

# Cucurbit Genetics Cooperative



1998

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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 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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## Cucurbitaceae '98: Evaluation & Enhancement of Germplasm

**“Cucurbitaceae '98: Evaluation and Enhancement of Cucurbit Germplasm”** will be held from 30 November to 4 December 1998 at the Asilomar Conference Center in Pacific Grove, California, USA. The meeting is sponsored by the *American Society for Horticultural Science* (ASHS) and the *USDA Agricultural Research Service*. Besides ASHS and USDA/ARS, conveners include the *Cucurbit Genetics Cooperative* and *The Cucurbit Network*.

There will be sessions on Germplasm Resources, Entomology, Pathology, Industry, Breeding, Genetics, and Mapping and Markers. In addition to the research and discussion sessions, there will be meetings of the Cucurbit Crop Germplasm Committee, the

National Melon Research Group, the Cucurbit Genetics Cooperative, the Watermelon Research and Development Group, the Squash Breeders, the National Cucumber Conference, and the Pickling Cucumber Improvement Committee.

The conference facility, Asilomar, is a U.S. National Historic Landmark located on 44 hectares (107 acres) of pristine forest, dunes and beach in Pacific Grove. It offers on-site recreational facilities, such as a heated pool, volleyball courts, and walking trails. Guests are free to walk myriad forest paths, miles of nearby beach, and the adjacent boardwalk that spans a sand dune restoration project. (The project ensures the preservation of

several rare and endangered species of plants.)

Social activities will include a “Taste of California” Reception, a Networking/Social Gathering, a Monterey Bay Aquarium Excursion, and a Special Farewell Luncheon. Optional Tours will be available to Big Sur and to “Steinbeck Country.”

The early registration deadline is 26 October 1998. On-site registration will also be available. A full brochure, including information on fees and payment, housing, meal service, and driving directions from San Francisco or Los Angeles, can be obtained by writing to:

**Cucurbitaceae '98, 600 Cameron Street, Alexandria, VA 22314-2562, USA** (Fax: 703.836.2024).

Alternatively, information can be downloaded from the web at <http://www.ashs.org/events/cucurbit/cucurbit.html>. We hope to see you there!

### 1997 Annual CGC Business Meeting

Tim Ng, Chair

The 21st Annual CGC Business Meeting was held on 25 July 1997 in Salt Lake City, Utah, in conjunction with the 94th Annual Conference of the American Society for Horticulture Science (ASHS). Twenty-six members and friends of CGC were in attendance.

Tim Ng opened the meeting by pointing out that the *original* organizational meeting for CGC was also held in Salt Lake City, at the 1977 ASHS meeting, and that CGC had grown considerably in the past 20 years. Updates on the CGC membership, reports and finances were then presented. Tim mentioned that CGC Report No. 20 (1997) was

### Comments . . . . .

***From the CGC Coordinating Committee:*** The Call for Papers for the 1998 Report (CGC Report No. 21) will be mailed in September 1997. Papers should be submitted to the respective Coordinating Committee members by 31 January 1998, although late submissions may be considered if received prior to our processing deadline. The Report will be published by June/July 1998. As always, we are eager to hear from CGC members regarding our current activities and future direction of CGC.

***From the CGC Gene List Committee:*** Lists of known genes for the *Cucurbitaceae* have been published previously in *HortScience* and in reports of the Cucurbit Genetics Cooperative. CGC is currently publishing complete lists of 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 (published in each CGC Report) were adopted in order to provide guidelines for the naming and symbolizing of genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

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

## NEWS

delivered to the printer on July 10, and that copies should be available and sent to CGC members within a few days. The timetable for soliciting reports for CGC Report No. 21 (1998) was briefly mentioned. Also, for the first time CGC members will be able to submit their manuscripts via encoded files on the Internet to the appropriate Coordinating Committee member.

Tim next gave an update on the CGC web page. Although CGC has decided not to make complete copies of CGC Reports less five years old available on the web, Tim is listing the table of contents and coding a few papers from each new issue for the website as an inducement for prospective new CGC members to join. Meanwhile, the volunteer digitization and OCR effort for back issues was going slowly, and two issues had been placed on the web in pdf format just to expedite the process. Priorities are now being given to back issues which are out of stock, or for which only a few copies are left in inventory.

CGC Coordinating Committee members serve 10-year terms, and **J. Brent Loy** (Univ. New Hampshire) rotated off in 1997 as the Coordinating Committee member for *Cucurbita* spp. **Linda Wessel-Beaver** (Univ. Puerto Rico) was elected as the new CGC Coordinating Committee member for *Cucurbita*. Thanks, Brent, and congratulations, Linda!

**Harry Paris'** request to change Rule #3 of the CGC Gene Nomenclature for the Cucurbitaceae (first presented at the 1996 CGC Business Meeting) was discussed further. Harry felt that since no standard or normal genotype has been established for any species of the Cucurbitaceae, it is inappropriate for us to automatically use the symbol "+" to indicate the "wild" type. Rather, we should change our rules to be consistent with those in the **Glossary of Genetics and Cytogenetics** (R. Rieger et al., 1976),

whereby the recessive allele is entirely in lower case and the dominant allele is capitalized. **Dick Robinson** pointed out that the cucurbit gene rules were first proposed in 1976 by a committee appointed by ASHS, and were based on the gene rules for the Tomato Genetics Cooperative. Prior to the establishment of these rules, cucurbit gene nomenclature was confusing, with incorrect gene symbols, duplicate symbols and names, multiple symbols and names, and unassigned symbols commonly found in the scientific literature. These and other discrepancies were subsequently eliminated through the work of the Cucurbit Gene List Committee and CGC. Dick felt that with the current proposal and other proposals that may arise to change the gene rules, and CGC should adopt whatever rules most members prefer. He also pointed out, however, that there are times when using the "+" designation might be convenient, such as with multiple gene stock studies where using the "+" would simplify the genotype description.

After much discussion, the members decided to change Rule 3 to conform with **Glossary of Genetics and Cytogenetics** nomenclature. This will be reflected in the future Gene Lists and Gene Nomenclature Rules in the CGC Report. However, the members also thought that researchers should be able to use whatever system they preferred for their own research reports, such as the "+" designation, as long as the usage is consistent and clear throughout the article. This will also be made evident in the revised CGC Gene Nomenclature Rules.

The proposal of having CGC compile a list of cloned genes of the Cucurbitaceae was again discussed. A committee consisting of **Gary Thompson**, **Mike Havey** and **Rebecca Grumet** (Chair), was asked to look further into the feasibility and need for the project.

Upcoming meetings were announced, such as those for the PCIC/PPI/CCGC

joint meeting (Las Vegas, October 1997) and Cucurbitaceae '98 (California, December 1998). The next CGC meeting will be held in July 1998 in Charlotte, NC, in conjunction with the 95th Annual ASHS Conference.

Under New Business, **Todd Wehner** asked whether we would be willing to pay a small "affiliate's fee" to ASHS as compensation for their scheduling the CGC meeting in conjunction with their Annual Conference. Tim mentioned that CGC was a small, non-profit organization run "on a shoestring," and that any additional expenses would have to be passed on to the membership. Several members remarked that if an "affiliate's fee" were to be charged, that perhaps we shouldn't try to be scheduled in the ASHS program, but should just show up anyway and try to find an empty room or meet in one of the local establishments in the conference town. After further discussion, it was decided not to entertain the idea of paying a fee any further.

As there was no further business, and the meeting was adjourned.

## 1998 Annual CGC Business Meeting

Tim Ng, Chair

The 22nd Annual CGC Business Meeting was held on 15 July 1998 in Charlotte, North Carolina, in conjunction with the 95th Annual Conference of the American Society for Horticulture Science (ASHS). Despite ASHS's scheduling of a cucurbit production research session and a cucurbit seed physiology presentation concurrent with the CGC meeting, 17 members and friends of CGC were in attendance.

After introductions, **Tim Ng** began the meeting by presenting updates on CGC memberships, reports, and finances. He also mentioned that CGC Report No. 21 (1998) would be delayed this year because of

unexpected demands on his time, including an earlier than usual ASHS meeting. He hoped to have it completed by the end of July. The Call for Papers for CGC Report No. 22 (1999) will be sent sometime in September 1998, with a suggested deadline of 30 January 1999 for submissions. With the millennium approaching, Tim also mentioned that the computers and software he used for CGC were all Y2K-compliant.

The issue of curating the cucurbit mutant collection was brought up. Of particular concern was the watermelon mutant collection, with one curator no longer at his former company (although the seed were) and the other curator recently discovering that some of his seedlots were contaminated with the watermelon fruit blotch organism. Several alternatives were discussed, including possibly having the USDA PI station handle the curation. However, it was pointed out that some of these mutants are difficult to cultivate and maintain, and that standard PI procedures for seed increases might not be sufficient for these mutants. After further discussion, it was decided that we should encourage Todd Wehner to revise his request for funding for a cucurbit gene center and resubmit it to the CCGC.

**Jim McCreight** provided an update for Cucurbitaceae '98 meeting. Brochures containing the second announcement were being distributed at the ASHS meeting. Tim mentioned that the ASHS website had been updated in the past week with this information also, and that he had provided a link from the CGC page to the ASHS information on Cucurbitaceae '98. (Editor's note: further information on Cucurbitaceae '98 can be found elsewhere in this CGC Report.)

Tim gave an update on the impact of the Internet on CGC. At least a dozen new members had joined after finding CGC on the web. As for the "digitization of back issues" project,

**Todd Wehner** has completed the OCR work on Reports 1 and 2. **Tom Andres** and **Jim McCreight** are nearly finished with Reports 3 and 4, respectively. **Bill Rhodes** has completed OCR work on Report 5. **Rebecca Brown** and **Jeff Adelberg** are still working on their reports. **Bruce Carle** volunteered to tackle Report 8, **Linda Wessel-Beaver** Report 9, **Todd Wehner** Report 10, **Jim McCreight** Report 11 and **Jack Staub** Report 12. They will be sent the originals for each of these reports. There was no further business, and the meeting was adjourned.

## Cucurbit Crops Germplasm Committee Update

James D. McCreight, Chair

The Cucurbit Crops Germplasm Committee (CCGC) met in Las Vegas, Nevada, on 30 October 1997 in conjunction with Pickle Packers International (PPI) and the Pickling Cucumber Improvement Committee (PCIC).

**Alan Stoner**, Director, Plant Genetic Resources Laboratory, reported that PCGRIN will be made available to countries for management of local germplasm collections. IPGRI is translating the documents into Spanish and other languages. Also, the General Accounting Office (GAO) survey of the National Plant Germplasm System (NPGS) is complete and will be sent to CGC chairs. It is also available on the web at <http://www.gao.gov> (request report GAO/RCED-98-20). The study was initiated by GAO, and the highest marks were given to GRIN system. Suggestions for improvements to GRIN need to be sent to CCGC to permit them to make the needed changes.

Alan also reported that images are now available on GRIN. **Todd Wehner** reported that he sent 35mm color slides of *Luffa* fruits to Griffin

for their database. A proposal was made by Todd to include photos of the cucumber collection as well. Ames has scanners for images, but it has been used heavily by the maize project.

**Kathy Reitsma**, *Cucumis* curator, reported that the Ames Plant Introduction collection has 3,054 melons, 1,350 cucumber, 347 wild, and 1,006 squash accessions, with 34% to 80% available, and 6% to 55% backed up at NSSL. Evaluation data ready to be loaded includes downy mildew of melon (**Claude Thomas**) and GSB in melon and squash (**Molly Kyle**). GRIN passport information continues to be updated from old reports and seed packets. The Committee voted to have the three *Bryonia* accessions transferred to Griffin. **Charles Block** (Ames) reported that original seeds of melon and squash are being tested by ELISA for squash mosaic virus. Accessions sent directly to NSSL may not be included in active collections, and the accessions at NSSL are available in multiple sets. Grow-outs need to be made to identify which accession is the most appropriate to include of the duplicate sets.

**Jack Staub** learned on a visit to India in September that many of the seeds that were collected in 1992 (Staub and McCreight, *Cucumis* expedition) had been misplaced or had a reduced level of germination. Thus, some of the accessions collected that year are only available in the USA.

**Germplasm Evaluation.** **Dave Wolff** is completing his *Monosporascus* resistance evaluation in melon. **Don Hopkins** is working on fruit blotch resistance in watermelon. **Mark Bohning** is developing procedures for tracking entry of evaluations into GRIN.

**Germplasm collection.** **Bob Jarret** would like to make a list of collection sites to visit in priority order. **Allan Stoner** would like to see planned exploration proposals to prioritize system-wide over crops. Ten

proposals were forwarded for review. Collection in India is now easier, and should be pursued.

**Germplasm exchange.** Exchanges are requested and often are sent. The Vavilov Institute in Russia is on the web, and accessible through the GRIN web site. The Vavilov Institute is being supported by Russia, but additional support has been requested from other countries, including the USA. Their records are being computerized, and seeds are being increased. IPGRI is involved. Other exchanges needed are: Beijing, Poland, Czech Republic. Although Russia and eastern Europe are exchanging, some countries have restrictions on germplasm use.

**Gene stocks.** Although tomato gene stocks are maintained formally by USDA through the *Charles Rick Tomato Gene Stock Center* (University of California, Davis), proposals by **Dick Robinson** and **Todd Wehner** for a cucurbit gene stock center have not been funded. Gene mutants are difficult to maintain, and need to be done separately from RPIS increases. A gene stock center proposal could be developed for submission in 1998.

The cucumber and watermelon subcommittee reports were updated, but the squash and melon reports were not.

### 18th Annual Meeting of the Watermelon Research and Development Group

Ray D. Martyn, Past Chairman

The Watermelon Research and Development Group (WMRG) met in Little Rock, AR, on Sunday, 1 February 1998, for its 18th annual meeting. The meeting was held at the Robinson Center in conjunction with The Southern Association of Agricultural Scientists (S.A.A.S), the Southern Region: American Society for Horticultural Sciences,

(SR:ASHS) and the Southern Division: American Phytopathological Society (SD:APS). This was one of the best attended meetings in a long time with over 50 scientists present. Nine research reports were presented with a lot of discussion. In addition, **Mr. William Watson**, Executive Director of the National Watermelon Promotion Board, was present and gave an update on the activities of the Board and their research support program. And lastly, **Dr. Benny Bruton**, was elected as the new Chairman for WMRG. Benny is a plant pathologist with the ARS-USDA laboratory in Lane, OK.

**I. Research Updates.** A number of research presentations were made by the scientists present. **George Boyhan**, University of Georgia, Statesboro, GA, [gboyhan@uga.cc.uga.edu], talked about developing ZYMV resistant watermelon cultivars. George has been evaluating different Equis and Egun-types for resistance to ZYMV and WMV. One of the most promising lines has been a AU- Producer x Egun cross. Several back crosses have been made with good results. The fruit is not yet uniform in color but it does have good resistance to both ZYMV and WMV. They are still several years from the finished product. **Charlie Main** and **Gerald Holmes**, North Carolina State University, Raleigh, NC, talked about long distance movement of downy mildew spores. Charlie and Gerald presented the computer model forecast system for tobacco blue mold (downy mildew) as a potential model for use in cucurbit diseases. The model is based point source of inoculum, wind speed and directions, and rain events. A network of spotters in various states report when the disease occurs in their area. From there, the model will predict when and where the next outbreak will occur. A good discussion took place about the potential for developing a similar model for DM of cucurbits. For more information see the Blue Mold Home Page at

<http://www.ces.nsu.edu/depts/pp/bluemold>.

**Don Maynard**, University of Florida, Bradenton, FL, [dmaynard@ufl.edu], discussed watermelon fruit defects. Don addressed the topic of fruit maladies of unknown cause. The first was a ring spot symptom on fruit and it was a consensus of the group that it was probably caused by papaya ring spot virus. The second symptom was a rind necrosis. There appeared to be varietal differences. It appeared similar to the bacterial rind necrosis but there was not a group consensus. The last major malady was hollow heart. Don asked the questions "When does it first begin to develop in the fruit? Is there a genetic basis for it occurring? Are seedless more prone than seeded varieties? Is it environmentally induced?." It was noted that a fluorescent microscopy technique can detect hollow heart when it is only 2-3 mm in size. **Dan Egel**, Purdue University, SW Purdue Agriculture Center, Vincennes, IN, [egel@purdue.edu] talked about fungicide application technology for melons. Dan reported on a series of tests in which he evaluated spray pressure and nozzle type for efficacy in spraying melons for *Alternaria* blight. His data indicated that there were no difference in effectiveness between flat fan and hollow cone nozzles at either 30, 60, or 90 psi. At higher pressures, differences may be evident.

**Bruce Carle**, University of Florida, Central Florida AREC, Leesburg, FL, [rbcw@gnv.ifas.ufl.edu], discussed new directions in watermelon breeding in Florida. Bruce is a new faculty member specializing in cucurbit breeding. His main research thrust is on improving disease resistance in diploid watermelons (*Fusarium* wilt race 2, ZYMV, WMV 2, fruit blotch, and anthracnose). He is working toward pyramiding resistance genes. A second emphasis is improving in vitro tetraploidy in seedless varieties. Bruce also



announced that the watermelon breeding program will move to the Apopka Station as the Leesburg Station will be closing down. **Dr. Todd Wehner**, North Carolina State University, Raleigh, North Carolina [todd\_wehner@ncsu.edu], gave an overview of the watermelon breeding program at N. C. State. Todd has renewed Warren Henderson's program on studying the inheritance of the inhibitor gene for yellow flesh. He is also working on disease resistance to gummy stem blight. He has identified a high level of resistance to this disease in PI 189225. He is trying to correlate results of seedling disease assays with mature plants in the field. Thus far, seedlings that appear resistant in the greenhouse are susceptible in the field. Todd is screening a number of accessions from the germplasm collection including 51 *Citrullus* accessions from China and 25 from South Africa.

**Joe Norton**, Auburn University, Auburn, AL, reported on the work of Dr. Fenny Dane in investigating molecular tagging for identifying resistance genes to Fusarium wilt and gummy stem blight and also the use of AFLPs and PCR for variety identification. Joe also reported that much of the honey bee population in Alabama has been destroyed by the predatory mite infestation. **Tony Keinath**, Clemson University, Clemson, SC [tknth@clemson.edu], discussed PCR detection of *Didymella bryoniae*. Tony has developed specific PCR RAPD primers that can distinguish between *D. bryoniae* and *Phoma medicaginis*. Both fungi produce a 650 bp band with the primers but they can be distinguished by their sequence. A second species of *Phoma*, *P. exuda*, produces a 450 bp band than can be used for identification. Thus far, this species has only been found in New York.

**Benny Bruton**, USDA-ARS, Lane, OK, [bbruton@ag.gov], gave an update on yellow vine disease. Using primers for the 16S RNA of

prokaryotes, they have identified a 640 bp fragment that is amplified from symptomatic plant tissue but is not amplified from healthy tissue. PCR has been able to detect this fragment from all parts of the plant. Also, transmission electron microscopy shows bacteria-like organisms in the phloem tissue of symptomatic plant tissue, but not in healthy tissue. There is also a distinct phloem necrosis associated with this malady.

