cb$_l$*	*cucumber beetle* resistance. Interacts with *Bi*, the nonbitter *Bi+_ cbcb* being the more resistant (in C922-174-B).		58
cl	-	*curled leaf.* Elongated leaves that curl upward and inward. Usually male and female sterile.		12
dc-1	-	*Dacus cucurbitae-1* resistance. One of two complementary recessive genes for resistance to the melon fruitfly. See *dc-2.*		76
dc-2	-	*Dacus cucurbitae-2* resistance. One of two complementary recessive genes for resistance to the melon fruitfly. See *dc-1.*		76
dl	-	*dissected leaf* (in URSS 4). **Highly indented leaves.**	10	20
dl^v	*cl*	*dissected leaf Velich.* **First described as *cut leaf* in Cantaloup de Bellegarde. Allelic to *dl*.**		81
dlet	*dl*	*delayed lethal.* Reduced growth, necrotic lesions on leaves and premature death.		87
dl-2	-	*dissected leaf-2.* First described as "hojas hendidas".		24
f	-	*flava.* **Chlorophyl deficient mutant. Growth rate reduced (in K 2005).**	8	64

Gene symbol		Character	Linkage group	Refer
Prefer.	Synonym			
fas	-	*fasciated* stem (in Vilmorin 104).		28
fe	-	*fe* (iron) inefficient mutant. Chlorotic leaves with green veins. Turns green when adding Fe in the nutrient solution.		57
Fn	-	*Flaccida necrosis*. Semi-dominant gene for wilting and necrosis with F pathotype of Zucchini Yellow Mosaic Virus (*Fn* in Doublon, *Fn*+ in Védrantais).	2	73
Fom-1	*Fom₁*	*Fusarium oxysporum melonis* resistance. Resistance to races 0 and 2 and susceptibility to races 1 and 1,2 of Fusarium wilt (*Fom-1* in Doublon, *Fom-1*+ in Charentais T).	5	72
Fom-2	*Fom₁.₂*	*Fusarium oxysporum melonis* resistance. Resistance to races 0 and 1 and susceptibility to races 2 and 1,2 of Fusarium wilt. (*Fom-2* in CM 17187, *Fom-2*+ in Charentais T).	6	72
Fom-3	-	*Fusarium oxysporum melonis* resistance. Same phenotype as *Fom-1* but segregates independently from *Fom-1*. (*Fom-3* in Perlita FR, *Fom-3*+ in Charentais T).		88
g	-	*gynomonoecious*. Mostly pistillate, fewer perfect flowers. Epistatic to *a*: $a^+\_ g^+\_$ monoecious; $a^+\_ gg$ gynomonoecious; $aa\ g^+\_$ andromonoecious; $aa\ gg$ hermaphrodite.		69
gf	-	*green flesh* color. Recessive to salmon. (*gf* in honeydew, *gf*+ in Smiths' Perfect cantaloupe).		36
gl	-	*glabrous*. Trichomes lacking (in Arizona glA).	3	26
gp	-	*green petals*. Corolla leaf like in color and venation.		55
Gs	-	*Gelatinous sheath* around the seeds. Dominant to absence of gelatinous sheath.		29
gyc	-	*greenish yellow corolla*.		86
gy	*n, M*	gynoecious. Interacts with *a* and *g* to produce stable gynoecious plants ($a^+\_ gg\ gygy$) (in WI 998).		41, 43
h	-	*halo* cotyledons. Yellow halo on the cotyledons, later turning green.	4	59
jf	-	*juicy flesh*. Segregates discretely in a monogenic ratio in segregating generations.		8
L	-	*Lobed* leaf. Dominant on non lobed, linked with *Acute leaf apex*. (*L* in Maine Rock, *L*+ in P.V. Green).		31
lmi	-	*long mainstem internode*. Affects internode length of the main stem but not of the lateral ones (in 48764).	8	51
Mc	-	*Mycosphaerella citrullina* resistance. High degree of resistance to gummy stem blight (in PI 140471).		70