#### II. News From the National Watermelon Promotion Board.

**William Watson**, Executive Director, NWPB, Orlando, FL, [H2Omelon@watermelon.org], announced that the research program sponsored by the NWPB had an increase in funding this year. In 1997 there was \$12,500 available to support research. This year, there was \$40,000. This is a big increase and shows the support that NWPB has for watermelon research. Twenty-one proposals were received this year as opposed to 12 proposals last year. The NWPB's web site won a first place national award from the National Agriculture Marketing Association (NAMA). This is nation-wide competition. They also won several other awards for marketing, as well as "best of show" for their Media Kit. Congratulations.

**III. New Business.** **George Boyhan**, University of Georgia, agreed to take the lead in setting up a web site for our group. Items that could be included on the site would be meeting summaries, dates, etc.; mailing addresses and e-mail lists; upcoming events; research notes, and links to many other watermelon related topics. Please send your comments to George. Also, we were asked by the Southern Region: American Society for Horticulture Sciences to consider changing our meeting time from Sunday afternoon to something different because of perceived conflicts with other events. A vote was taken and it was agreed that a Sunday afternoon time was good and

we would not change at this time. The meeting time for the WMRG will remain Sunday at 1 p.m.

**IV. New WMRG Chairman.** **Benny Bruton**, Plant Pathologist with the USDA-ARS in Lane, OK, was duly elected the new chairman of the Watermelon Research Group. Benny is a long-time participant of the group and conducts research on soilborne disease of melons and watermelons. Benny officially took over the chairmanship at the close of this meeting.

**V. Upcoming Meetings** The 19th Annual Meeting of the Watermelon Research and Development Group will be 30 January 1999 in Memphis, TN.

## Upcoming Meetings of Interest to Cucurbit Researchers

<u>MEETING</u>	<u>DATE</u>	<u>LOCATION</u>	<u>CONTACT</u>
<b>Pickle Packers Fall Business Conference</b>	14-16 October 1998	Sheridan Center, Toronto, Ontario, Canada	Pickle Packers Intl. (630) 584-8950
<b>Cucurbitaceae '98</b>	30 November - 4 December 1998	Asilomar Conference Center, Pacific Grove, California	James D. McCreight (831) 755-2684 <a href="mailto:jmccreig@asrr.arsusda.gov">jmccreig@asrr.arsusda.gov</a>
<p>Note: the following groups will also meet at this conference:</p> <p><i>Cucurbit Crop Germplasm Committee</i>  <i>National Melon Research Group</i>  <i>Cucurbit Genetics Cooperative</i>  <i>Watermelon Research &amp; Development Group</i>  <i>Squash Breeders</i>  <i>National Cucumber Conference</i>  <i>Pickling Cucumber Improvement Committee</i></p>			
<b>Watermelon Research and Development Group</b>	30 January 1999	Memphis, Tennessee	Benny D. Bruton (404) 889-7395 <a href="mailto:bbruton@ag.gov">bbruton@ag.gov</a>
<b>Cucurbit Genetics Cooperative</b>	July 1999	Minneapolis, Minnesota	Timothy J Ng (301) 405-4345 <a href="mailto:tn5@umail.umd.edu">tn5@umail.umd.edu</a>
<b>Pickling Cucumber Improvement Committee</b>	27-29 October 1999	Opryland Hotel Nashville, Tennessee	Jack E. Staub (608) 262-0028 <a href="mailto:jestaub@facstaff.wisc.edu">jestaub@facstaff.wisc.edu</a>

# Relationships Among Putative Botanical Varieties in Cucumber

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**Introduction.** Research using populations developed from diverse breeding lines indicates low genetic variance for fruit yield in processing cucumber (*Cucumis sativus* var. *sativus* L.) (Wehner, 1989). This low variance is at least partially due to the narrow genetic base of pickling cucumber. The results of previous studies suggest that selection for yield in elite adapted populations would likely be a slow and costly process.

Incorporation of quantitatively inherited characteristics into commercially adapted cultivars from exotic germplasm can be an effective way to obtain greater genetic variation and response to selection (Wehner, 1989). *Cucumis sativus* var. *hardwickii* (R.) Alef. is a potential source of exotic germplasm for increasing the genetic variation for yield in cucumber because of its multiple lateral, sequential fruiting habit (Kupper and Staub, 1998, Fredrick and Staub, 1989). Nevertheless, var. *hardwickii* has many undesirable characteristics which have caused problems during the incorporation of desirable characteristics. Other botanical varieties of *Cucumis sativus* exist and should be evaluated for their breeding potential.

The USDA North Central Plant Introduction Station (NCPIS), Ames, Iowa received 12 accessions which were classified as botanical varieties of *C. sativus* (Table 1). These accessions by definition of the variety status were presumed to be different from *C. sativus* var. *sativus*, and were given the taxonomic classification of var. *anatolicus* (1; PI 504559), var. *cilicicus* (2; PI 504561), var. *europaeus* (3; PI 504562), var. *falcatus* (4; PI 504563), var. *indo-europaeus* (5; PI 504565), var. *iranoturanieus* (6; PI 504566), var. *izmir* (7; PI 504567), var. *sikkimensis* (8; PI 504568), var. *squamosus* (9; PI 504570), var. *testudaceus* (10; PI 504571), var. *tuberculatus* (11; PI 504572), and var. *vulgatus* (12; PI 504573) at their source prior to transmittal to NCPIS. Therefore, a study was designed to evaluate the genetic diversity

among these accessions, and between these accessions and an accession of var. *hardwickii* (13; PI 183967) and var. *sativus* (Line GY14) (14) used in previous studies (Kupper and Staub, 1998, Fredrick and Staub, 1989).

**Material and Methods.** Twelve accessions of putative botanical varieties of *C. sativus* were received from NCPIS (Table 1). These accessions along with *C. sativus* var. *hardwickii* and *C. sativus* var. *sativus* (line GY14) and F<sub>1</sub> progeny (derived from crosses with these accessions and USDA WI 2870) were evaluated for isozyme variation at 21 loci according to Meglic et al. (1996) and five morphological characteristics [days to flower, lateral branch number (primary), cumulative fruit number, weight, and length:diameter ratio over three harvests]. For morphological analysis, varieties and F<sub>1</sub> hybrids were planted in treatment rows 6.1 m long spaced 1.5 m apart at Hancock, WI in 1993. Plant spacing within a row was about 12 cm. Border rows were placed between main plots and at plot ends. For electrophoresis, accessions (~15 seedlings bulked) were characterized using starch gel electrophoresis. ANOVA and mean separation and cluster analyses were performed according to Steel and Torrie (1980). Mean separations for all variables in all ANOVAs were performed with Fisher's protected LSD at the 5% level and coefficients of variation were calculated.

**Results and Discussion.** Isozyme and morphological analysis resulted in similar, but not identical dendrogram depictions. Variation at nine polymorphic enzyme loci (*Ak-2*, *Ak-3*, *Fdp-1*, *Mdh-3*, *Mpi-2*, *Pgi*, *Pgm*, *Pgd-2*) was important in defining varietal differences (Table 2). Variety *hardwickii* and var. *falcatus* were most dissimilar from all other varieties examined (cluster analysis not presented).

Botanical varieties differed in their morphological characteristics (Table 1). Mean comparisons of morphological characteristics among the F<sub>1</sub> progeny are given in

Table 1. Comparisons among putative *Cucumis sativus* L. botanical varieties for sex expression, days to anthesis, lateral branch number (primary), and fruit number, weight and length:diameter ratio (L:D).

Botanical variety	Origin	Sex <sup>1</sup>	Days to anthesis	Lateral branch number	Three harvest yield	Three harvest weight (kg)	L:D ratio
<i>var. anatolicus</i>	Russia	M	36.8	2.5	58.8	36.1	4.0
<i>var. cilicicus</i>	Russia	M	19.0	1.0	81.5	29.5	1.9
<i>var. europaeus</i>	Russia	M	32.3	3.0	99.5	33.3	3.3
<i>var. falcatus</i>	Japan	M	44.5	1.5	30.0	18.6	11.6
<i>var. indo-europaeus</i>	Russia	M	35.3	2.8	86.5	31.8	2.1
<i>var. irano-turanicus</i>	Russia	M	35.0	2.8	82.3	27.5	2.1
<i>var. izmir</i>	Russia	M	35.8	1.5	93.5	29.2	2.1
<i>var. sikkimensis</i>	Russia	M	58.3	4.5	60.0	24.5	2.0
<i>var. squamosus</i>	Russia	M	38.0	2.8	81.8	17.5	1.8
<i>var. testudaceus</i>	Russia	M	38.5	1.0	92.8	31.9	2.1
<i>var. tuberculatus</i>	China	M	35.0	0.0	45.0	19.4	3.2
<i>var. vulgatus</i>	India	M	39.5	3.3	48.3	23.6	2.4
<i>var. hardwickii</i>	India	M	77.5	6.0	2.0	0.2	1.5
<i>var. sativus</i> (Gy14)	USA	G	38.8	0.0	97.5	25.0	2.6
LSD			2.7	0.6	16.3	6.9	0.3
C.V. (%)			25	25	15	17	10

<sup>1</sup> M = monoecious and G = gynoecious.

Figure 1. Comparisons among F<sub>1</sub> hybrids derived from matings between putative botanical varieties of cucumber (*Cucumis sativus* L.) and USDA line WI 2870 (var. *sativus*) [Vertical bar 1= var. *anatolicus*, 2 = var. *cilicicus*, 3 = var. *europaeus*, 4 = var. *falcatus*, 5 = var. *indo-europaeus*, 6 = var. *irano-turanicus*, 7 = var. *izmir*, 8 = var. *sikkimensis*, 9 = var. *squamosus*, 10 = var. *testudaceus*, 11= var. *tuberculatus*, 12 = var. *vulgatus*, 13 = var. *hardwickii*, and 14 = var. *sativus* (Gy14)].

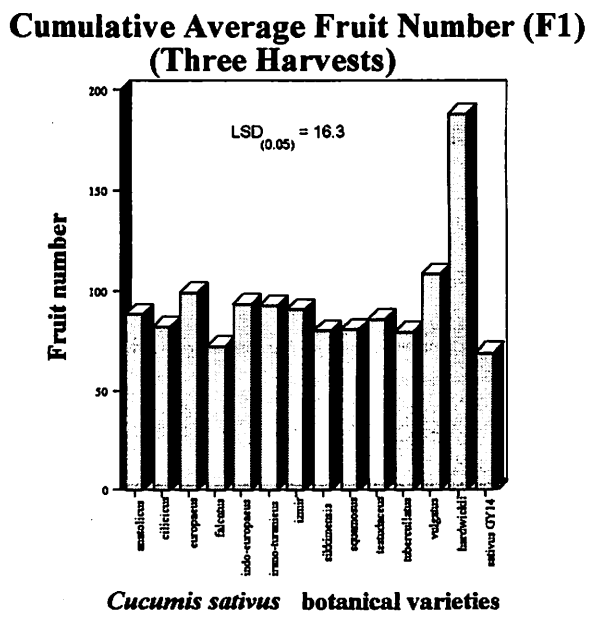
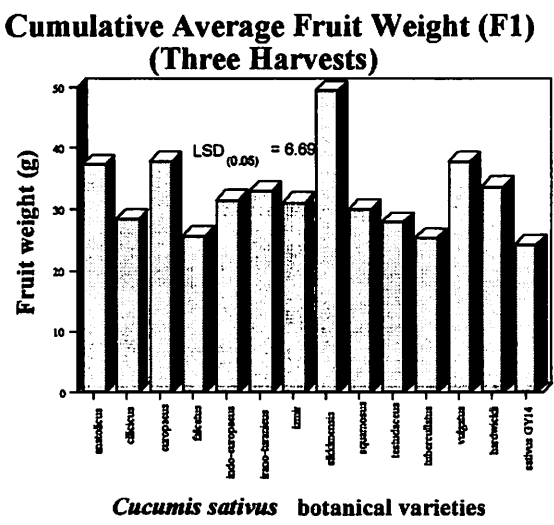
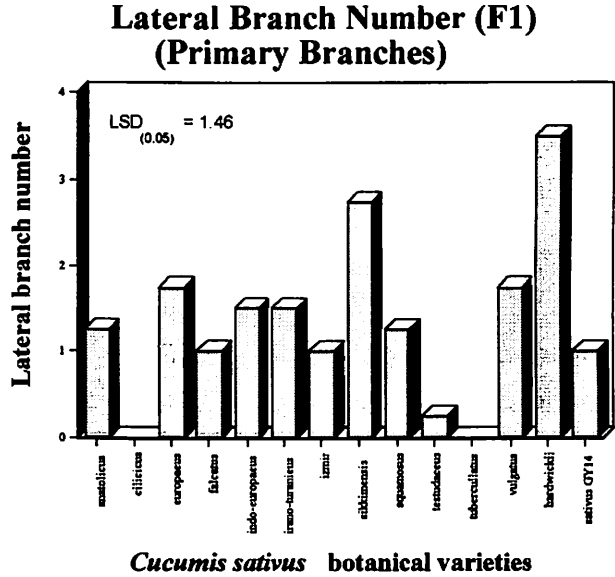
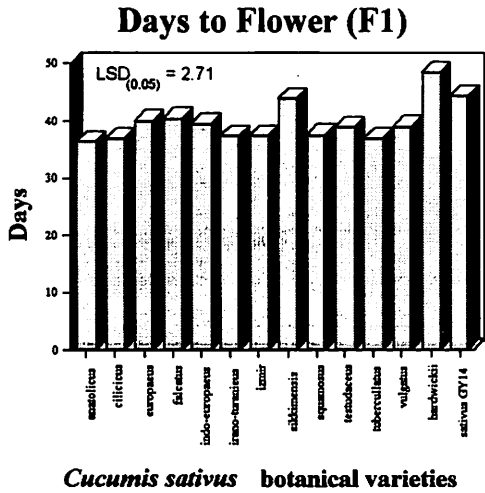


Table 2. Isozyme genotypes at nine loci of 14 *Cucumis sativus* botanical varieties<sup>1</sup>.

Botanical variety	Ak2	Ak3	Fdp1	Fdp2	Mdh3	Mpi2	Pgl	Pgm	Pgd2
<i>sativus</i>	22	11	22	11	11	22	22	11	11
<i>anatolicus</i>	12	22	22	11	11	22	22	12	22
<i>cilicicus</i>	22	12	12	11	11	11	22	12	12
<i>europaeus</i>	22	12	12	11	11	12	12	22	11
<i>falcatus</i>	22	11	11	11	11	11	22	22	11
<i>hardwickii</i>	11	11	12	11	11	22	22	22	11
<i>indo-europaeus</i>	22	22	11	22	11	11	11	12	22
<i>irano-turanieus</i>	22	22	22	22	12	11	11	22	22
<i>izmir</i>	22	22	12	22	11	22	11	12	22
<i>sikkimensis</i>	22	11	22	11	11	22	22	22	11
<i>squamosus</i>	12	22	11	22	11	12	22	22	22
<i>testudaceus</i>	22	11	22	11	11	11	11	11	11
<i>tuberculatus</i>	12	11	12	11	11	12	22	22	11
<i>vulgatus</i>	22	11	12	11	11	11	22	22	11

<sup>1</sup> only polymorphic loci shown

Figure 2. Relationships of cucumber (*Cucumis sativus* L.) accessions based on cluster analysis of morphological characteristics.

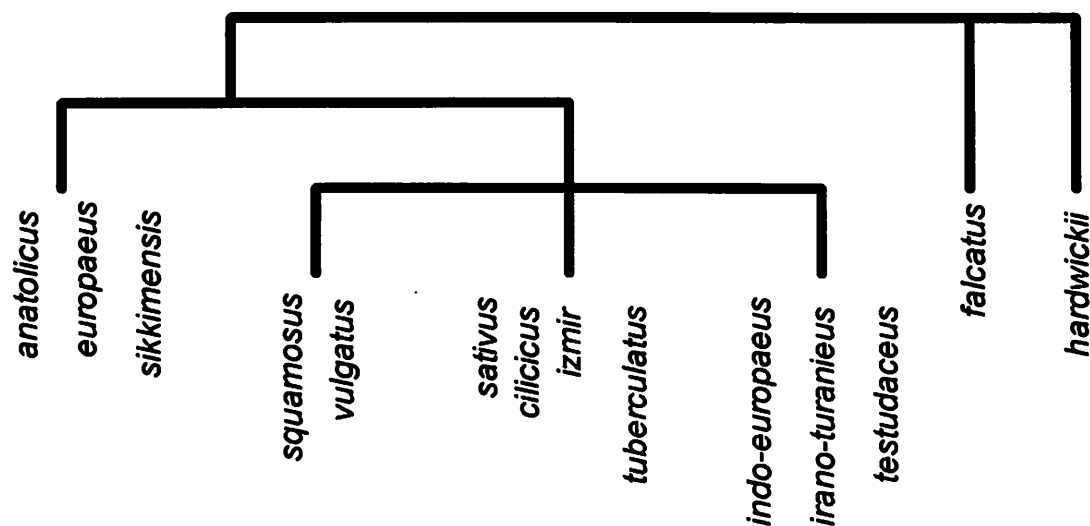


Figure 1. Relative relationships based on morphology are depicted by cluster analysis in Figure 2. The following observations can be made: 1) var. *testudaceus* was early flowering when compared to *sativus*; 2) var. *sikkimensis* produced more laterals than did *sativus*; 3) var. *europaeus* and *vulgatus* yielded higher than *sativus*; 4) var. *sikkimensis* had larger fruit than did all other varieties; and 5) var. *falcatus* produced longer fruit than did any other variety. The following observations can be made based on F<sub>1</sub> performance: 1) var. *hardwickii* is probably the best source for increasing fruit number in variety *sativus* among the accessions examined in this study; 2) the varieties *sikkimensis*, *vulgatus*, and *europaeus* are similar but distinct from variety *sativus* which is most like *cilicicus*, *izmir*, and *tuberculatus*; and 3) since varieties *sikkimensis* and *vulgatus* produce an abundant number of lateral branches and have genetic factors which condition relatively long and large fruit, these should be considered as having potential for increasing yield potential in adapted cucumber. The variety *europaeus* also may have some potential in this regard. Varieties *sikkimensis*, *falcatus*, and *hardwickii* might have potential in plant improvement based on yield performance.

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# Paternal Inheritance of Mitochondrial DNA in Cucumber: Confirmation by PCR Method

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**Introduction.** In cucumber, we have reported that mitochondrial DNAs are inherited paternally in sexual propagation (1). Since this was revealed by RFLP analysis, we could not neglect the possibility of bi-parental inheritance. In order to examine the above point, we investigated the DNA sequence of a mitochondrial DNA clone which was inherited paternally, and PCR analysis was performed subsequently using reciprocal hybrids.

**Methods.** One of the mitochondrial clones, B-174 (1.0kb), was sequenced. Based on this nucleotide sequence data, we applied the IPCR (Inverse PCR) for the self-ligated EcoRI digested total DNA of the cucumber lines, and the IPCR products were cloned by TA-cloning system and sequenced. The DNA sequence was determined by the dideoxy sequencing method. The parameters for PCR reaction were 30 cycles of heating at 94 C for 15 sec, at 60 C for 15sec and at 72 C for 2 min.

**Results and Discussion.** Southern blot hybridization analysis of EcoRI- digested genomic DNAs that were isolated from several cucumber varieties and the wild strain indicated five polymorphic single band patterns, 2.4kb (Type 1), 2.6kb (Type 2), 3.0kb (Type 3), 3.2kb (Type 4) and 3.7kb (Type 5), when the blots were probed with B-174. We have completed the cloning and se-

quencing for the three types: Type 1, Type 2 and Type 3 (part of the nucleotide sequence data have been submitted to the DDBJ, EMBL and GenBank with accession number D17360). The restriction maps of the three types of mitochondrial clones suggest that the polymorphisms resulted from the deletion at the neighbor region of the B-174 fragment (Fig.1). Two sets of primers, primers A and K for Type 2 and primers W and K for Type 3, were designed which could detect the deletion sequence, and PCR detections were performed for the cucumber lines and their reciprocal hybrids. As shown in Fig. 2, the specific PCR amplification product was observed in each of the parental lines. In the reciprocal hybrids, PCR products were transmitted only from the paternal plant. These results strongly suggest that the mitochondrial DNA is inherited strictly in a paternal manner in cucumber.

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Figure 1. Restriction enzyme maps of the three types of mitochondrial clones. [B = BamHI; E = EcoRI; P = PstI; S = StyI; X = XbaI; → = primer A; ⇒ = primer W; ← = primer K; - = homologous regions; ≈, ~ = non-homologous regions.]

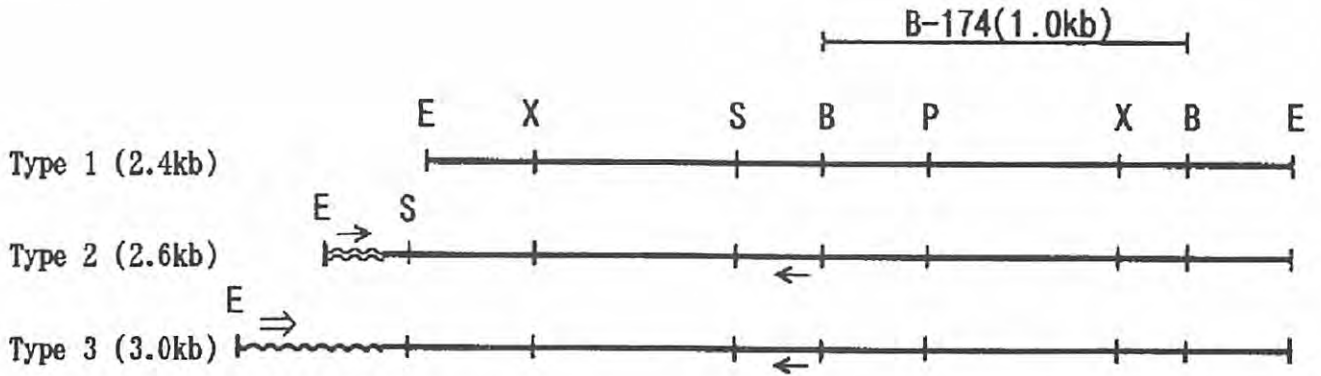
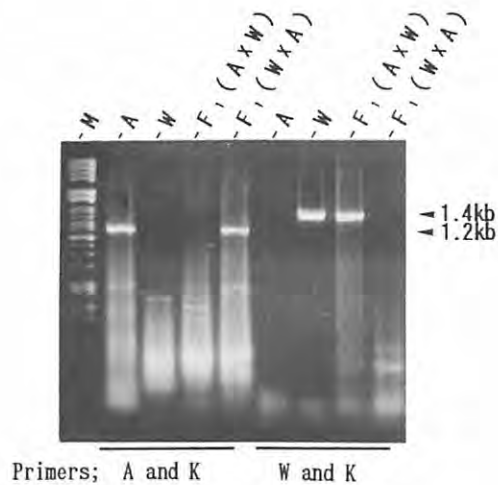


Figure 2. Product of the PCR reaction from cucumbers lines and their reciprocal hybrids. [A = Aofushinari (Japanese local variety, Type 2); W = W103 (*hardwickii*, Type 3); M = molecular marker ( $\lambda$  Hind III / Hinc II)]



# Effects of Copper Seedcoat Agent on Cucumber Germination and Seedling Growth

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**Introduction.** Copper, one of the trace elements in plants (1), is essential to the normal growth of vegetables. Cucumber is one of the vegetables which is very sensitive to copper (2). Some research has shown that soaking seeds in CuSO<sub>4</sub> (copper sulfate) solution may enhance plant growth and development, and even improve fruit nutritive quality in cucumber.

Disease has proven to be a major threat to vegetable production worldwide. Seeds and soil are the two major initial sources of disease causing pathogens in vegetable production. Research has showed that soaking seeds of pepper and tomato in Cu<sup>2+</sup> solution can control some diseases (3,4).

Seed coat techniques have proven to be a convenient and effective way to improve seed quality and seedling growth, and to control seedling diseases (5,6,7). In China, we are now conducting a project entitled, "Seed Technology Industrialization." Seed coat evaluation is a major part of that project. Our research was conducted to evaluate the effects of Cu<sup>2+</sup> in seedcoat agents on seed germination, seed storage, seedling growth, and seedling disease control in cucumber.

**Materials and Methods.** Cucumber seeds of cultivar Jin 4-3-1 and chemically pure copper sulfate were used in the experiment. Copper was added to the cucumber seedcoat agent at the concentration of 1.0% or 4.0%, and a no-copper control was added to the seed coating mixture. The seedcoat agent was applied to cucumber seeds at 1:10 (w:w) proportion of seedcoat agent to seed.

Germination testing of the coated seeds was conducted at the constant temperature of 25 C. One hundred seeds for each test plot (treatment) were sowed on a plastic foam bed (1 cm thick) in a culture dish. There were three replications of each treatment. The germinated seeds were counted 8 times from 24 hours to 108 hours after sowing in an interval of 12 hours (when the root length of the seeds exceeded half of the seed length). The germinating percentage was calculated according to the total seeds (100 seeds) sowed and the total germinated seeds at the end of germination. The germination energy

was calculated using the total seeds sowed and the total germinated seeds counted at the first three data collection intervals (i.e., 48 hours after sowing). The germinating index (GI) was calculated with the following formula:

$$GI = \sum (G_t/D_t)$$

where G<sub>t</sub> is the number of germinated seeds at the data collection interval, and D<sub>t</sub> is the corresponding count of the data collection interval.

The storage ability of coated seeds was examined by an artificial aging method. The seeds were artificially aged at an air-tight container with a container relative humidity of ~99% and a temperature of 42 C for 4 days. Germination was examined after this treatment.

The effects of copper-coated seeds on seedling growth were investigated by observation of seedlings under field conditions. Seeds were sowed in a vermiculite medium in a container 8 cm high and 8 cm in diameter. Four seeds were sowed in each container and after emergence seedling were thinned to one plant. There were 10 containers in each plot with 3 treatment replications. Twenty-five days after sowing, seedling height, stem diameter (2 cm above ground), leaf area and fresh weight of roots and tops were measured. Leaf area was calculated as the sum of the cross-products of the length width of each leaf. Seedling index (SI) was calculated by the following formula:

$$SI = (\text{stem diam}/\text{seedling height}) \times \text{seedling weight}$$

The effects of copper-coated seeds on disease resistance of seedlings were tested by sowing the coated seeds in infected vermiculite medium and nursing seedlings under outdoor, natural conditions for 25 days. The percentages of the sprout rot and the wilted seedlings (caused by diseases) were calculated.

**Results.** Seed coating with either 1.0% or 4.0% of Cu<sup>2+</sup> improved seed germination. The germinating percentage, germinating energy, germinating index, and the root length of coated seeds were increased by 10.7%, 3.2%, 12.4% and 2.5%, respectively. The SSR test showed that the germinating percentage, germinating energy, and

Table 1. Effects of copper in seedcoat agent on germination of cucumber seeds.

Cu <sup>2+</sup> concentration (%)	Germinating percentage (%)	Germinating energy (%)	Germinating index	Root length (cm)	Root fw (g)
0 (ck)	68.0 bA	66.7 bA	13.86 b	1.88	0.037
1.0	82.7 aA	80.7 aA	18.34 a	2.05	0.039
4.0	75.3 abA	68.7 abA	15.58 ab	2.35	0.036

Table 2. Germination of artificially-aged copper coated cucumber seeds.

Cu <sup>2+</sup> concentration (%)	Germinating percentage (%)	Germinating energy (%)	Germinating index
0 (ck)	24.1 aA	22.7 aA	3.74 aA
1.0	16.0 abA	16.0 abA	2.28 abA
4.0	9.3 bA	9.3 bA	1.45 bA

Table 3. Effects of copper in seedcoat agent on seedling growth of cucumber.

Cu <sup>2+</sup> concentration (%)	Seedling height (cm)	Stem diameter (cm)	Seedling fw (g)	Root/top ratio	Seedling index	Leaf area (cm <sup>2</sup> )
0 (ck)	10.0 a	0.36 a	3.91 bB	0.43 aA	1.848 a	46.4 a
1.0	9.3 a	0.34 a	4.18 abAB	0.40 abB	1.840 a	46.4 a
4.0	11.4 a	0.35 a	4.45 aA	0.35 bA	1.690 a	47.8 a

Table 4. Copper in seedcoat agent on seedling disease control of cucumber.

	Cu <sup>2+</sup> concentration (%)	Emergence (%)	Rotten pre-emergence (%)	Fallen seedling (%)	Total incidence (%)
Expt. I	0 (ck)	8.3	91.7	2.7	94.4 aA
	1.0	97.2	2.8	0.0	2.8 bB
	4.0	100.0	0.0	2.8	2.8 bB
Expt. II	0 (ck)	0.0	100.0	0.0	100.0 aA
	1.0	88.7	11.1	5.6	16.7 bB
	4.0	88.7	11.1	0.0	11.1 bB

germinating index of 1% Cu<sup>2+</sup> coated seeds were significantly higher than those of the control (Table 1).

In contrast, copper in the seedcoat agent was not an advantage to the storage ability of coated seeds, especially with the higher copper concentration. However, the differences in germinating percentage, germinating energy and germinating index between the 1.0% Cu<sup>2+</sup> treatment and the control were not significant (Table 2). These data suggest that the coating of cucumber seeds with 1.0% Cu<sup>2+</sup> will not significantly affect seed storage ability.

With regard to seedling growth, the copper seedcoat treatment showed some advantages. However, the effects of the copper treatment were related to the concentration applied. The 4.0% Cu<sup>2+</sup> treatment increased seedling height, seedling fresh weight and leaf area by 14.4%, 14.5%, and 3.0%, respectively. The increase of seedling fresh weight was most significant. The 1.0% Cu<sup>2+</sup> treatment appeared to have no significant effect on seedling growth. Because copper treatment improved root growth more than top growth of the seedlings, both 1.0% and 4.0% Cu<sup>2+</sup> treatments decreased the ratio of root fresh weight to top fresh weight of seedlings (Table 3).

Copper in the seedcoat agent showed the most significant effect on seedling disease control. Both 1.0% Cu<sup>2+</sup> treatment and 4.0% Cu<sup>2+</sup> treatment had almost the same effect (Table 4). Because of disease infection, 91.7% of the control seeds (no copper in the seedcoat agent) failed to produce seedlings, and either rotted in the germination stage or in pre-emergence. Also, some of the seedlings produced from the control treatment seeds suffered from post-emergence disease and died early in their growth. Therefore, the total incidence of the disease in the control reached as high as 94.9%. For the copper treatments,

only 11.1% of the seeds were rotted during pre-emergence, and the total incidence of post-emergence disease was only 2.8%.

In conclusion, different concentrations of copper in seedcoat agent may have different effects. Generally speaking, the 1.0% Cu<sup>2+</sup> treatment not only improved germination but also had fewer detrimental effects on seed storage. Results indicate that the 4.0% Cu<sup>2+</sup> treatment was best for seedling growth, and had no significant effect on seed storage ability. For disease control, the 1.0% Cu<sup>2+</sup> treatment had almost the same effect as 4.0% Cu<sup>2+</sup> treatment.

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# The Relationship Between Low-temperature Germination and Chilling Tolerance in Cucumber

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**Introduction.** It is very important to improve chilling tolerance in cucumber (*Cucumis sativus* L.). In China, chilling tolerant varieties will not only decrease the production cost of winter-grown greenhouse cucumbers and raise their competitiveness in the market, but also save energy and reduce air-pollution (1). One of the main difficulties during cold-tolerant breeding is that selection requires large plant populations for screening. This is often expensive. In addition, the accuracy of selection is lowered when large populations are used. Thus, breeders have been searching for a convenient and cost-effective indirect selection method to identify cold tolerant cucumber varieties. In this paper, the relationship between low-temperature germination and chilling tolerance of cucumber seedlings is reported, and the possibility of indirect selection in cucumber chilling tolerance is discussed.

**Materials and Methods.** Fifteen cucumber lines with differing chilling sensitivity were used. The seeds of all lines were produced at the Vegetable Experiment Station of Northwestern Agricultural University in July 1993.

**I. Germination test.** Seed germination research was conducted in growth chambers with one chamber set at 15 C and another at 25 C (both without light). The experimental design was a randomized complete block with three replications (Test I). Seeds of each line were tested in 100 mm diameter petri plates into which two pieces of filter paper, 10 ml of distilled water, and 100 seeds were added. Three replications of 100 seeds each were used for testing in each of the two test temperatures.

After the second day of experiment initiation, the number of germinating seeds were counted daily. Seeds were considered germinated when a radicle reached half of the seed in length. The end of germination was taken when seeds no longer germinated. The mean germination days

(MGD) and germination index (GI) were calculated as follows:

$$MGD = \Sigma (G_t * D_t) / \Sigma G_t$$

$$GI = \Sigma (G_t / D_t)$$

where  $G_t$  = number of seed germinating at time  $t$ , and  $D_t$  = corresponding days of germination.

**II. Test of seedling cold tolerance.** The germinated seeds in Test I were sown in plastic pots filled with manure and soil (manure:soil=1:1; v:v). Seedlings at the third-leaf stage were moved to the two controlled environment (chambers). Plants in both experimental chambers were arranged in a completely randomized block design with three replications, with 12 plants per replication. In order to allow for seedling acclimation to the chamber environment, both chambers were kept in 25 C/15 C (day/night temperature) for 24 hr before the testing commenced. The temperature in one chamber was normal (25 C/15 C for 5 days, and 3 C for 1 day). In another chamber, the temperature was lower (20 C/10 C for 5 days, and 3 C for 1 day). All other conditions in both chambers were similar (irradiance was 33.7 w/m, 10 hours per day, RH was between 80 to 90%).

When the three treatments ended, temperatures in both chambers were returned to 25 C/15 C. Two days later, chilling injury of seedlings was investigated. Injury rankings (data not shown) were according to Li (3). The formula of chilling index (CI) was as follows :

$$CI = \Sigma (r * n) / (r_{max} * N)$$

where  $r$  = rank of injury,  $n$  = number of plants,  $r_{max}$  = the largest rank, and  $N$  = number of total plants examined.

**Results.** A variance analysis showed that no differences in MGD and GI existed at 25 C, but differences in MGD (X1) and GI (X2) at 15 C were detected (X1:F = 4.59\*\* ; X2:F = 6.71\*\*). This indicated that there was no differ-

ence in germinating ability among the various cucumber lines at 25 C, but that significant differences in low-temperature germination among cucumber lines existed at 15 C. The variances of chilling index at 20 C/10 C (X3) and that at 25 C/15 C (X4) were both significant (X3:F = 10.29\*; X4:F = 8.84\*\*). Data show that differences in chilling tolerance existed among the 15 cucumber lines tested. The results of genetic correlation analysis are presented in Tables 1-3.

Table 1. Phenotypic correlation matrix.

X1	1.000			
X2	-0.635**	1.000		
X3	-0.652**	-0.184	1.000	
X4	0.480**	-0.043	0.729**	1.000
	X1	X2	X3	X4

Table 2. Genetic correlation matrix.

X1	1.000			
X2	-0.738**	1.000		
X3	0.883**	-0.288	1.000	
X4	0.285	-0.092	0.361	1.000
	X1	X2	X3	X4

Table 3. Environmental correlation matrix.

X1	1.000			
X2	0.066	1.000		
X3	-0.335	0.299	1.000	
X4	0.091	0.168	0.355	1.000
	X1	X2	X3	X4

The phenotypic correlation matrix (Table 1) showed that strong correlations existed among 15 mean germinating days (X1), 20/10 CI (X3), and 25/15 CI (X4). The correlations between 15 MGD (X1), and 15 GI (X2), and between 20/10 CI (X3) and 25/15 CI (X4) were significant. The correlations between 15 GI (X2) and 20 C/10

C CI (X3) or 25/15 CI (X4) were, however, not significant.

The genetic correlation matrix showed that genetic correlations existed between 15 MGD (X1) and 15 CGI (X2) or 20/10 CI (X3). The genetic correlation coefficients between 25 C/15 CI (X4) and 15 MGD (X1) or 20/10 CI (X3), however, were not significant. Because the degree of chilling injury of cucumber seedlings under low-temperature stress can be measured by CI, CI can be used to determine the chilling tolerance of cucumber seedlings. Accordingly, CI with lower values indicate better chilling tolerance. It is evident that although chilling tolerance of cucumber seedlings growing under 20 C/10 C correlated with that of seedlings grown under 25 C/15 C, they did not correlate with each other. The same situation also existed between X1 and X4.

**Discussion.** Although it would be important to have the ability to select for chilling tolerance of cucumber indirectly, the present study does not completely clarify such selection. The research at the former IVT (now CPRO) showed that the proportion of leaf area of cold-tolerant cucumber lines was higher than that of controls (cold-sensitive lines) under 20 C/10 C, so it is possible to increase the ability of cold-tolerance through selecting fast-growing plants in seedlings stage under low temperature (2). The results of another study in former Soviet Union showed that cold-tolerance in cucumber could be increased significantly during selection by using dry matter content and leaf-area after chilling treatment as selection parameters. In this study, no difference in germination ability among 15 cucumber lines at 25 C existed, but significant differences in germination ability were detected at 15 C. Genetic correlation analysis indicated that MGD at 15 C positively correlated with CI at 20 C/10 C. This suggests that the slower cucumber seeds germinate under low temperature (15 C), the more severe cucumber seedlings injured by chilling. With regards to the genetic correlation, the association between CI at 20 C/10 C and CI at 25 C/15 C was not significant. A possible reason for this observation was that chilling acclimation at 25 C/15 C was ineffective, and thus the genetic potential of chilling tolerance of cucumber seedlings was not expressed.

The initial results of this study were: 1) that 15 C could be used to distinguish the low-temperature germinating ability of cucumber lines; 2) the mean germination days of cucumber and the cold tolerance of seedlings was

negatively correlated, and; 3) it is possible to screen the cold-tolerant cucumber germplasms and to select cold-tolerance of cucumber lines indirectly by testing their low-temperature germinating ability at 15 C. These results, however, require further investigation.