Gene symbol				
Prefer.	Synonym	Character	Linkage group	Refer
Mc-2	*Mcⁱ*	*Mycosphaerella citrullina* resistance. Moderate degree of resistance to gummy stem blight (in C-1 and C-8)		70
Mca	-	*Macrocalyx.* Large, leaf like structure of the sepals in staminate and hermaphrodite flowers (*Mca* in makuwa, *Mca⁺* in Annamalai).		30
Me	-	*Mealy* flesh texture. Dominant to crisp flesh. (*Me* in *C. callosus*, *Me⁺* in makuwa).		29
ms-1	*ms¹*	*male sterile-1.* Indehiscent anthers with empty pollen walls in tetrad stage.	3	6
ms-2	*ms²*	*male sterile-2.* Anthers indehiscent, containing mostly empty pollen walls, growth rate reduced.	6	5
ms-3	*ms-L*	*male sterile-3.* Waxy and translucent indehiscent anthers, containing two types of empty pollen sacs.	12	53
ms-4	-	*male sterile-4.* Small indehiscent anthers. First male flowers abort at bud stage (in Bulgaria 7).	9	49
ms-5	-	*male sterile-5.* Small indehiscent anthers. Empty pollen (in Jivaro, Fox).	13	47
Mt	-	*Mottled* rind pattern. Dominant to uniform color. Epistatic with *Y* (not expressed in *Y⁺y⁺*) and *st* (*Mt_ stst* and *Mt_ st⁺st⁺* mottled; *Mt⁺Mt⁺ stst* striped, *Mt⁺Mt⁺ st⁺st⁺* uniform). (*Mt* in Annamalai, *Mt⁺* in makuwa).		29
Mu	-	*Musky* flavour (olfactory). Dominant on mild flavour (*Mu* in *C. melo callosus*, *Mu⁺* in makuwa or Annamalai).		29
n	-	*nectarless.* Nectaries lacking in all flowers (in 40099).		2
Nm	-	*Necrosis* with *Morocco* strains of Watermelon Mosaic Virus (*Nm* in Védrantais, *Nm⁺* in Ouzbèque).		71
nsv	-	Melon *necrotic spot virus* resistance (in Gulfstream, Planters Jumbo).	7	14
O	-	*Oval* fruit shape. Dominant to round; associated with *a*.	4	82
p	-	*pentamerous.* Five carpels and stamens; recessive to trimerous (in Casaba).		75
Pa	-	*Pale* green foliage. *PaPa* plants are white (lethal); *PaPa⁺* are yellow (in 30567).	3	52
Pc-1	-	*Pseudoperonospora cubensis* resistance. One of two complementary incompletely dominant genes for downy mildew resistance (in PI 124111). See *Pc-2*.		11, 78

Gene symbol				Linkage	
Prefer.	Synonym	Character		group	Refer
Pc-2	-	*Pseudoperonospora cubensis* resistance. One of two complementary incompletely dominant genes for downy mildew resistance (in PI 124111). See *Pc-1*.			**11, 78**
Pc-3	-	*Pseudoperonospora cubensis* resistance. Partial resistance to downy mildew (in PI 414723).			**22**
Pc-4		*Pseudoperonospora cubensis* resistance. One of two complementary genes for downy mildew resistance in PI 124112. Interacts with *Pc-1* or *Pc-2*.			**44**
Pgd-1¹	*6-PGDH-2¹* *Pgd-2¹*	*Phosphoglucodehydrogenase-1¹*. One of two codominant alleles that regulates 6-phospho-glucodehydrogenase, each regulates one band.The heterozygote has one intermediate band. See *Pgd-1²*.			25
Pgd-1²	*6-PGDH-2²* *Pgd-2²*	*Phosphoglucodehydrogenase-1²*. One of two codominant alleles that regulates 6-phospho-glucodehydrogenase, each regulates one band. The heterozygote has one intermediate band. See *Pgd-1¹*.			25
Pgi-1¹	*PGI-1¹*	*Phosphoglucoisomerase-1¹*. One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See *Pgi-1²*.			25
Pgi-1²	*PGI-i²*	*Phosphoglucoisomerase-1²*. One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See *Pgi-1¹*.			25
Pgi-2¹	*PGI-2¹*	*Phosphoglucoisomerase-2¹*. One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See *Pgi-2²*.			25
Pgi-2²	*PGI-2²*	*Phosphoglucoisomerase-2²*. One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See *Pgi-2¹*.			25
Pgm-1¹	*PGM-2¹* *Pgm-2¹*	*Phosphoglucomutase-1¹*. One of two codominant alleles, each regulating two bands. The heterozygotes has three bands. See *Pgm-1²*.			25
Pgm-1²	*PGM-2²* *Pgm-2²*	*Phosphoglucomutase-1²*. One of two codominant alleles, each regulating two bands. The heterozygotes has three bands. See *Pgm-1¹*.			25
Pm-1	*Pm¹* *Pm-A?*	*Powdery mildew* resistance-1. Resistance to race 1 of *Sphaerotheca fuliginea* (in PMR 45).			**38, 39**
Pm-2	*Pm²* *Pm-C?*	*Powdery mildew* resistance-2. Interacts with *Pm-1*. **Resistance to race 2 of** *Sphaerotheca fuliginea* **(in PMR 5 with** *Pm-1*).			**7**
Pm-3	*Pm³*	*Powdery mildew* resistance-3. Resistance to race 1 of *Sphaerotheca fuliginea* (in PI 124111).		7	**33,34**

Gene symbol				
Prefer.	Synonym	Character	Linkage group	Refer
Pm-4	*Pm^4*	*Powdery mildew* resistance-4. Resistance to *Sphaerotheca fuliginea* (in PI 124112).		33, 34
Pm-5	*Pm^5*	*Powdery mildew* resistance-5. Resistance to *Sphaerotheca fuliginea* (in PI 124112).		33, 34
Pm-6	-	*Powdery mildew* resistance-6. Resistance to *Sphaerotheca fuliginea* race 2 (in PI 124111).		42
Pm-E	-	*Powdery mildew* resistance-E. Interacts with *Pm-C* in PMR5 for *Erysiphe cichoracearum* resistance.		23
Pm-F	-	*Powdery mildew* resistance-F. Interacts with *Pm-G* in PI 124112 for *Erysiphe cichoracearum* resistance.		23
Pm-G	-	*Powdery mildew* resistance-G. Interacts with *Pm-F* in PI 124112 for *Erysiphe cichoracearum* resistance.		23
Pm-H	-	*Powdery mildew* resistance-H. Resistance to *Erysiphe cichoracearum* and susceptibility to *Sphaerotheca fuliginea* (in 'Nantais oblong').		23
Pm-w	*Pm-B?*	*Powdery mildew* resistance in WMR 29. Resistance to *Sphaerotheca fuliginea* race 2.	2	63
Pm-x	-	*Powdery mildew* resistance in PI 414723. Resistance to *Sphaerotheca fuliginea*.	4	63
Prv^1	*Wmv*	*Papaya Ringspot Virus* resistance. Resistance to W strain of Papaya ringspot Virus (formerly Watermelon Mosaic Virus 1) (in B 66-5, WMR 29, derived from PI 180280). Dominant to *Prv^2*.	5	66, 83
Prv^2	-	*Papaya Ringspot Virus* resistance. Allele at the same locus as *Prv^1* but different reaction with some strains of the virus (in 72-025 derived from PI 180283). Recessive to *Prv^1*, dominant to *Prv$^+$*.	5	40, 66
Px-1^1	*PRX-1^1*	*Peroxidase-1^1*. One of two codominant alleles, each regulating a cluster of four adjacent peroxidase bands. The heterozygote has five bands. See *Px-1^2*.		25
Px-1^2	*PRX-1^2*	*Peroxidase-1^2*. One of two codominant alleles, each regulating a cluster of four adjacent peroxidase bands. The heterozygote has five bands. See *Px-1^1*.		25
Px-2^1	*Px$_{2A}$* *Prx2*	*Peroxidase-2^1*. One of two codominant alleles, each regulating a cluster of three adjacent peroxidase bands. The heterozygote *Px-2^1 Px-2^2* has 4 bands. See *Px-2^2*.		9, 18
Px-2^2	*Px$_{2B}$* *Prx2*	*Peroxidase-2^2*. One of two codominant alleles, each regulating a cluster of three adjacent peroxidase bands. See *Px-2^1*.		9, 18