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# Ethylene Production and the Evaluation of Tolerance to Low-temperature in Cucumber (*Cucumis sativus* L.)

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**Introduction.** An apparently universal response to stress by plants is the production of ethylene. In addition to "normal" production of ethylene, as in fruit ripening, plants produce ethylene when they are stressed by low temperature (1). Stress ethylene is generally produced before visible symptoms appear, and appears to be a transitory phenomenon (1,2). When stress is removed, ethylene production normally reverts back to normal within 24 hr. Many studies have shown that the extent to which ethylene is released after chilling could provide a selection tool which would allow the ranking of different genotypes for tolerance to low temperature (1,2,3), although this application may be limited to some degree. The serviceability of this approach was investigated in the current study.

**Materials and methods.** Three cucumber (*Cucumis sativus* L.) accessions were used in the trials: 'Pingli' (chilling-tolerant), 'Xinong-145' (moderately tolerant) and 'Jinyan-4' (chilling-susceptible). Eight-day-old cucumber seedlings were acclimated under 12 hr day length with day and night temperatures of 15 C and 10 C, respectively, for 2 days. Seedlings were then chilled at 2.5 C for 24, 48 or 72 hr, and transferred to a 12 hr day length "resuming period" (day/night temperatures of 25 C and 18 C, respectively) prior to ethylene measurements. Four uniform seedlings per sample were sealed in 20 ml glass jars for 2 hr before ethylene measurement. Gas samples of 1 ml were taken from the jars and measured by gas chromatography. All tests were replicated twice.

**Results.** During the resuming period, 'Xinong-145' did not produce abnormal amounts of ethylene after chilling (2.5 C) for 24 hr. With low temperature treatment for 48 hr or 72 hr, during the resuming period there appeared two peaks of ethylene production within 72 hr. The two peaks appeared after resuming for 4 hr and 36-48 hr respectively. The first ethylene peak for the 72 hr treat-

ment was higher than for the 48 hr treatment. However, the 2nd ethylene peak for the 72 hr treatment was lower than the 48 hr treatment, but its duration was longer. Therefore, the optimum stress condition for the detection of stress ethylene production appears to be 2.5 C for 72 hr (Fig. 1).

Similar results were obtained when seedlings of the three cucumber accessions were chilled for 72 hr (Fig. 2). There were bursts of ethylene production during the resuming period, and peaks measured during the resuming periods of 4 hr and 36-48 hr corresponded well with the tolerance ability to low temperature in cucumber. The most tolerant cultivar, 'Pingli', had the highest ethylene peak value.

Based upon these experiments, when ethylene is to be used in the identification of low temperature tolerance ability in cucumber, the standardized detection program should be as follows: treat 8-day-old seedlings at 2.5 C for 72 hr, then detect ethylene production after resuming for 4 hr or 36-48 hr.

**Discussion.** Ethylene production was induced by low temperature, and may be an adaptation of the plant to chilling temperatures. During the resuming period after chilling, there were two peaks of ethylene production within 72 hr, and the second peak was accompanied by the physical breakdown of the seedling. It has been suggested that the amount of ethylene produced after chilling could serve as a selection indicator for tolerance to low temperature. If so, this technique could have many advantages, such as ease of measurement, high sensitivity, lack of interference from other metabolites, and non-destructiveness to the seedlings. The further development of this technique may result in increased efficiency for breeding for low-temperature tolerance in cucumber.



Figure 1. Ethylene dynamic state after chilling for different time.

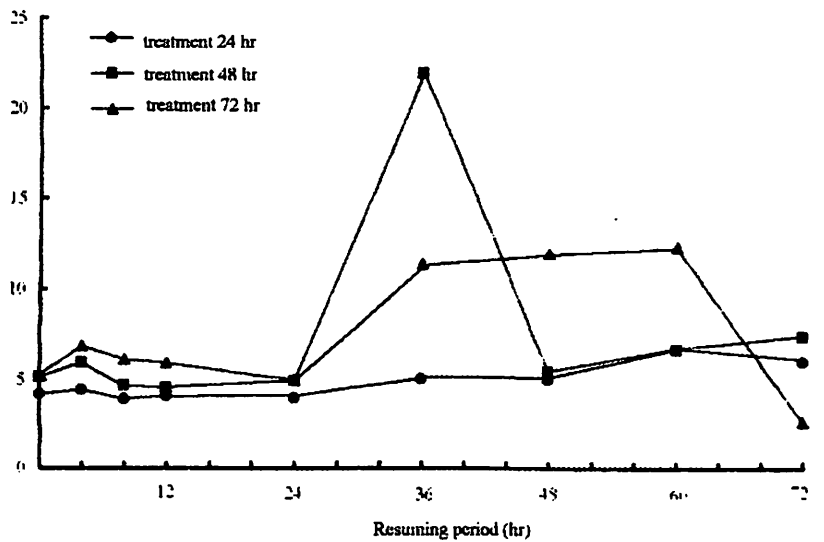
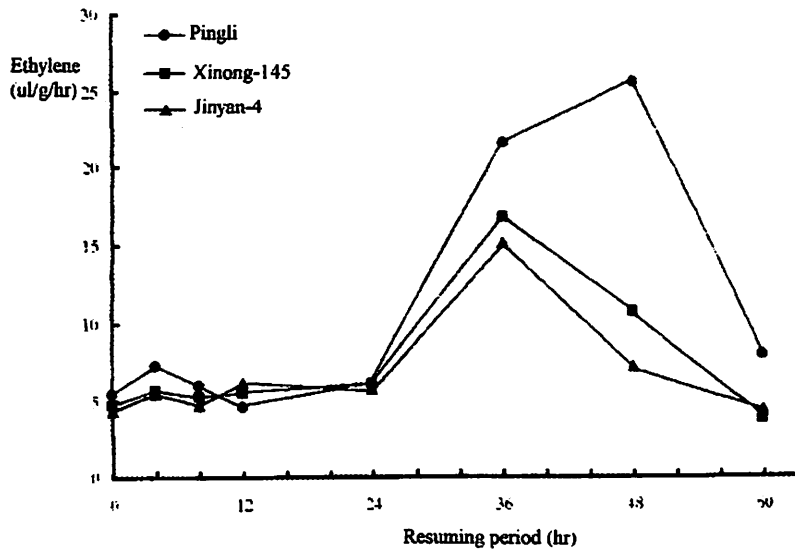


Figure 2. Ethylene dynamic state after chilling 72 hr in different cucumber cultivars.



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# Free Proline Contents and Catalase Activity in Cucumber Leaves At Elevated Temperatures

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**Introduction.** Heat tolerance of cultivars in cucumber is a very important character for mid-summer and early autumn production in north China (1,2). If one could distinguish the tolerance of cultivars and breeding lines by an index measure at some developmental stage, phenotypic selection could be possible. The purpose of our research was to identify a physiological index associated with heat tolerance in cucumber.

**Materials and Methods.** Five cultivars with different heat-tolerance sensitivities were selected, [Erzaози (No.1), Heidan-1 (No.2), 8113 (No.3), Jin-4 (No.4) and 3511 (No.5)] in order to study heat tolerance. Plants of each cultivar cultured 25 days in the three-leaf stage were heat treated at the temperature of 36 C and compared to control plants grown at 28 C. The free proline concentration and the activity of catalase in treated and controlled leaves of each cultivar were measured after 1 day, 3 days, and 5 days. Relative increment percentage (RIP) indicated relative plant reaction under heat stress and was calculated as:

$$\text{RIP} = \frac{[(\text{contents/activity at } 36 \text{ C}) - (\text{contents/activity at } 28 \text{ C})]}{(\text{contents/activity at } 28 \text{ C})} \times 100$$

**Results.** 1. *Free proline contents in leaves.* The RIP of each cultivar after 1, 3 and 5 days is shown in Table 1. The positive sign of all RIP values indicates that the contents of free proline in leaves accumulated after heat-treatment. Generally, RIP increased first and reached its maximum after 3 days under heat-treatment, and then

decreased after 5 days. At Days 1 and 5 after heat treatment, the RIP of heat-tolerant cultivars No.5 and No.1 was evident. At Day 3 the RIP of cultivars was variable and the RIP of cultivar No.5 was highest, followed by cultivar No.4 and No.3. The RIP of cultivar No.1 was lowest and RIP of cultivar No.2 was slightly higher than that of No.1. The more tolerant the cultivar was, the higher the observed RIP value. The RIP of free proline after 3 days of heat treatment was discriminating, and coincided completely with the heat tolerance ranking the cultivars examined.

2. *Activity of catalase.* The RIP of each cultivar is shown in Table 2. Data revealed that catalase activity decreased after 1 day of heat treatment, and the decrease in RIP of heat tolerant cultivars No.5 and No.4 was less than the other cultivars tested. Activity was restored gradually after Days 3 and 5 with the exception that cultivar No.4 which decreased again after 5 days of treatment. These results show that the heat tolerance of the cultivars examined was not consistent.

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Table 1. RIP of free proline contents in cucumber leaves subjected to heat stress (%).

Treatment	Cultivars				
	No.1	No.2	No.3	No.4	No.5
After 1 day	3.9	18.5	40.9	74.5	55.0
After 3 days	74.3	95.0	655.0	673.0	953.2
After 5 days	14.8	117.5	122.0	129.3	142.8

Table 2. RIP of catalase activity in cucumber leaves subjected to heat stress (%).

Treatment	Cultivars				
	No.1	No.2	No.3	No.4	No.5
After 1 day	-93.1	-93.2	-55.2	-11.8	-49.7
After 3 days	-44.8	-81.7	-31.5	105.5	-9.7
After 5 days	13.8	46.1	109.7	43.7	209.5

# Stand Correction Methods for Cucumber Fruit Yield

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**Introduction.** Field germination of cucumber (*Cucumis sativus* L.) seeds is highly dependent on environmental conditions. Cool, wet conditions during the spring planting season slows cucumber seed germination and increases seedling losses. Poor seed germination results in uneven seedling stands within and between plots and within and between fields. Yield comparisons are difficult to make when large differences in stand exist among plots. Several studies have determined fruit yield at different planting densities (5,8). However, a correction for yield based on plant spacing has not been published for cucumber. Cramer and Wehner (1,2,3) corrected cucumber plant stand for 15 to 30 plants per 3.1 m test plot. They assumed a linear increase in fruit yield per plot as stand increased linearly. That correction method may not be the best, since increases in fruit yield may not be linear at all plant densities. The objective of this research was to evaluate several methods for stand correction that could be used in studies of cucumber yield. The methods were judged on their ability to increase the amount of variance explained by the model (coefficient of determination values), and decrease the amount of variation in the data (coefficient of variability).

**Methods.** In previous studies (1,2,3,4), eight cucumber populations were evaluated for fruit yield per se, and for combining ability for yield when lines were crossed to elite testers. For the studies of yield *per se*, 3.1 m plots were thinned to 30 plants while for the combining ability studies, 1.2 m plots were thinned to 16 plants. In this study, we examined several stand correction methods as follows: 1) no stand correction, 2) analysis of covariance using stand as a covariate, 3) elimination of data from plots with 50% or less stand from the analysis, 4) elimination of data from plots with 50% or less stand from the analysis combined with analysis using stand as a covariate, and 5) elimination of data from plots with 50% or less stand from the analysis combined with linear correction of yield to a full stand based on yield per plant at the current density. Traits studied were total and early fruit

number per plot, percentage of marketable fruits per plot, fruit shape rating (6), and a simple weighted index, SWI (7). The analysis of variance model assumed replications as random effects and populations and cycles as fixed effects. Analyses were conducted using the different stand correction methods and the coefficient of determination values ( $R^2$ ) and coefficient of variability values (CV) were compared for each correction method.

**Results.** For both studies and all traits, stand correction methods increased the coefficient of determination values ( $R^2$ ) and decreased the coefficient of variability (CV) values when compared to the same values for no correction (Tables 1 and 2). The correction method which gave the highest  $R^2$  values and lowest CV values varied depending upon the trait for the fruit yield per se data (Table 1). Analyzing total yield using stand as a covariate increased  $R^2$  values and decreased CV values when compared to other correction methods. The method of eliminating data from plots with low stand counts and conducting a linear correction for yield based on 30 plants per plot,  $R^2$  and CV values were the best for both early yield and SWI. One particular correction method was no more effective than another method for improving the  $R^2$  and CV values for the percentage of marketable fruits per plot. Correction methods which involved eliminating data from plots with low stand counts increased the  $R^2$  values for fruit shape, but had little effect on the CV values.

When the number of replications was significantly increased (as in the studies where populations were crossed with a tester), a stand correction method which eliminated data from plots having low stand counts and analyzed data using stand as a covariate provided the highest  $R^2$  values and lowest CV values for all yield traits (Table 2). In addition, this correction method may give a more accurate representation of trait means since low stand plots eliminated from the analysis.

Table 1. Coefficient of determination ( $R^2$ ) and coefficient of variability (CV) values from an analysis of variance of five yield traits from eight cucumber populations using five methods of stand correction on data (1, 2).<sup>z</sup>

Correction method	Total fruits/plot	Early fruits/plot	% marketable fruits/plot	Fruit shape	SWI
<b>Coefficient of determination (<math>R^2</math>)</b>					
None	0.67	0.60	0.82	0.67	0.64
Stand covariate	0.70	0.62	0.82	0.67	0.67
Low stand plots eliminated	0.68	0.62	0.83	0.70	0.65
Low stand plots eliminated and stand covariate	0.70	0.62	0.83	0.70	0.66
Low stand plots eliminated and stand corrected	0.67	0.65	0.83	0.70	0.71
<b>Coefficient of variation (CV)</b>					
None	34	80	21	15	32
Stand covariate	33	78	21	15	31
Low stand plots eliminated	33	78	22	15	31
Low stand plots eliminated and stand covariate	32	77	22	15	31
Low stand plots eliminated and stand corrected	33	73	22	15	22

<sup>z</sup> The model included replications (16), populations (8) as whole plots, and cycles of selection (3) as sub plots. For stand correction methods 1 and 2, the number of observations per traits were 381 (total and early fruits/plot), 379 (fruit shape), and 378 (percentage of marketable fruits/plot, SWI). For stand correction methods 3, 4 and 5, the number of observations per traits were 353 (total and early fruits/plot), 352 (fruit shape), and 351 (percentage of marketable fruits/plot, SWI).

Table 2. Coefficient of determination ( $R^2$ ) and coefficient of variability (CV) values from an analysis of variance of five yield traits from eight cucumber populations using five methods of stand correction on data (3, 4).<sup>z</sup>

Correction method	Total fruits/plot	Early fruits/plot	% marketable fruits/plot	Fruit shape	SWI
<b>Coefficient of determination (<math>R^2</math>)</b>					
None	0.70	0.72	0.57	0.52	0.70
Stand covariate	0.76	0.73	0.57	0.52	0.72
Low stand plots eliminated	0.70	0.72	0.57	0.52	0.70
Low stand plots eliminated and stand covariate	0.77	0.75	0.63	0.54	0.74
Low stand plots eliminated and stand corrected	0.59	0.62	0.57	0.52	0.60
<b>Coefficient of variation (CV)</b>					
None	34	56	15	16	22
Stand covariate	31	55	15	16	21
Low stand plots eliminated	34	56	15	16	22
Low stand plots eliminated and stand covariate	28	52	13	16	20
Low stand plots eliminated and stand corrected	41	70	15	16	29

<sup>z</sup> The model included replications (88), populations (8) as whole plots, and cycles of selection (3) as sub plots. For stand correction methods 1 and 2, the number of observations per traits were 747 (total and early fruits/plot), 738 (percentage of marketable fruits/plot, fruit shape), and 737 (SWI). For stand correction methods 3, 4 and 5, the number of observations per traits were 702 (total and early fruits/plot), 696 (fruit shape), and 695 (percentage of marketable fruits/plot, SWI).

Stand correction methods helped to explain further the variance observed for particular traits while accounting for variation based solely on plant stand. Researchers should also examine the trait means using each correction method to determine which method accurately represents the means observed. A correction method based on a linear increase in fruit yield as stand increases linearly may inflate yields at certain planting densities. Further research is needed to determine the regression response of fruit yield to planting density.

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# Correlations Among Yield Components in Exotic Cucumber Germplasm

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**Introduction.** The genetic relationships among 13 botanical varieties of *Cucumis sativus* L. are presented in a companion paper in this issue. It is evident from that study that the botanical varieties examined differ in their morphology. Phenotypic correlations among morphological characteristics (fruit number, lateral branch number, fruit length and diameter and number of female nodes) of *C. sativus* var. *hardwickii* accessions has been documented (Kupper and Staub, 1988). Such correlations have also been examined in lines derived from var. *hardwickii* (Fredrick and Staub, 1989). Since *C. sativus* var. *hardwickii* differs considerably from var. *sativus* (commercial cucumber), it has been used as an outgroup in various diversity assessments in cucumber (Dijkhuizen et al., 1996; Meglic et al., 1996). Correlations between yield components can be important in breeding of cucumber using exotic germplasm. Therefore, a study was designed to investigate the association of yield components observed in a study of putative botanical varieties (companion article) and relate them to previous studies using *C. sativus* var. *hardwickii* and its derived inbred lines. This was done in order to determine whether the correlations for yield components observed among botanical varieties could be used in developing strategies for cucumber improvement.

**Material and Methods.** Parents and F<sub>1</sub> progeny are those given in a companion article in this issue. Phenotypic correlation coefficients were calculated for each character according to Becker (1992) and Steel and Torrie (1980). These were compared to data from Kupper and Staub (1988) and Fredrick and Staub (1989).

**Results and Discussion.** Cross-progeny in this study exhibited different values for all variables measured when compared to their parents (companion article) and themselves (Table 1). Many parents and progeny exhibited similarities with the var. *sativus* accessions examined. Although flowering dates were close to the

common var. *sativus* parent, the values of the variables were generally closer to the var. *sativus* inbred parent (WI 2780) than the botanical variety used in a particular cross. Values were higher in cross-progeny where the botanical variety was extreme for a particular trait (e.g., var. *falcatus* for L:D ratio, var. *sikkimensis* for lateral branch number).

Significant positive correlations were detected between lateral branch number and three-harvest yield, lateral branch number and three-harvest fruit weight, days to anthesis and three-harvest fruit weight, and three-harvest yield and fruit weight (Table 2). In studies by Kupper and Staub (1988) using var. *hardwickii* accessions crossed to var. *sativus* elite lines, moderate to large positive correlations were observed between fruit number and lateral branch number (Table 2). The positive relationship found between these two characters confirmed other studies (Horst and Lower, 1978; Hutchins, 1940). Moderate to large negative correlations occurred between fruit number and fruit size in studies by Kupper and Staub (1988). In that study, large numbers of fruit tended to occur on plants with considerable lateral branching, but the size of the fruit borne on those plants were relatively small. In a study using var. *hardwickii*-derived lines mated with var. *sativus* lines, Fredrick and Staub (1989) found that correlation coefficients were moderate to large between three-harvest yield and lateral branch number (Table 2). A high positive correlation was detected between fruit length and fruit L:D ratio. Negative correlations of intermediate range were detected between L:D ratio and three-harvest yields and lateral branch number.