Gene symbol				Linkage	
Prefer.	Synonym	Character		group	Refer
r	-	*red* stem. Red pigment under epidermis of stems, especially at nodes; tan seed color (in PI 157083).		3	3, 52
ri	-	ridge. Ridged fruit surface, recessive to ridgeless. (*ri*+ in Pearl, *ri* in C68).			77
s	-	*sutures*. Presence of vein tracts ("sutures"); recessive to ribless.			1
Sfl	*S*	*Subtended floral leaf*. The floral leaf bearing the hermaphrodite flowers is sessile, small and enclose the flower. (*Sfl* in makuwa, *Sfl* in Annamalai).			30
si-1	*b*	*short internode-1*. Extremely compact plant habit (bush type) (in UC Topmark bush).		1	19
si-2	-	*short internode-2*. Short internodes from 'birdnest' melon (in Persia 202).			60
si-3	-	*short internode-3*. Short internodes in 'Maindwarf' melon.			45
Skdh-1^1	-	*Shikimate dehydrogenase-1*. One of two codominant alleles, each regulating one band (see *Skdh-1^2*).			9
Skdh-1^2	-	*Shikimate dehydrogenase-1*. One of two codominant alleles, each regulating one band (see *Skdh-1^1*).			9
So	-	*Sour* taste. Dominant to sweet.			46
sp	-	*spherical* fruit shape. Recessive to obtuse; dominance incomplete.			1, 50
st	-	*striped* epicarp. Recessive to non-striped.			32
v	-	*virescent*. Pale cream cotyledons and hypocotyls; yellow green foliage (mainly young leaves).		11	35
v-2	-	*virescent-2*.			21
Vat	-	*Virus aphid transmission* resistance. Resistance to the transmission of viruses by *Aphis gossypii* (in PI 161375).		2	65
w	-	*white* color of mature fruit. Recessive to dark green fruit skin. (*w* in honeydew, *w*+ in Smiths' Perfect cantaloupe).			36
wf	-	*white flesh*. Recessive to salmon. *wf*+ epistatic to *gf*+*gf*.			37, 10
Wi	-	White color of *immature* fruit. Dominant to green.			46
Wt	-	*White testa*. Dominant to yellow or tan seed coat color.			32
Y	-	*Yellow* epicarp. Dominant to white fruit skin.			32

| Gene symbol | | | Linkage | |
Prefer.	Synonym	Character	group	Refer
yg	*y*	*yellow green* leaves. Reduced chlorophyll content.	6	84
yg^W	*lg*	*yellow green Weslaco*. First described as *light green* in in a cross Dulce x TAM-Uvalde. Allelic to *yg*.		13
yv	-	*yellow virescence*. Pale cotyledons; yellow green young leaves and tendrils; bright and yellow petals and yellow stigma; etiolated; older leaves becoming green.	1	85
yv-2	-	*yellow virescence-2*. Young leaves yellow green, old leaves normal green		68
Zym	-	*Zucchini Yellow Mosaic* Virus resistance. Resistance to pathotype 0 of this virus (in PI 414723).	4	67

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Muskmelon :	M. Pitrat
Watermelon :	B.B. Rhodes
Cucurbita spp. :	R. W. Robinson
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Other genera :	R. W. Robinson

Linkage groups in *Cucumis melo* L.

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Thirteen independent linkage groups have been described until now in muskmelon but no relation has been identified between a linkage group and a chromosome.

Group 1: **si-1** (short internode-1) - **yv** (yellow virescence).

Group 2: **Vat** (Virus aphid transmission resistance) - **Pm-w** (a gene for powdery mildew resistance in WMR 29, allelism tests have not been made with the 6 described powdery mildew resistance genes) - **Fn** (Flaccida necrosis).

Group 3: **ms-1** (male sterile-1) - **r** (red stem) - **gl** (glabrous) - **Pa** (Pale).

Group 4: **a** (andromonoecious) - **O** (Oval fruit shape) - **h** (halo cotyledons) - **Pm-x** (Powdery mildew resistance in PI 414723, allelism tests have not been made with the 6 described powdery mildew genes) - **Zym** (Zucchini yellow mosaic virus resistance). The order in this group is unknown.

Group 5: **Prv** (Papaya Ringspot virus resistance) - **Fom-1** (*Fusarium oxysporum* f. sp. *melonis* resistance 1)

Group 6: **ms-2** (male sterile-2) - **yg** (yellow green foliage) - **Fom-2** (*Fusarium oxysporum* f. sp. *melonis* resistance 2)

Group 7: **nsv** (necrotic spot virus resistance) - **Pm-3** (Powdery mildew resistance -3)

Group 8: **f** (flava) - **lmi** (long mainstem internode)

Group 9: **ms-4** (male sterile-4)

Group 10: **dl** (dissected leaf)

Group 11: **v** (virescent)

Group 12: **ms-3** (male sterile-3)

Group 13: **ms-5** (male sterile-5)

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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.

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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Provvidenti, Rosario Cornell Univ., Dept. Plant Pathology, NY State Agric. Experiment Sta., Geneva NY, 14456-0462. Ph.: (315) 787-2316, FAX: (315) 787- 2389. Breeding & genetics of resistance to viral diseases of cucumber, squash, melon, watermelon & other cucurbits.

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Ray, Dennis Department of Plant Sciences, University of Arizona, Tucson AZ, 85721. Ph.: (602) 621-7612, FAX: (602) 621-7186, E-mail: *dtray@ccit.arizona.edu*. Genetics and cytogenetics of *Cucumis melo* and *Citrullus* spp.

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Scheirer, Douglas M. The Nestle Food Co./Baking/Libby, 216 N. Morton Ave., P.O. Box 198, Morton IL, 61550. Ph.: (309) 263-2133. Breeding, cultural practices, etc., associated with processing pumpkin (*Cucurbita moschata*) Dickinson.

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Shifriss, Oved 21 Walter Avenue,, Highland Park NJ, 08904. Ph.: (908) 246- 0028. Regulation of the $\underline{B}$ genes in *Cucurbita*.

Shiga, Toshio Plant Biotech. Ctr., Sakata Seed Corp., 358 Uchikoshi, Sodegaura, Chiba, 299-02, Japan. Ph.: 0438-75-2369, FAX: 0438-75-2594. Cell biology.

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Staub, Jack E. USDA-ARS, Dept. Horticulture, Univ. Wisconsin, Madison WI, 53706-1590. Ph.: (608) 262-0028, FAX: (608) 262-4743. Cucumber breeding & genetics, physiology, biochemical genetic markers, evolution, environmental stress.

Stephenson, Andrew G. 208 Mueller Lab, Penn State University, University Park PA, 16802. Ph.: (814)863-1553, FAX (814)865-9131, E-mail: *as4@psuvm.psu.edu*.

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Whiteaker, Gary Nunhems Seed Corporation, 221 East Main Street, Lewisville ID, 83431. Ph.: (208) 754-8666, FAX: (208) 754-8669.

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Zitter, Thomas Andrew Cornell Univ., Dept. Plant Pathology, 334 Plant Science Building, Ithaca NY, 14853-5908. Ph.: (607) 255-7857, FAX: (697) 255-4471, E- mail: *taz1@cornell.edu*. Fungal and viral diseases; disease resistance.

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Lusike Wasilwa

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Wei Ouyang
Ken Owens
Lawrence Pierce
Vickie Pierce
Robert Harold Schroeder
Joseph Stern
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Anna Trulson
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Terrence Walters

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Mike Meadows
Baldwin Miranda
Tom V. Williams
Richard P. Wunderlin

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David Groff
Greg Tolla

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Gary Whiteaker
Paul Yorty

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Robert M. Skirvin

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Laura C. Merrick

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Charles A. McClurg
Timothy J Ng

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Dale E. Marshall
Hector Quemada

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Edward M. Croom

Nebraska
Dermot P. Coyne

New Hampshire
J. Brent Loy

New Jersey
Oved Shifriss

New York
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Mark Hutton
Molly Kyle
H.M. Munger
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R.W. Robinson
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W.R. Henderson
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E. Van Wann