Data from these studies and the present study indicate that positive correlations exist between yield and lateral branch number, and negative correlations exist between days to anthesis, lateral branch number and yield and L:D ratio in exotic germplasm (i.e., using documented and

Table 1. Comparisons among F<sub>1</sub> progeny of putative *Cucumis sativus* L. botanical varieties mated with a *C. sativus* var. *sativus* line (WI 2870) for sex expression, days to anthesis, lateral branch number (primary), and fruit number, weight and length:diameter ratio (L:D).

F1 hybrid/line <sup>1</sup>	Origin	Sex <sup>2</sup>	Days to anthesis	Lateral branch number	Three harvest yield	Three harvest weight (kg)	L:D ratio
var. <i>anatolicus</i>	Russia	G	23.5	1.3	88.3	47.0	2.5
var. <i>cilicicus</i>	Russia	G	23.8	0.0	82.3	50.1	2.5
var. <i>europaeus</i>	Russia	G	27.0	1.8	99.0	41.1	2.6
var. <i>falcatus</i>	Japan	PF	27.5	1.0	72.0	32.5	4.3
var. <i>indo-europaeus</i>	Russia	PF	26.5	1.5	93.3	38.3	2.1
var. <i>irano-turanieus</i>	Russia	PF	24.3	1.5	92.5	40.6	2.0
var. <i>izmir</i>	Russia	G	24.3	1.0	90.8	52.7	1.8
var. <i>sikkimensis</i>	Russia	PF	30.8	2.8	79.8	18.2	2.7
var. <i>squamosus</i>	Russia	PF	30.3	1.0	51.0	24.3	2.3
var. <i>testudaceus</i>	Russia	G	25.8	0.3	85.5	44.5	2.1
var. <i>tubercullatus</i>	China	G	23.8	0.0	79.3	58.7	2.2
var. <i>vulgatus</i>	India	PF	35.3	1.8	108.3	37.3	2.4
var. <i>hardwickii</i>	India	PF	35.3	3.5	188.3	29.1	1.9
var. <i>sativus</i> (Gy14)	USA	G	31.3	1.0	69.0	27.8	2.3
var. <i>sativus</i> (WI 2870) <sup>3</sup>	USA	G	24.3	0.0	76.5	47.0	2.4
LSD			2.7	0.6	16.3	6.9	0.3
C.V. (%)			25	25	15	17	10

<sup>1</sup>Hybrid made by crossing each botanical variety with the USDA pickling cucumber line WI 2870.

<sup>2</sup>M = monoecious, PF = predominantly female, and G = gynoecious.

<sup>3</sup>WI 2870 observed as a line.



Table 2. Correlation coefficients of morphological traits of F<sub>1</sub> hybrids of *Cucumis sativus* botanical varieties and *Cucumis sativus* var. *sativus* lines in three studies.

Study	Trait	Days to anthesis	Lateral branch number	Three harvest yield	Three harvest weight (kg)	L:D ratio
Kupper & Staub (1988) <sup>1</sup>	Days to anthesis		-0.02	-0.15	---	-0.12
	Lateral branch no.			0.74**	---	-0.48**
	Three harvest yield				---	-0.39*
	L:D ratio					---
Fredrick & Staub (1989) <sup>2</sup>	Days to anthesis		0.34	0.27	---	-0.33**
	Lateral branch no.			0.57*	---	-0.47*
	Three harvest yield				---	-0.65*
	L:D ratio					
Present Study <sup>3</sup>	Days to anthesis		0.41	0.41	0.68**	---
	Lateral branch no.			0.68**	0.67**	---
	Three harvest yield				0.50**	---
	L:D ratio					---

<sup>1</sup>Cross-progeny derived from matings between six var. *hardwickii* accessions and three inbred lines of var. *sativus*.

<sup>2</sup> Cross-progeny derived from matings between five var. *hardwickii* x var. *sativus*-derived lines and four var. *sativus* lines.

<sup>3</sup>Cross-progeny derived from matings between 13 botanical varieties of *C. sativus* and a var. *sativus* line WI 2870 .

putative botanical varieties of *C. sativus*). We suggest that these relationships be considered when developing germplasm enhancement strategies using exotic germplasm in cucumber.

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## Application of Inter-SSR Markers in Melon (*Cucumis melo* L.)

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**Introduction.** Molecular marker techniques are beginning to have an impact on plant breeding and plant genetic resource management. A variety of methods have been employed to evaluate polymorphism in melon (*Cucumis melo* L.), including RFLP, RAPD, SSR and AFLP (1,3,4,5,6). Our studies on SSR have demonstrated that microsatellite repeats are abundant in the melon genome. We estimate that a GA/CT repeat occurs at approximately 200kb intervals (Danin-Poleg et al., in preparation). This finding has led us to test the capacity of Inter-Simple Sequence Repeat (ISSR) markers (7) to discriminate melon cultivars. ISSR methods involve PCR amplification of DNA using a single primer composed of a microsatellite sequence anchored at the 3' or 5' end by 2-4 arbitrary, often degenerated, nucleotides. These primers target SSRs that are abundant throughout the genome and do not require prior knowledge of a DNA sequence. Amplification products can be separated on a polyacrylamide or an agarose gel. Here we report the use of ISSR markers in distinguishing melon genotypes representing both wide and narrow genetic backgrounds.

**Materials and Methods.** Eight *Cucumis melo* L. genotypes were tested: PI 414723, 'Dulce', 'Freeman's Cucumber', 'Noy Yizre'el', 'Galia', 'Arava', and 'C8' (Hegala) (the last three are F<sub>1</sub> Galia types all bred at Newe Ya'ar). DNA was isolated from the bulked leaf or root tissue of 10 plants of each genotype using a mini preparation procedure described by Fulton et al. (2).

PCR reaction mixtures for separation on agarose gel (non-radiolabeled) contained: 30 ng of plant genomic DNA, 2mM of Mg<sup>2+</sup>, 7.5 pmole of primer (Kit #9, USA), 166 mM of each of the dNTPs, 1x Taq Buffer (Advanced Biotechnologies, UK), 0.5 unit of Taq DNA polymerase (Advanced Biotechnologies, UK), in a total volume of 25 ml.

The amplification program was as follows: 7 min at 94 C, 30 s at 94 C, 45 s at 45 C, and 2 min at 72 C for 35 cycles on a thermocycler (PTC-100 MJ Research Inc.). PCR products were separated by electrophoresis in a 1.2% agarose gel (Pechcomp LTD 9201) for 4-5 hours and

stained with ethidium bromide. The size marker used was PBR 322 Alw441/MVA1 (MBI Fermentas). PCR reaction mixtures for separation on sequencing gel (radiolabeled) contained: 30 ng of plant genomic DNA, 1mM of Mg<sup>2+</sup>, 7.5 pmole of primer, 277mM of dATP, dTTP, dGTP, 3.3mM of dCTP, 0.1 ml of 3000 Ci/mmol [<sup>32</sup>P] dCTP or 3000 Ci/mmol [<sup>33</sup>P] dCTP, 1 x Taq Buffer (Advanced Biotechnologies, UK), 1 unit of Taq DNA polymerase (Advanced Biotechnologies, UK), in a total volume of 15 ml. The PCR conditions were the same as above but for 27 cycles. PCR products (3.0 ml/lane) were separated on a DNA sequencing gel containing 6% polyacrylamide, 8M urea and 1x TBE, at 60 W constant power for 3.5-5.5 h. After drying, the gels were exposed to a Kodak XAR-5 film (Eastman Kodak). Forty-two ISSR primers were used to amplify DNA from the eight melon genotypes.

**Result and Discussion.** The majority of the primers (26 primers, 62%) detected polymorphism among the eight melon genotypes. Eight primers (19%) did not differ between melon genotypes, giving a monomorphic pattern, and eight (19%) primers failed to amplify a clear product.

An average of 10 amplification products per primer for each genotype was separated on an agarose gel. Of these products 1 to 5 were clearly polymorphic. Amplification products of the primers that had yielded polymorphic patterns on an agarose gel were also tested on sequencing gels. The resolution of the sequencing gel was three times greater than that of the agarose gel. An average of 30 amplification products per primer for each genotype was detected on a sequencing gel with a minimum of 10 polymorphic products. Figure 1 and 2 depict separation using an agarose and sequencing gel, respectively.

ISSR was applied to assess polymorphism between parental lines of a mapping population developed between PI 414723 and 'Dulce' (*Cucumis melo* var. *acidulus* Naud. and *Cucumis melo* var. *cantalupensis* Naud.) (3). Twenty one (50%) of the 42 ISSR primers that were tested distinguished between PI 414723 and 'Dulce'. For

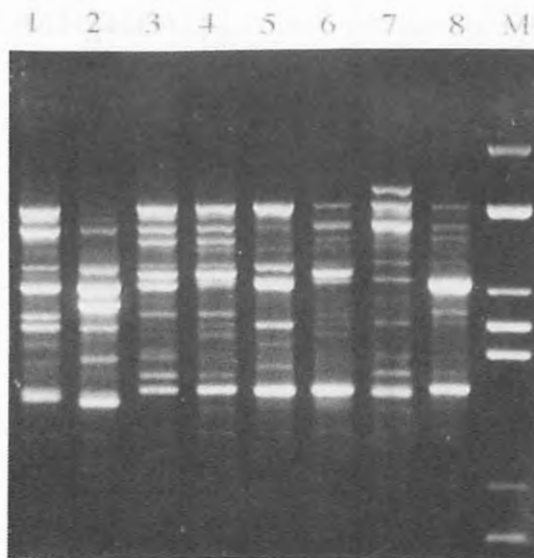


Figure 1. ISSR PCR polymorphism detected on an agarose gel using primer 807. Lanes 5 and 6 are PI 414723 and 'Dulce', respectively.

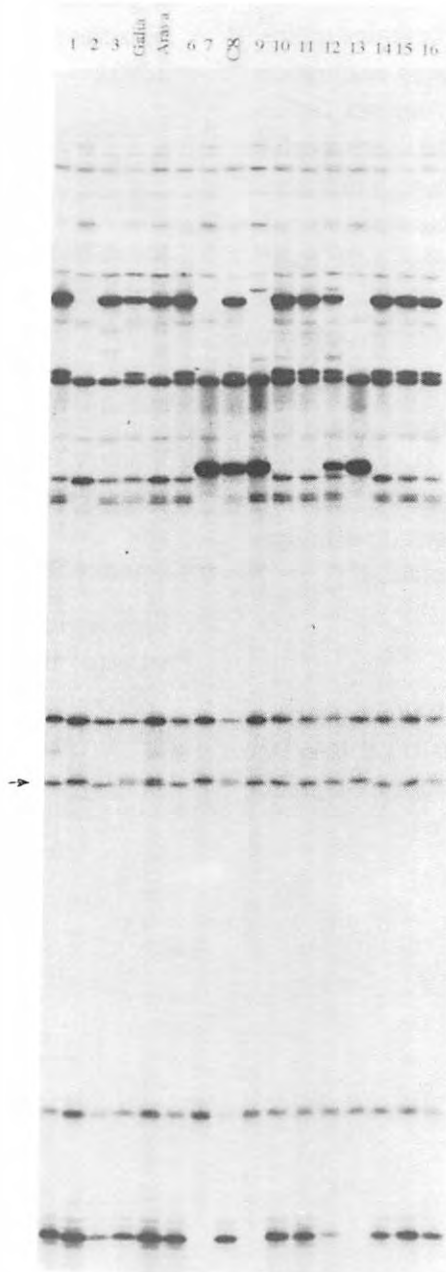


Figure 2. ISSR PCR polymorphism detected on a sequencing gel using primer 841.

comparison, 16 (59%) of 27 melon SSR markers and 25 of 104 (24%) RAPD primers distinguished between the same two genotypes (Danin-Poleg, in preparation).

The efficiency of the ISSR method was further demonstrated by distinguishing among closely related genotypes. Twelve of the 42 primers (29%) detected polymorphism (yielding 21 polymorphic products) between two breeding lines within the Galia group, representing a narrow genetic background. Using one primer we can clearly distinguish among the cultivars Galia, Arava and C8 (Fig. 2). The majority of the bands amplified using ISSR primers segregate as dominant markers, yet some segregate as codominant markers when scored in a segregating population (for example see the band marked by an arrow in Fig 2, where lanes 6 & 7 demonstrate the parental alleles while C8, the F<sub>1</sub> between them clearly demonstrate the heterozygote).

The results here suggest that ISSR markers are highly polymorphic and informative in melon. They are especially valuable for diversity studies and legal protection where multi-loci markers are advantageous. In addition they may be efficiently used for map saturation.

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# A Source of a High Level of Tolerance to Squash Mosaic Virus in a Melon from China

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Squash mosaic virus (SqMV) is one of the major viral agents affecting melons (*Cucumis melo* L.). It is seed transmitted, hence it can be found in every area of the world where this cucurbit is cultivated. In nature, it is spread by striped and spotted cucumber beetles (*Acalymna* and *Diabrotica* spp.) (1). Strains of this virus have been classified into two pathotypes. Type I includes melon strains, which cause prominent symptoms on melons and mild symptoms on squashes. Type II includes squash strains, which incite prominent symptoms on squashes and mild symptoms on melons (3).

In the last 35 years, our efforts to locate a melon source of resistance to both pathotypes of SqMV in cultivars, plant introductions, and landraces have been mostly unsuccessful. Some landraces from India, Afghanistan and Pakistan appeared to possess a low level of tolerance (4). Some tolerance was found also in a Chinese melon and a few landraces from the Indian subcontinent (7), but none were considered to be suitable for breeding programs. Four years ago we obtained seeds of a melon found in the Fujian Province of China in 1951 from a California gardener. For years, he grew it in his garden and sold some of the fruits in local Chinese market. He also claimed that it was very productive and resistant to diseases. After our seed increase, plants of this melon, designated 'China 51' (*C. melo* var. *makuwa*) were tested with each of the following cucurbit viruses: cucumber mosaic (CMV), papaya ringspot (PRSV), squash mosaic (SqMV), watermelon mosaic (WMV), and zucchini yellow mosaic (ZYMV). 'China 51' was found to be susceptible to PRSV, WMV and ZYMV, but it appeared to be uniformly resistant to CMV. A few plants failed to develop systemic symptoms with SqMV. Enzyme-linked immunosorbent assays (ELISA) and recovery tests confirmed the resistance to CMV. Plants that had remained symptomless with SqMV were found to be infected by the virus. The progenies of these few plants were highly tolerant to strains of SqMV belonging to both pathotypes. Considering that in melon, sources of resistance to CMV have been previously reported (2,5,6), our priority was directed to determine whether

SqMV was seedborne in 'China 51', and if the high level of tolerance could be retained through breeding.

Individual 'China 51' plants were inoculated with one of four SqMV strains of which two belong to the melon pathotype and the others to the squash pathotype. Seeds from these plants were individually planted in sterilized clay pots containing pasteurized soil. The resulting plants (200 for each strain) remained symptomless, but were periodically assayed to detect any viral infection. All the ELISA tests which were conducted using tissue from the 2<sup>nd</sup>, 10<sup>th</sup>, and 20<sup>th</sup> leaves of each plant, were negative. Hence, it appeared that none of the four strains of SqMV was able to infect any of the embryos of 800 seeds.

For genetic studies, 'China 51' was crossed with 'Honeydew'. One group of 20 plants of the resulting F<sub>1</sub> ('China 51' x 'Honeydew'), after two distinct inoculations with a melon pathotype (SqMV-NY93), developed persistent and prominent systemic foliar mosaic and plant stunting. Conversely, a second group of 20 F<sub>1</sub> plants of the same cross, reacted with a mild systemic mottling when infected with a squash pathotype (SqMV-NY88). One hundred twenty-eight plants of the F<sub>2</sub> ('China 51' x 'Honeydew') tested with the melon pathotype segregated in a ratio of 1 highly tolerant (30) to 3 susceptible (98). One hundred eighteen plants of the same F<sub>2</sub> cross inoculated with the squash pathotype segregated in a ratio of 1 highly tolerant (28), 2 partially tolerant (58), and 1 susceptible (32). Thus, the high level of tolerance displayed by 'China 51' with the melon pathotype appeared to be inherited recessively, whereas for the squash pathotype, it appeared to be partially dominant. However, considering that the partially tolerant plants developed some fruit symptoms, the high level of tolerance possessed by 'China 51' should be classified as recessive. More genetic studies are contemplated, including backcross populations. Preliminary studies on the inheritance of resistance to CMV involving crosses of 'China 51' x 'Honeydew' indicate that the resistance to this virus is also recessive.

Of particular interest were the fruits of the F<sub>1</sub> ('China 51' x 'Honeydew'), which externally, closely resembled those of the Honeydew, although the flesh was salmon, similar to 'China 51'.

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# Ethylene Production in Ripening Fruits of *Cucumis melo* var. *cantalupensis*, *C. melo* var. *inodorus*, and Their Hybrids

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**Introduction.** Melon (*Cucumis melo* L.) is a polymorphic species with regard to fruit characteristics, exhibiting diversity for shape, external and internal color, surface netting, sweetness, flavor, ripening pattern and storability. Fruits of the *cantalupensis* subspecies of melon typically exhibit a climacteric rise in ethylene and carbon dioxide production during ripening, while *inodorus* fruits produce little or no detectable ethylene as they approach maturity (Kendall and Ng, 1988; Wolff and Dunlap, 1995). The objective of the current study was to investigate ethylene production in fruits from crosses between *cantalupensis* and *inodorus* melons.

**Materials and Methods.** Two *cantalupensis* genotypes (MD 8725 and MD 8654) and two *inodorus* genotypes (MD 921HD and 'MaryGold') were used. Both MD 8725 and MD 8654 were netted, orange-fleshed cultigens, while MD 921HD was a green-fleshed honeydew and 'MaryGold' was a white-fleshed casaba. The *cantalupensis* lines abscised at fruit maturity and exhibited a short storage life, while the *inodorus* lines ripened slowly and failed to develop a distinct abscission layer.

F<sub>1</sub> hybrid seeds were obtained through controlled pollinations in the winter of 1995 in the greenhouse. Five hybrids were obtained: (1) MD 921HD x 'MaryGold', (2) MD 8725 x MD 8654, (3) MD 921HD x MD 8725, (4) MD 921HD x MD 8654, and (5) 'MaryGold' x MD 8654. Seeds were sown in a greenhouse and transplanted to the field on 30 June 1996. Plants were spaced 1.8 m within the row, and 1.9 m between rows. The experimental design was a RCB with three replications, each replicate consisting of four plants.

When fruits were ca. 25 days old (25 days post-anthesis), they were surface sterilized, and a sterilized stainless steel needle (12 gauge) fitted with a rubber septum cap was inserted into the cavity and secured to the rind with cyanocrylate ester glue. This allowed repetitive sampling of internal ethylene, beginning when the fruits were ca. 27 days old and still pre-climacteric. A 1 ml gas sample was extracted from each fruit at 9 a.m. each day, then analyzed by gas chromatography (Hewlett-Packard

5830A). Fruits which abscised at maturity were maintained in the field with additional sampling.