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John P. Navazio
Philipp W. Simon
Jack E. Staub
Gary Taurick

International CGC Members

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 Anthony E. Rumsey
 David A. Shann
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 Johanna Winkler
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 Roni Levy
 Wilson Roberto Maluf
 Hiroshi Nagai
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 S. van Deursen
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Covenant and By-Laws of the Cucurbit Genetics Cooperative

ARTICLE I. Organization and Purposes

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

ARTICLE II. Membership and Dues

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

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

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

ARTICLE III. Committees

1. The Coordinating Committee shall govern policies and activities of the CGC. It shall consist of six members elected in order to represent areas of interest and importance in the field. The Coordinating Committee shall select its Chairman, who shall serve as a spokesman of the CGC, as well as its Secretary and Treasurer.

2. The Gene List Committee, consisting of at least five members, shall be responsible for formulating rules regulating the naming and symbolizing of genes, chromosomal alterations, or other hereditary modifications of the cucurbits. It shall record all newly reported mutations and periodically report lists of them in the Report of the CGC. It shall keep a record of all information pertaining to cucurbit linkages and periodically issue revised linkage maps in the Report of the CGC. Each committee member shall be responsible for genes and linkages of one of the following groups: cucumber, *Cucurbita* spp., muskmelon, watermelon, and other genera and species.

3. Other committees may be selected by the Coordinating Committee as the need for fulfilling other functions arises.

ARTICLE IV. Election and Appointment of Committees

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

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

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

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

ARTICLE V. Publications

1. One of the primary functions of the CGC shall be to issue an Annual Report each year. The Annual Report shall contain sections in which research results and information concerning the exchange of stocks can be published. It shall also contain the annual financial statement. Revised membership lists and other useful information shall be issued periodically. The Editor shall be appointed by the Coordinating Committee and shall retain office for as many years as the Coordinating Committee deems appropriate.

2. Payment of biennial dues shall entitle each member to a copy of the Annual Report, newsletters, and any other duplicated information intended for distribution to the membership. The aforementioned publications shall not be sent to members who are in arrears in the payment of dues. Back numbers of the Annual Report, available for at least the most recent five years, shall be sold to active members at a rate determined by the Coordinating Committee.

ARTICLE VI. Meetings

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

ARTICLE VII. Fiscal Year

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

ARTICLE VIII. Amendments

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

ARTICLE IX. General Prohibitions

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

1. The CGC shall be organized and operated exclusively for scientific and educational purposes.

2. No part of the net earnings of the CGC shall or may under any circumstances inure to the benefit of any individual.

3. No part of the activities of the CGC shall consist of carrying on propaganda or otherwise attempting to influence legislation of any political unit.

4. The CGC shall not participate in, or intervene in (including the publishing or distribution of statements), any political campaign on behalf of a candidate for public office.

5. The CGC shall not be organized or operated for profit.

6. The CGC shall not:

    (a) lend any part of its income or corpus without the receipt of adequate security and a reasonable rate of interest to;

    (b) pay any compensation in excess of a reasonable allowance for salaries or other compensation for personal services rendered to;

    (c) make any part of its services available on a preferential basis to;

    (d) make any purchase of securities or any other property, for more than adequate consideration in money's worth from;

    (e) sell any securities or other property for less than adequate consideration in money or money's worth; or

    (f) engage in any other transactions which result in a substantial diversion of income or corpus to any officer, member of the Coordinating Committee, or substantial contributor to the CGC.

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

ARTICLE X. Distribution on Dissolution

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

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 1993

Balance (31 December 1992)   **$4,481.27**

Receipts:

Dues & CGC back issue orders	$2,102.00	
Interest on savings	$122.97	
Total receipts		$2,224.97

Expenditures:

CGC Report No. 15 (1992)		
Printing	$1,832.88	
Mailing	$478.94	
Call for papers (Report No. 16)	$116.81	
Miscellaneous (envelopes, postage, etc.)	$9.57	
U.S. FDIC bank fees	$9.39	
Total expenses		$2,447.59

Balance (31 December 1993)   **$4,258.65**