**Results and Discussion.** There were significant differences in ethylene peak day among the different genotypes (Figure 1). The earliest peak days (38-39 days) were exhibited by MD 8725 and its hybrids with MD 8654 and MD 921HD. 'MaryGold' had the longest period to peak ethylene production at 48 days. The MD 921HD x 'MaryGold' F<sub>1</sub> had the second longest period, at a value midway between the two parents.

Peak ethylene values for the nine cultigens are illustrated in Figure 2. On the average, the ethylene peak of the *cantalupensis* lines was 6 times that of the *inodorus* lines (137 ppm vs. 23 ppm). Ethylene peak values between the subspecies was intermediate at 66 ppm. The ethylene peak of MD 8725 (184 ppm) was twice that of MD 8654 (90 ppm), while the ethylene peaks of MD 921HD and 'MaryGold' were 30 ppm and 15 ppm, respectively.

Based upon these and other characteristics measured (data not shown), fruits of the *cantalupensis* lines reached the onset of the climacteric and initiated ripening earlier than the *inodorus* lines. They ripened and abscised rapidly and were highly perishable, whereas the *inodorus* fruits ripened slowly and could be stored for a longer time. The peak value of ethylene production appeared to be the most useful index for determining storage life of the cultigens and their hybrids.

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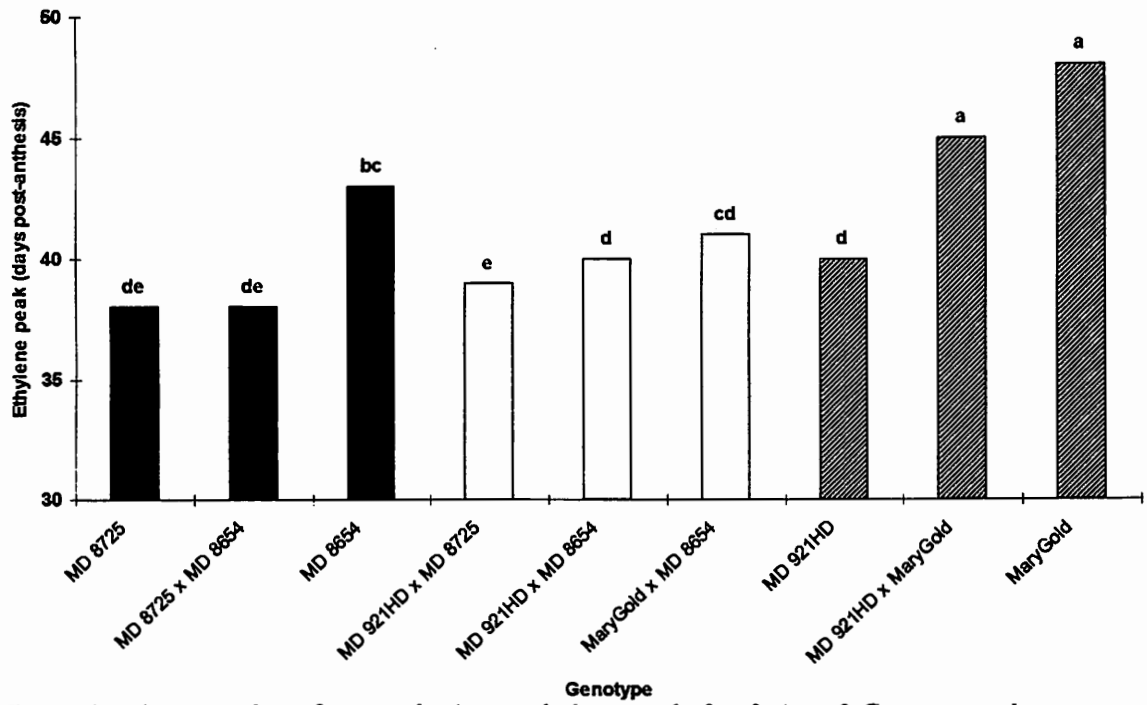


Figure 1. Average days from anthesis to ethylene peak for fruits of *Cucumis melo* var. *cantalupensis*, *C. melo* var. *inodorus*, and their hybrids.

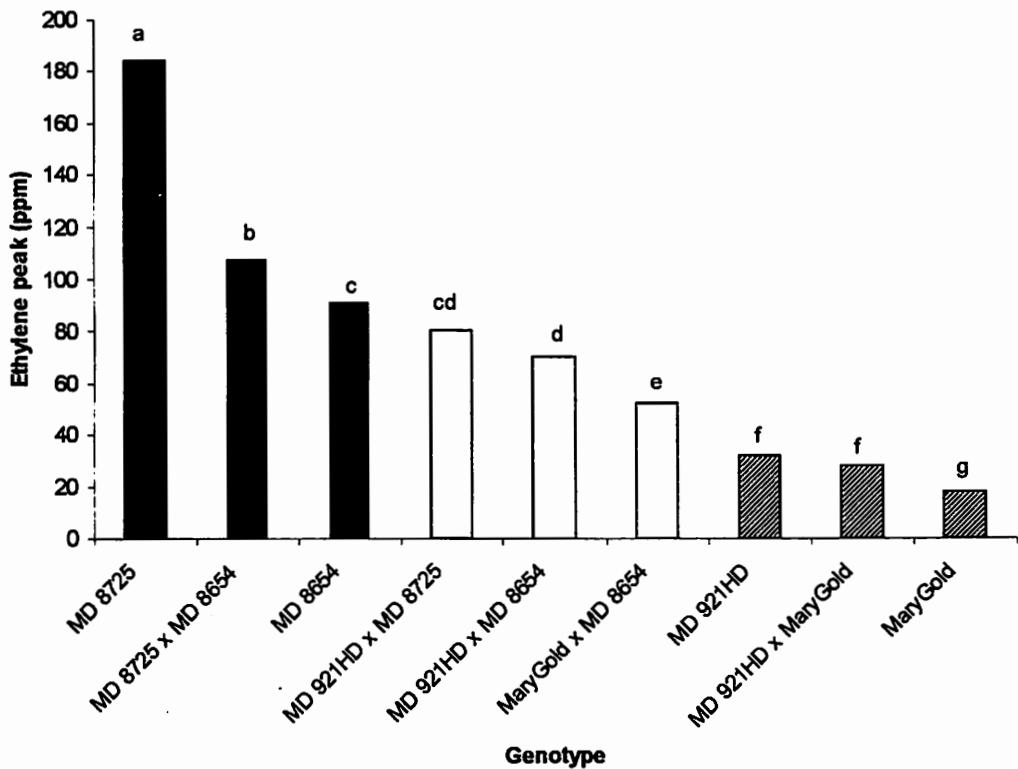


Figure 2. Average peak ethylene values for fruits of *Cucumis melo* var. *cantalupensis*, *C. melo* var. *inodorus*, and their hybrids.

# Identification and Distribution of Races of *Fusarium oxysporum* f. sp. *niveum* on Watermelon in Korea

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**Introduction.** *Fusarium* wilt in watermelon, caused by *Fusarium oxysporum* f. sp. *niveum* (E.F.Sm.) Snyder & Hans., which occurs throughout the world, is one of the most serious production problems confronting watermelon growers. The disease has been found wherever the crop has been grown in Korea. All commercial cultivars have been found to be susceptible to *Fusarium* wilt. Disease control in Korea has been accomplished by grafting watermelon on squash, but grafting is accompanied by high labor costs and reduced fruit quality.

Three pathogenic races (0, 1 and 2) of *Fusarium oxysporum* f. sp. *niveum* have been identified based on pathogenicity to a set of different cultivars (2,6). The races may also be distinguished by vegetative compatibility tests. Races 0 and 1 were first classified in 1972 by Citrulli, while race 2 was first described in Israel by Netzer (8). Many commercial cultivars have resistance to races 0 and 1, while race 2 is more aggressive and overcomes all currently available wilt resistant watermelon cultivars, except PI 296341-FR (3,5,7). Race 2 is now established in Texas, Oklahoma and Florida (1,4) and is becoming more common in Mississippi. This research was carried out to identify race distribution in Korea as part of a watermelon *Fusarium* disease resistance breeding program.

**Materials and Methods.** Single spore isolates of *F. oxysporum* f. sp. *niveum* were obtained in 1994 from stem sections of naturally infected plants cultured on Potato Dextrose Agar (PDA) medium. Isolates were tested for pathogenicity on a susceptible watermelon cultivar and stored on PDA. Inoculum of each isolate was prepared by growing the isolate for one week at 25 C in Potato Dextrose broth (200g potato, 20g dextrose

per liter) under continuous fluorescent light. Cultures were filtered through four layers of sterile cheese cloth, and suspensions were adjusted  $1 \times 10^5$  micro conidia/ml. Sterilized seeds of the following indicator plants, 'Sugar Baby', 'Charleston Grey', and 'Calhoun Grey' (Table 1), were planted in flats containing a 1:1:1 (v/v) mixture of vermiculite, perlite and peat. Three-week-old seedlings were root-dip inoculated for 5 minutes in isolate suspensions adjusted to  $1 \times 10^5$  microconidia/ml and transplanted into larger pots (12 cm diameter) containing the described potting mix. Seven to 12 plants were used per indicator cultivar and the experimental design was a random arrangement. Control plants were root-dip inoculated with distilled water. Wilt symptoms were recorded after 3 weeks. The following index was used: resistance 20 % wilt; susceptibility 80% wilt.

**Results and Discussion.** A total of 55 isolates of *F. oxysporum* f. sp. *niveum* were collected from 22 commercial watermelon fields in the South and Central areas of South Korea from March to October 1994 (see Figure 1). All commercial cultivars were susceptible to the disease. Pathogenicity tests conducted on 'Sugar Baby' indicated that 22 isolates were pathogenic, while 33 isolates were nonpathogenic. Three pathogenic races could be identified (Figure 1 and Table 2): race 0 at 2 locations; race 1 at 8 locations; and race 2 in 11 fields. However, only one isolate (race 2) was recovered per location.

This study documents the existence and distribution of three races of *F. oxysporum* f. sp. *niveum* in south Korea and the need to develop resistance in commercial cultivars to the three races.

Table 1. Watermelon differentials used to separate the three races of *Fusarium oxysporum* f. sp. *niveum*.

Host differential	Race <sup>2</sup>		
	0	1	2
Sugar Baby	S	S	S
Charleston Grey	R	S	S
Calhoun Grey	R	R	S

<sup>2</sup> R = Resistant; S = Susceptible

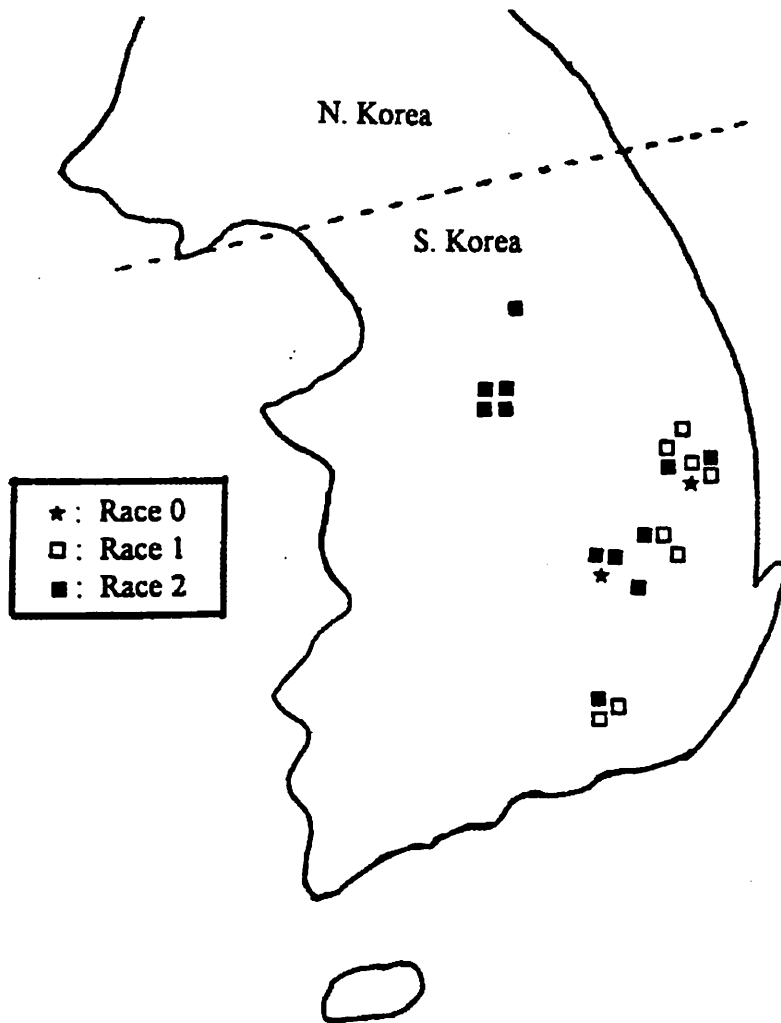


Figure 1. Distribution of races of *Fusarium oxysporum* f. sp. *niveum* on watermelon in Korea.

Table 2. Pathogenicity and race identification of isolates on *Fusarium oxyspoum* f. sp. *niveum* collected in watermelon fields in S. Korea.

Isolate number	Date (1994)	Pathogenicity <sup>y</sup>	Identification of races <sup>z</sup>			Race
			Sugar Baby	Charleston Grey	Calhoun Grey	
Fon-1	March 15	S	S	S	S	2
2	March 15	S	S	S	R	1
3	June 29	S	S	S	R	1
4	June 29	S	S	S	R	1
5	June 29	S	S	S	S	2
6	June 30	S	S	S	S	2
7	June 30	S	S	S	R	1
8	June 30	W	S	R	R	0
9	June 30	S	S	S	R	1
10	May 14	M	S	S	R	1
11	August 17	M	S	S	R	1
12	August 17	S	S	S	R	1
13	August 17	M	S	S	S	2
14	August 18	S	S	R	R	0
15	August 18	W	S	S	S	2
16	Sept. 14	S	S	S	S	2
17	Sept. 14	S	S	S	S	2
18	October 4	S	S	S	S	2
19	October 4	M	S	S	S	2
20	October 4	S	S	S	S	2
21	October 4	S	S	S	S	2
22	October 14	M	S	R	R	0

<sup>y</sup> S = Strong, M = Medium, W = Weak

<sup>z</sup> R = Resistant, S = Susceptible

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## New Resistance to Race 2 of *Fusarium oxysporum* f. sp. *niveum* in Watermelon

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**Introduction:** Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (E.F. Sm.) Snyder & Hans., which occurs throughout the world, is one of the most serious production problems confronting watermelon growers. Once a field is infested the Fusarium wilt pathogen may survive for many years, thus long-term crop rotations (5-10 years) are often used. One approach to controlling Fusarium wilt is grafting (3), which is efficient but requires intensive labor demands and results in reduced quality. However, genetic resistance has consistently proven to be the most effective and efficient means of control (1,2). At present, three races of *F. oxysporum* f. sp. *niveum* have been reported: race 0, race 1, and race 2 (8). The most recently described is race 2, which is a more aggressive pathogen than race 0 or race 1 (4). It was first observed in Israel in 1973 and the United States in 1981 (5,8). All currently known Fusarium wilt resistant cultivars are susceptible to race 2. One plant introduction, PI 296341-FR, has been found to be resistant to all 3 races of the pathogen (6,7).

**Material and Methods.** Isolates of the pathogen, collected in a commercial watermelon fields in South Korea, were identified and used for inoculation studies. An active culture of each isolate was incubated in liquid potato dextrose broth medium (200 potato, 20g dextrose per liter) on a shaker (80 rpm) at 25 C for one week under continuous fluorescent light. The race 2 suspension was filtered through four layers of sterile cheesecloth, and adjusted to  $10^5$  microconidia per milliliter. One liter of the microconidial suspension was mixed with 10 liters of autoclaved soil (1 vermiculite: 1 perlite: 1 peat). For disease screening, 15 seeds, pretreated with Benlate-T, from each of 124 watermelon lines or cultivars were planted in the Fusarium microconidia-soil mixture. Fusarium wilt resistance was scored 4 weeks after planting. The index of resistance was arbitrarily designated as follows: 1= 20% wilt, 2= 20-40% wilt, 3= 40-60% wilt, 4= 60-80% wilt, and 5= 80% wilt. In a second test, isolates of race 1 (18467) and race 2 (62939) from ATCC

(Rockville, MD) were used. Each isolate streaked onto PDA medium was incubated for 2 weeks under 12 hr fluorescent light at room temperature. Microconidial suspensions were adjusted to  $10^5$  microconidia per milliliter. Seeds were planted in 48 cell trays with Fafard No. 3-B soil mix (peat 45%, perlite and vermiculite) and grown for 3 weeks in a greenhouse. The 3-week old seedlings were root-dip inoculated with the microconidial suspension. Disease ratings were conducted 3 weeks after inoculation.

**Results and Discussion.** One hundred and twelve lines were screened for resistance to *F. oxysporum* f. sp. *niveum*, race 2. Most of the lines were susceptible to race 2, but PI 271769 was resistant (Table 1). Although PI 482261 and PI 482299 showed some resistance, both had wilt indices of 33.3% and were not considered resistant. PI 271769 plants survived 4 weeks of inoculation with the pathogen, while 74 lines showed more than 80% wilt. Although PI 271769 was not 100% resistant, field tests will be conducted to verify field resistance. In the second inoculation test, using the root-dip method, PI 271769 was completely resistant to race 1 and showed only 8% wilt after inoculation with race 2 (Table 2). 'Royal Sweet' showed 8% wilt to race 1 and 83% wilt to race 2. 'Sugar Baby' and 'Charleston Grey' were 87% and 92% wilt to races 1 and 2. Although cultivars known for their susceptibility to race 1 and race 2 failed to show 100% wilt symptoms, the results of both tests were comparable. In the race 2 root-dip inoculation test, PI 271769 showed 8% wilted. This could be due to genetic segregation of the resistance gene or experimental error. This new source of resistance should be useful in watermelon disease resistance breeding programs and as a host differential for race identification.

**PI Description.** PI 271769, collected in South Africa, can be recognized by large vines with large, light green leaves with shallow divisions at the leaf edge. The PI is andromonoecious, fruit are small (about 800g), round,

Table 1. Screening of watermelon cultivars and PIs in South Korea for resistance to race 2 of *Fusarium oxysporum* f. sp. *niveum*.

Index of Resistance <sup>z</sup>	No. of Lines	Line or cultivar names
1	1	PI 271769
2	2	PI 482261, PI 482299
3	9	PI 189317, PI 271773, PI 271779, PI 346787, PI 482291, Petite Sweet, Sunshade, 4N-Y, Chunryung,
4	26	PI 164708, PI 167126, PI 171392, PI 179662, PI 179878, PI 186490, PI 244019, PI 271363, PI 271778, PI 288232, PI 299378, PI 299379, PI 381752, PI 388770, PI 482322, Klondike, Deawang, T4-11-15, Sugar Doll, Indonesia, Egusi, Picnic, Jubilee, Candy Red, Warpaivt, Sunny Boy,
5	94	PI 164804, PI 164977, PI 179884, PI 182934, PI 183398, PI 185635, PI 186975, PI 189225, PI 189316, PI 203551, PI 234603, PI 244018, PI 248178, PI 249008, PI 255137, PI 269677, PI 270550, PI 271775, PI 296341, PI 306782, PI 346082, PI 381703, PI 381731, PI 381740, PI 381742, PI 386015, PI 386016, PI 386018, PI 386019, PI 386024, PI 386025, PI 432337, PI 457916, PI 481871, PI 482247, PI 482250, PI 482252, PI 482253, PI 482256, PI 482258, PI 482264, PI 482269, PI 482273, PI 482275, PI 482286, PI 482289, PI 482293, PI 482308, PI 482316, PI 482318, PI 482319, PI 482321, PI 482333, PI 482334, PI 482341, PI 494527, PI 494528, PI 494529, PI 494530, PI 494532, PI 500349, PI 500352, PI 512350, Sindaewha 2, Gabo, Deawhacream, Sugar Baby, Shindungtewha, Chunseo, New Hampshire Midget, T4-11-5, Kiwon, Family Fun, Buyeon, Egypt, T4-11-49, Noungwoomanri, Trial 351, New Asca, Moodungsan, China-1, Ukdeawha, Peacock, Chilean Black, Crimson Sweet, Charleston Grey, Congo, Calhoun Grey, Wimmera, All Sweet, Shindeawha 3, Klondike Striped, Jubilee II, Daegam,

<sup>z</sup> 1 = 0-20% wilt, 2 = 20-40% wilt, 3 = 40-60% wilt, 4 = 60-80% wilt, 5 = 80-100% wilt



Table 2. Screening of cultivars and PI for resistance to *Fusarium oxysporum* f. sp. *niveum* using the root-dip inoculation technique.

Cultivar	Percentage of wilt	
	Race 1 (ATCC 18467)	Race 2 (ATCC 62939)
Sugar Baby	92 %	92 %
Charleston Grey	87	92
Royal Sweet	8	83
PI 271769	0	8

light green with a spot of yellow, flesh is white, inedible, and has a very low sugar content (about 2.6° Brix), seeds are light green to brown. Contact the PI station at Griffin, GA, or YS Kwon for seed availability.

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## Combining Ability in Summer Squash (*Cucurbita pepo* L.)

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**Introduction:** Diallel analyses have been used extensively in plant species for genetic interpretation, which requires certain assumptions, and in a more practical way in the estimation of general combining ability (GCA) and specific combining ability (SCA), which are useful in devising breeding strategies (1,6). In the Cucurbitaceae GCA and SCA estimates have been reported mainly in *Cucumis sativus* L. and *Cucumis melo* L. (2,3,5) among others. In *Cucurbita pepo* L., which has been adapted also to hybrid production (9), information regarding combining abilities is far from complete. The objective of this report is to evaluate GCA and SCA in lines of summer squash, and to use this information when planning breeding programs.

The experimental material consisted of ten selected inbred lines (UR 4, 7, 18, 19, 30, 33, 34, 38, 52 y 53) derived from the highly variable summer squash population 'Tupungato Magnif INTA' (caserta type) and the diallel crosses including reciprocals. The lines were selected at an early stage for yield (one cycle top-cross) and light colored fruits. The experiment was established on 18 October 1996 at the Experimental Field of the Fac. de Cs Agrarias, UNR, located at Zavalla (33° 01' S; 60° 53' W), Argentina, in a randomized complete block design with three replication of eight plants, in a plant spacing of 1.4 m between rows and 0.80 m within plants in the row. The following variables were evaluated on a plot basis: Total fruit number (TFN) (12 harvests), precocious fruit number (PFN) (first three harvests), days from sowing to first harvest (DFH), number (LN) and diameter (LD) (cm) of expanded leaves and height of plants (PH) (cm). These last three vegetative characters were evaluated in three plants 30 days after sowing. The analyses were performed considering Griffing's model, method 1, model 1 (4) using the DIALLEL computer program (6). The weight of 100 seeds was used as a covariable. The relative importance of GCA and SCA was evaluated following (1). In order to meet normal distribution of residuals TFN, PFN and DFH were transformed by square root and LN by loge.

The GCA mean square was highly significant ( $p < 0.01$ ) for all variables except plant height, for which it was significant ( $p < 0.05$ ). The SCA and reciprocal mean squares were highly significant for all variables except for days to first harvest for which they were significant (Table 1). The ratio  $2\Phi_g / (2\Phi_g + \Phi_s)$  which is a measure of the relative importance of GCA (values close to unity) or SCA (values close to zero) was 0.45, 0.36, 0.50, 0.17, 0.37 and 0.19 for TFN, PFN, DFH, LN, LD and PH respectively. Non-additive gene actions were of major relative importance in the vegetative characters and precocious production. For total fruit number and days to first harvest additive and non-additive genetic variation accounted for nearly the same amount of the total genetic variation. In other cucurbits the GCA seems to play a major role (2,3,5). The best hybrids cannot be predicted simply on the basis of GCA of the parents alone. Breeding strategies should consider both selection *per se* of the lines (maybe in an early stage) and the testing of a wide range of hybrid combinations.

Differences between reciprocals were attributed in early reports (7,8) to maternal seed size. In our study some other maternal and/or non-maternal (specific reciprocal) effect would be responsible for such differences since the covariable weight of 100 seeds had failed to explain any significant variation of traits. The ratio  $\Phi_r / (2\Phi_g + \Phi_s)$  which is a measure of the relative importance of the reciprocal quadratic component in relation to genetic variation was 0.28, 0.23, 0.31, 0.30, 0.38 and 0.35 for TFN, PFN, DFH, LN, LD and PH respectively. With the same resources, not considering reciprocals, the number of lines to evaluate in diallel crosses can be increased approximately 37-41%. It seems reasonable to increase the chance of better combinations, leaving the evaluation of reciprocals to a latter stage of the breeding program, only in the outstanding experimental hybrids. Finally the quadratic components estimated in this report may be biased upward due to genetic x environment interaction. In this species the importance of this interaction is yet to be proven.

Table 1. ANOVA Griffing's analysis for total fruit number(TFN), precocious fruit number (PFN), days to first harvest (DFH), leaf number (LN), leaf diameter (LD) (cm) and plant height (PH) (cm) in a 10 lines complete diallel crosses.

Source of variation	df	Total fruit number		Precocious fruit number		Days to first harvest	
		M.S.	Sig	M.S.	Sig	M.S.	Sig
Genotypes	99	1.8258	**	1.3511	**	0.04015	**
G.C.A.	9	5.2287	**	3.4905	**	0.10885	**
S.C.A.	45	1.4697	**	1.2052	**	0.03231	*
Reciprocal	45	1.5009	**	1.0691	**	0.03443	*
Covariable <sup>2</sup>	1	0.4503	ns	0.3417	ns	0.01498	ns
Error	197	0.9561		0.7041		0.02382	

Table 1 cont.

Source of variation	df	Leaf number		Leaf diameter		Plant height	
		M.S.	Sig	M.S.	Sig	M.S.	Sig
Genotypes	99	0.034836	**	14.6830	**	24.2072	**
G.C.A.	9	0.053583	**	34.5032	**	35.5655	*
S.C.A.	45	0.035160	**	12.2111	**	23.6690	**
Reciprocal	45	0.030767	**	13.1938	**	22.4807	**
Covariable <sup>2</sup>	1	0.001208	ns	4.4192	ns	20.8769	ns
Error	197	0.018717		7.6084		14.7638	

\*\* p<0.01

\* p<0.05

<sup>2</sup> 100-seed weight

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## Heterosis in Summer Squash (*Cucurbita pepo* L.)

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**Introduction.** Cultivated *Cucurbita* species have not followed the general pattern of inbreeding and heterosis of other cross-pollinated crops like maize and onions (9). In early reports (1,4,8) it was noticed that inbreeding may not necessarily decrease vigor and yielding capacity and that selected inbreds could equal or outyield commercial checks. Nevertheless significant heterosis have been reported in crosses between certain varieties of *C. pepo* L. and *C. maxima* L. (2,5), encouraging hybrid production.

It is convenient to specify a reference population when dealing with heterosis (6). The heterosis ( $h_I$ ) in the cross between two inbred populations is an expression of  $h_I = h_0 + I$ . The first component,  $h_0$ , is the heterosis expressed in crosses between non-inbred populations (interpopulation level). The second,  $I$ , is any inbreeding effect in the populations and will increase heterosis in the same amount (6). Heterosis in this sense (intrapopulation level) has rarely been reported in *C. pepo* L. The present reports aims to evaluate heterosis in lines derived from a summer squash population (*Cucurbita pepo* L.).

The experimental material consisted of ten selected inbred lines (UR 4, 7, 18, 19, 30, 33, 34, 38, 52 and 53) derived from the highly variable summer squash population 'Tupungato Magnif INTA' (caserta type) and the diallel crosses including reciprocals. The experiment was established on 18 October 1996 at the Experimental Field of the Fac. de Cs Agrarias, UNR, located at Zavalla (33° 01' S; 60° 53' W), Argentina, in a randomized complete block design with three replication of eight plants, in a plant spacing of 1.4 m between rows and 0.80 m within plants in the row. The following variables were evaluated on a plot basis: total fruit number (TFN) (12 harvests), precocious fruit number (PFN) (first three harvests), days from sowing to first harvest (DFH), number (LN) and diameter (LD) (cm) of expanded leaves and height of plants (PH) (cm). These last three vegetative characters were evaluated in three plants 30 days after sowing. An ANOVA was performed, partitioning the genotype source of variation into parents, crosses and the contrast parents vs. crosses. The significance of this contrast was used as a test of heterosis (3). The CON-

TRAST statement of GLM, SAS, was used (7). In order to meet normal distribution of residuals TFN, PFN and DFH were transformed by square root and LN by loge.

The contrast parents vs. crosses was highly significant ( $p < 0.01$ ) for LN, LD and PH ( $F = 10.21, 11.8$  and  $10.54$  respectively; 1, 203 df) and significant ( $p < 0.05$ ) for TFN and PFN ( $F = 4.75$  and  $6.02$ ; 1, 203 df). For these characters mean values and heterosis (best parent) of the five outstanding crosses are shown in Table 1. On average heterosis was superior for precocious fruit number, followed by total fruit number, leaf number and plant height. These results confirm, as it was reported in variety crosses, that at an intrapopulation level significant heterosis could be achieved, especially for early production. In breeding programs, in order to meet maximum hybrid yield, both of the above components of heterosis should be pursued. In a population breeding scheme, where fruit color and plant habit traits can be fixed in the first selection cycles, the inbreeding and crossing method could be a useful tool in increasing population means for yield traits through hybrid or synthetic variety production.

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Table 1 Means and heterosis (expressed as percentage of the best parent of each cross) of five outstanding crosses for total fruit number (TFN), precocious fruit number (PFN), leaf number (LN), leaf diameter (LD) and plant height (PH).

Total fruit number			Precocious fruit number			Leaf number		
Cross	Mean (fruits/ha)	Heterosis %	Cross	Mean (fruits/ha)	Heterosis %	Cross	Mean (No.)	Heterosis %
19 x 53	136,272	38.4	19 x 52	13,616	162.9	53 x 34	8.64	34.3
18 x 53	132,109	34.2	18 x 53	12,734	375.4	33 x 53	8.26	19.7
33 x 19	130,412	32.8	30 x 33	12,131	24.9	18 x 53	8.24	27.3
19 x 52	130,412	41.1	7 x 4	10,926	129.2	19 x 33	7.80	9.24
53 x 19	127,778	29.8	33 x 30	10,825	11.5	53 x 7	7.75	20.5
Average	131,396	35.2		12,046	140.7		8.13	22.20

Leaf diameter			Plant height		
Cross	Mean (cm)	Heterosis %	Cross	Mean (cm)	Heterosis %
34 x 19	28.20	14.5	30 x 18	35.10	36.0
18 x 33	28.10	22.5	33 x 18	33.83	29.1
7 x 18	27.76	20.9	18 x 19	33.20	-4.4
18 x 52	27.30	13.4	34 x 53	33.06	25.1
53 x 7	27.16	18.3	33 x 34	32.60	24.4
Average	27.70	17.9		33.55	22.04

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# A Simple Protocol for Isolating DNA from Fresh *Cucurbita* Leaves

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**Introduction.** Many DNA isolation protocols have been published, and there are many kits available from scientific suppliers. However, no protocols have been published specifically for use with the *Cucurbita* species. Most of the available kits are based on the alkaline lysis method of DNA extraction. This method has been found to be unsatisfactory for leaf tissue from all but the youngest squash plants because of the presence of secondary compounds. Many CTAB-based protocols have been published, however most require the leaf tissue to be frozen in liquid nitrogen or lyophilized, resources that are not always available. While polysaccharides have been the most commonly reported problem in DNA extraction from cucumber and melon (1,3), other compounds that bind to the DNA during extraction and interfere with PCR seem to be more of a problem in squash. The PVP-40 in the extraction buffer effectively binds these compounds (4).

The following protocol was adapted from published CTAB protocols (2,5) with the goals of being able to use fresh tissue and having a relatively fast and simple protocol. The protocol is given for a sample size of approximately 0.5 g of fresh leaf tissue but it can be readily scaled up or down. This is a convenient sample size because it is readily obtained from a seedling 10-14 days after planting without killing the plant, and it yields sufficient DNA for many PCR reactions.

**Materials and Methods.** For extraction of squash genomic DNA one can use partially expanded leaves from plants of any age. The leaves are ground fresh, without freezing or drying. The best results are obtained if DNA is extracted immediately after the leaves are harvested, but they can be stored in plastic bags in the refrigerator for up to two weeks. The leaves can be ground in grinding buffer in sample grinding pouches (Agdia). The pouches are convenient in that they are sterile, can also be used for collecting and storing samples, and avoid the need to filter the homogenate.

## Solutions:

1. *Grinding buffer:* 0.5 M Tris (pH 8.0), 20 mM EDTA (pH 8.0), 2.0 M NaCl.
2. *DNA extraction buffer:* 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 2 M NaCl, 2% (w/v) CTAB (acetyltrimethylammonium bromide), 2% (w/v) PVP-40 (polyvinylpyrrolidone). (Note: It is important to add the extraction buffer ingredients in the order shown, and to fully dissolve the CTAB before adding the PVP-40, or the mixture will clump. Dissolve the CTAB and the PVP-40 by stirring gently on a hotplate. Store the extraction buffer above 15 C to avoid precipitating the CTAB. Most protocols add  $\alpha$ -mercaptoethanol to the extraction buffer, however it appears to be unnecessary for squash.)
3. *Washing buffer:* 80% ethanol, 10 mM ammonium acetate.
4. *Other reagents:* chloroform:isoamyl alcohol (24:1), isopropanol, TE buffer, RNase A, 95% ethanol, 3 M sodium acetate.

## Protocol:

1. Preheat the extraction buffer to 65 C. Chill the grinding buffer to 0 C. Grind approximately 0.5g fresh leaf tissue in 2ml grinding buffer. Transfer to a 10ml centrifuge tube. Hold samples on ice until adding 2ml extraction buffer. Shake the tubes gently to mix the buffers and place in a 65 C water bath for 30 minutes with periodic shaking. (Note: It is essential that the extract (sap + buffers) remain at pH 8.0 to prevent precipitation of the DNA.)
2. Add 3.5 ml chloroform:isoamyl alcohol at room temperature and mix for 10 minutes by gentle inversions.
3. Centrifuge for 10 minutes at 11,000 x g to separate the phases.



4. Using a sterile plastic transfer pipette, transfer the aqueous layer to a fresh tube containing 2.5 ml ice-cold isopropanol.
5. A layer of DNA may be immediately visible at the top of the sample. The DNA can be spooled out, but yields will increase if the tubes are inverted gently to mix the sample, and the DNA allowed to precipitate overnight at room temperature.
6. Centrifuge for 5 minutes at 650 x g to pellet the DNA. The pellet should be off-white or brown.
7. Pour off the supernatant, drain the tubes on a tissue, and add 8 ml washing buffer. Invert gently to wash the pellet until it is white.
8. Centrifuge for 10 minutes at 650 x g. Carefully pour off the supernatant and drain the tubes. Air-dry the pellet until no smell of ethanol remains.
9. Resuspend the pellet in 500  $\mu$ l of TE buffer. Transfer to a 1.7 ml centrifuge tube. The sample will contain a mixture of DNA and RNA.

If the presence of RNA in the sample will interfere with quantification or use of the DNA, follow steps 10-13:

10. Add 3  $\mu$ l RNase A to each sample. Mix well, and incubate for 15 minutes at 37 C with occasional shaking.
11. Add 50  $\mu$ l 3M sodium acetate and 1 ml 95% ethanol to each tube and mix thoroughly. The DNA should be clearly visible.
12. Centrifuge for 1 minute at maximum speed in a microcentrifuge to pellet the DNA. Pour off the supernatant, drain the tubes and air-dry the pellet.
13. Resuspend the DNA in 500  $\mu$ l of TE buffer. Store at 4 C (short term) or at -80 C (long term).

This protocol has been used with leaf tissue from *Cucurbita pepo* and interspecific *C. pepo* x *C. moschata* hybrids. It should be effective with any *Cucurbita* species, although the addition of 0.25 mg activated charcoal per gram of tissue may be necessary with species containing higher levels of secondary compounds. Average yield was 240  $\mu$ g of DNA per gram of leaf tissue with a ratio A260/A280 between 1.8 and 2.0. These yields are lower than those reported by Baudracco-Arnas for melon (1), but much of that can be attributed to the use of fresh rather than dried tissue. The DNA is not degraded, is in pieces of approximately 4000 bp each, digests to completion and amplifies in the PCR process.

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# Genetic Diversity in Pumpkins (*Cucurbita pepo* L.) as Revealed in Inbred Lines Using RAPD Markers

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**Introduction.** Pumpkin is grown in Austria on approximately 8000 ha. The specific hull-less form *Cucurbita pepo* var. *styriaca* ( $2n=2x=40$ ) has facilitated easy seed-oil production since the beginning of this century. Pumpkin seed oil enjoys a growing popularity. Testing inbreds for combining ability in the field is especially expensive for pumpkins, and therefore indirect selection methods would be of great advantage.

Molecular markers have been used for predicting heterosis in maize and rape seed with encouraging results (2,3,4,5). Compared with all other marker types, RAPD markers stand out for quick and simple handling and low costs. Outbreeding crops often show a high level of polymorphism (e.g. 1,6).

Here we report the results of a preliminary study on the use of RAPD markers to estimate the extent of polymorphism and calculate genetic diversity in 20 inbred lines of pumpkin. The data obtained could aid in the selection of parents for heterosis breeding.

Twenty inbred lines (selfed for six generations) were provided by a private plant breeding company, Saatzucht Gleisdorf, Styria, Austria. Six seeds of each genotype were grown in the greenhouse.

**Materials and Methods.** *DNA isolation:* Leaves were frozen in liquid nitrogen and ground to fine powder in a mortar, transferred to a centrifuge tube with 10 ml extraction buffer (50 mM TrisHCl pH 8.0, 50 mM EDTA pH 8.0, 0.7 M NaCl, 1% CTAB, 0,1%  $\beta$ -mercaptoethanol); 20  $\mu$ l proteinase K and 1 ml SDS (10%) were added. After 30 min incubation at 65 C and cooling to room temperature 10 ml chloroform/isoamylalcohol (24:1) were added and the mixture was centrifuged (15 min, 3500 rpm). Isopropanol (7 ml) and 1 ml NaAc (3 M) were added to the upper phase and the nucleic acids were precipitated at -20 C for one hour. It was followed by centrifugation (15 min, 3500 rpm), redissolving of the dry pellet in 5 ml TE (pH 8.0, 10 mM TrisHCl, 1 mM EDTA), addition of 0.5  $\mu$ l RNase and incubation at 37 C

(20 min), precipitation with 5 ml isopropanol and 0.5 ml NaAc (3 M) 30 min at -20 C and centrifugation (15 min, 3500 rpm). The resulting pellet was redissolved in 2 ml TE (pH 8.0). After adding 5 ml ethanol (96%) and 0.2 ml NaAc and 15 min centrifugation the pellet was washed in 70% ethanol and the final pellet again was redissolved in TE (pH 8.0).

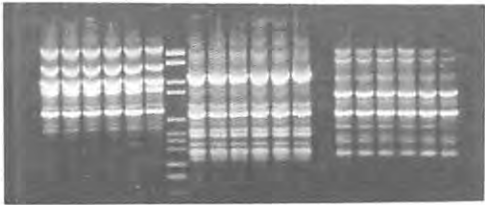
*PCR conditions:* 100 ng of genomic DNA were used in 25  $\mu$ l-volume amplification reactions containing 0.3  $\mu$ M (0.6  $\mu$ M in reactions that had to be repeated) 10mer random primer (Advanced Biotechnologies Ltd., kit 2 and 3), 1x reaction buffer, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTP and 1 Unit Taq polymerase.

*Temperature program of the thermal cycler (PCR machine PTC-100, MJ Research):* Initial denaturation of 60 seconds at 94 C followed by 34 cycles of 60 seconds at 94 C, 45 seconds at 36 C and 30 seconds at 72 C. After a final extension step of 5 minutes at 72 C the amplified DNA samples were stored at a temperature of 4 C.

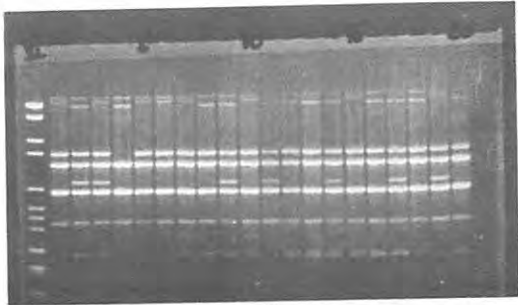
Fragments were separated in 1.5% agarose gels stained with ethidium bromide and photographed with a polaroid camera. After testing the lines for homogeneity (Fig. 1a), DNA of the six individual plants was pooled for further analysis.

**Results and Discussion.** *Data analysis:* Out of 40 primers tested three gave no amplification product at all (2,10, 3,2, 3,3), and three produced only monomorphic bands (3,12, 3,19, 3,20). With the remaining 34 primers we obtained a total of 116 polymorphic loci (Fig. 1b). According to band intensity, data were arranged in three data sets. The total number of bands represented data set 3. Data set 1 contained 56 strong bands only; 100 bands, strong and intermediate, were allocated to data set 2. The number of polymorphisms detected per primer was 3.4. Between the distance data based on data set 1 and 2 we found a correlation of 0.91 compared with a correlation coefficient of 0.72 between distance data derived from set 1 and 3. Although this correlation is high enough to

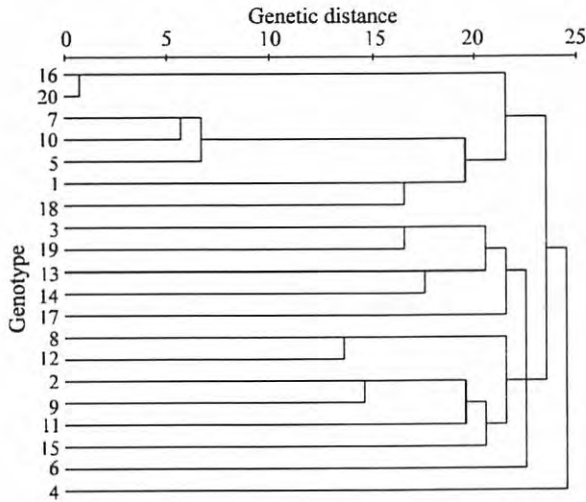
Fig. 1: Testing an inbred line for homogeneity using six individual plants (a); example for polymorphism among the 20 inbreds with one primer (b); UPGMA clustering of 20 inbred genotypes by RAPD (c); comparing F1s (in the middle) with parental inbreds (d).



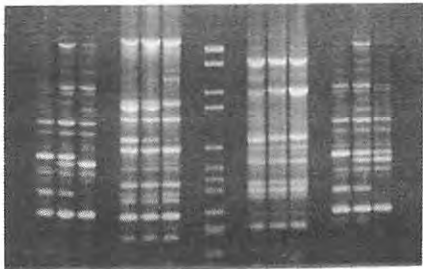
a



b



c



d

permit the use of faint bands if the total number of amplified loci is limited, in this study data set 2 was considered for the cluster analysis.

With an average genetic distance of 0.36 the distance data ranged from 0.009 and 0.537. The cluster analysis following the UPGMA method classified the 20 pumpkin genotypes in three main groups (Fig. 1c). Details of this dendrogram agree with pedigree information received from the breeder. For example, lines 16 and 20, located very close to each other in the dendrogram, represent two genotypes which were separated late in the inbreeding process. This also applies to the lines 5, 7 and 10. In contrast, line 4 - being the most distant to the rest in the dendrogram - was a genotype of recent US origin.

Comparing the banding patterns of F<sub>1</sub> individuals with their parental lines gave good indication on the stability of RAPD markers in general. It confirmed that hybrids and their corresponding parents can clearly be identified by RAPD analysis (Fig. 1d). Exceptions in conformity of the banding patterns could be explained by residual heterogeneity in the inbred line.

We found that RAPDs represent a fast and cost-efficient simple technology for studying the pumpkin genome. RAPD appears to be highly suitable for the construction of a linkage map and for marker-aided selection.

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## Some Observations Concerning Diversity in the Subspecies and Horticultural Groups of *Cucurbita pepo*

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*Cucurbita pepo* is a highly polymorphic species. On the basis of seed morphology and allozyme variation, this species has been divided into two subspecies, *pepo* and *ovifera* (1). The eight horticultural groups of the species (7) can easily be recognized as belonging to one or the other of the subspecies. The pumpkin, vegetable marrow, cocozelle and zucchini groups belong to subspecies *pepo* whilst the scallop, acorn, crookneck, and straight-neck groups belong to subspecies *ovifera*.

Besides the differences in seed morphology and allozyme variation, the subspecies are differentiated from each other by an entire set of phenotypic characteristics. The horticultural groups belonging to ssp. *pepo* almost always have larger fruits, larger flowers, larger seeds, and thicker peduncles than those belonging to ssp. *ovifera*. The fruits of ssp. *pepo* are smooth or grooved to very prominently ribbed (protrusion of the vein tracts) whereas those of ssp. *ovifera* are smooth to very prominently furrowed (depression of the vein tracts). Flecking in the fruit rind color is larger and denser in ssp. *pepo* than in ssp. *ovifera*. Silver leaf mottling, conferred by gene *M*, is common in ssp. *pepo* but almost non-existent in ssp. *ovifera*. Wartiness of the fruits, conferred by gene *Wt*, is rare in ssp. *pepo* but common in ssp. *ovifera*. Yellow fruit color, conferred by gene *Y*, occurs only in cultivars of ssp. *ovifera*. The horticultural groups of ssp. *pepo* contain a much larger number of cultivars and more phenotypic variation than do those of ssp. *ovifera*.

In addition to the fruit shape differences defining them, it can be seen that the horticultural groups of *C. pepo* ssp. *pepo* are differentiated from one another fairly well on the basis of various other phenotypic characteristics, some of which are conferred by known genes. For example, seeds of zucchini cultivars are almost always shorter and more plump than those of the other groups whereas seeds of pumpkin cultivars are almost always longer and thinner. The seeds are distributed in a cavity along nearly the entire length of the fruit in the pumpkins, zucchini, and vegetable marrows but in the cocozelles

the seed cavity is restricted to approximately one-third of the length, near the stylar end. Cocozelles tend to have the largest pistillate flowers, except for some of the largest-fruited cultivars of pumpkins (9). The top-dominant *L-1* allele is present in nearly all zucchini cultivars, but uncommon in the other groups. Alleles for fruit striping at the *l-1* locus are common in the cocozelle group but otherwise infrequent. The recessive *l-2* allele, otherwise rare, is common in the vegetable marrow group. Thus, most pumpkins are *l-1/l-1 L-2/L-2*; most vegetable marrows are *l-1/l-1 l-2/l-2*; most cocozelles are *l-1<sup>St</sup>/l-1<sup>St</sup> L-2/L-2*; and most zucchini are *L-1/L-1 L-2/L-2*.

Pumpkins, being round or nearly so, deviate the least in overall fruit shape from that of wild and feral forms of the species, and therefore are probably the oldest group. Pumpkins range up to 25 kg and can be smooth, with or without longitudinal grooves, or slightly to strongly ribbed. Although the familiar Halloween and pie pumpkins of the U.S.A. and Canada turn intense orange at maturity, some cultivars from Europe and Asia Minor remain green through maturity. Pumpkins from these locations have non-lignified rinds and thus are sliced easily, but most Mexican forms and other pumpkins used in the manner of summer squash have lignified rinds.

Vegetable marrows are probably the next oldest group of ssp. *pepo*, being the next closest to the ancestral fruit shape. Several forms of *C. pepo* having fruits similar in overall shape to vegetable marrows appeared in Europe prior to 1600. Writings in England from the 19th- and early 20th-century indicate that this group then contained a great deal of variability. Some old-time, vining cultivars of this group, for example, 'Table Dainty', still exist. However, the vegetable marrows have suffered much genetic erosion, as many of the original variable stocks of vegetable marrows that were grown in the Middle East, generally known as 'Baladi', have been replaced by hybrids developed and offered mostly by American and French seedsmen (4).

Cocozelles are the next oldest group, having long, but bulbous fruits. They were depicted in the 18th century and perhaps were described as early as the 17th century. 'Striato D'Italia' (syn. 'Napoli') is an old, variable cultivar and the likely source from which various cocozelle cultivars of Europe and the U.S.A. were derived. Many other cocozelle cultivars have been locally distributed in Italy for a very long time. Differing greatly from one another in fruit coloration, ribbing, and flecking, these local cultivars present an economically difficult situation for foreign seed companies in their attempts to introduce new cultivars. Presently, this group may harbor more genetic variation than any other group of summer squash.

A zucchini cultivar was first described around 1900. Quickly, the zucchini has become the economically most important group of the species. Although it therefore contains very many named cultivars, most of these are recently derived hybrid combinations of inbreds maintained by seed companies.

Zhiteneva (10) presented photographs of *C. pepo* collected in Mexico and Asia Minor. Comparing the photos, it is easily seen that the Mexican material does not contain the long-fruitedness found in the Asian material. This would seem to indicate that in the Old World, *C. pepo* had been selected more rigorously for long-fruitedness and adaptation of the young fruits to culinary purposes. Possibly, selection may have been easier in Asia because of isolation from primitive, weedy or wild round-fruited forms occurring in the native habitat, Mexico. Another possibility, not exclusive of the first, is that use of *C. pepo* ssp. *pepo* for seed consumption was more important in Mexico.

Most pumpkins, of course, are viney. Many vegetable marrows of yore were viney (2) and at least two vining vegetable marrow cultivars are extant. 'Table Dainty' and 'Vegetable Spaghetti'. Naudin (5) knew of at least one vining cocozelle. Some cocozelle stocks, while not viney, have distinctly longer internodes than others. I have not encountered, in the literature or personally, a zucchini cultivar that was vining, or that even had long internodes. As Pangalo (6) noted, bush growth habit and short internodes are characteristics derived under cultivation. That zucchini cultivars are never viney or long-internoded could only indicate that they were recently derived.

Other observations provide further insight concerning the history and relationships within *C. pepo* ssp. *pepo*. Landraces of *C. pepo* have been collected in remote regions of Italy by Hammer, Perrino, and colleagues (3, 8). I have obtained seeds of 14 of the *C. pepo* samples they collected. All belong to ssp. *pepo*. Of the 14, four are pumpkins (including summer pumpkins), three are cocozelles, three are vegetable marrows, one has a unique fruit shape and three are mixed stocks. There is not a single zucchini among these, again indicative of the relatively recent origin of the zucchini group. All of the accessions are bushy, but some have noticeably longer internodes than the others.

Tapley et al. (9) wrote that the zucchini originated in Italy and was introduced into the United States around 1920. All of the open-pollinated zucchini cultivars of Italy known to me contain "Milano" in their names. As at least three, 'Nero di Milano', 'Verde di Milano', and 'Verde Lungo di Milano', are quite distinct from one another, it seems that Milan and its environs are the specific locality for origination of the zucchini group. This geographic origin is strikingly more restricted and very different from that of the cocozelles, whose local Italian cultivars bear the names of a number of cities and provinces located further south and east, indicating a much wider distribution: Florence, Tuscany, Rome, Faenza, Genoa, Naples, Puglia, and Sicily.

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# Adventitious Flower and Shoot Formation by Various Cucurbits In Vitro

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**Introduction.** Growth and early flowering are important aspects of crop breeding. The development of efficient methods to speed flowering can reduce expenses for both growers and customers. The effects of various growth regulators on flowering have been studied. There have also been several reports concerning in vitro flowering of various crops (Scorza, 1982) including cucumber, *Cucumis sativus* (Rasjasekran et al., 1983; Msikita et al., 1990; Ali et al., 1991). According to Msikita et al. (1990), flowering in 'Burpless Hybrid' cucumber cultures varied depending on explant type and media combinations. They reported that flower initiation occurred by the seventh week of culture in vitro and the best adventitious shoot regeneration was obtained from medium supplemented with 2 mg/liter of BA (6-benzylaminopurine). Tissarat and Galletta (1993) used this in vitro system to produce cucumbers for use as food in space.

To facilitate studies of flower development in vitro, it is important that cultivars with high rates of flower development be identified. This study was initiated to screen a number of commercially available cucurbits for their ability to differentiate adventitious flowers in vitro.

**Materials and Methods.** Seeds of several cucurbits (Table 1) were sorted for uniformity and surface sterilized in 10% sodium hypochlorite (p.525% v/v) with 0.1% Triton X-100 added as a surfactant for 15 minutes. The seeds were rinsed three times with sterile distilled water (5 min/rinse). The seeds were left in the final rinse bottle until they were ready for transfer. Sterilized seeds were prepared for culture by making a single cut at each end of the seed. The seed coats (outer and inner testa) were then removed. Care was taken to remove the embryonic axes so explants consisted of only cotyledons.

The medium used in these studies was the modification of the Murashige and Skoog high mineral salts (1962) used previously by Msikita et al. (1990). The medium was prepared using standard procedures, pH was adjusted to 5.7, agar was added at 7.5 g l<sup>-1</sup>, and melted. The

liquid medium was dispensed into 25 X 150 mm culture tubes and autoclaved for 15 minutes at 1.06kg/cm<sup>2</sup>.

The explants were grown in a culture room maintained under 16 h days (cool white fluorescent light) between 20 and 22 C. The photosynthetically active radiation at the level of the agar surface was 131 μmol m<sup>-2</sup>s<sup>-1</sup>.

**Results.** Flower development was observed in *Citrullus lanatus* (watermelon), *Cucumis sativus* ('Hybrid Cucumber, Burpless'), and *Cucurbita melopepo* ('Zucchini, Dark Green' and 'Summer Squash, Crookneck'). The most flower development was observed in 'Hybrid Cucumber, Burpless' where 30% of the seeds developed flowers; it was the only cultivar of *Cucumis sativus* to develop flowers. Two of the five *Cucurbita melopepo* cultivars flowered: 'Zucchini, Dark Green' (4.8%) and 'Summer Squash, Crookneck' (10.5%).

Some of the seed lots were completely contaminated by bacteria. Other lots had no contamination. The consistent contamination of some lots and lack of contamination in others suggests that some seeds harbored internal contaminants.

At least some cotyledons of each cultivar became green and expanded, indicating the seeds were alive. The cultivars of *Cucumis sativus* had the best germination rates (87.5% to 100%). The high rate of germination is consistent with the low rate of contamination (20% or less) in the *Cucumis sativus* seeds. In most cultivars with low germination rates, contamination rates were high.

Root growth was observed in four of the six cucurbit species: *Cucumis sativus* ('Hybrid Cucumber, Burpless' and 'Burpless Hybrid Cucumber'), *Cucurbita melopepo* ('Zucchini, Dark Green' and 'Summer Squash, Crookneck'), and *Cucurbita pepo* ('Squash, Table Queen'). The highest rates of root growth were found in the cucumber and summer squash cultivars.

Adventitious shoot growth was recorded for some cultivars. All of the cultivars that formed flowers also had



Table 1. Growth and development of cotyledons from various cucurbit species in vitro

Genus species (common Name)	Source	Contaminatio n (%)	# Seeds	Cotyledon expansion & greening (%)	Flower formation (%)	Root formation (%)	Adventitious shoot formation (%)
<b><i>Citrullus lanatus</i> (watermelon)</b>							
Watermelon	Michael Leonard	90%	20	10%	5%	5%	N/A
<b><i>Cucumis melo</i> (cantaloupe)</b>							
Cantaloupe, Hale's Best	Michael Leonard	25%	20	40%	0%	0%	N/A
<b><i>Cucumis sativus</i> (cucumber)</b>							
Hybrid Cucumber, Burpless	Henry Fields	20%	20	90%	30%	25%	N/A
Cucumber, SMR-58	Northrup-King	15%	20	90%	0%	0%	35%
Cucumber, National Pickling	American Seed	25%	8	88%	0%	0%	13%
Cucumber, Early Spring Burpless	Henry Fields	0%	20	100%	0%	0%	40%
Cucumber, Straight 8	Michael Leonard	5%	20	90%	0%	0%	30%
Burpless Hybrid Cucumber	Michael Leonard	0%	6	100%	0%	33%	50%
<b><i>Cucurbita</i> spp. (pumpkin)</b>							
Pumpkin, Prize Winner Hybrid	Burpee	0%	6	100%	0%	0%	17%
Pumpkin, Jack O'Lantern	Michael Leonard	30%	10	80%	0%	0%	10%
<b><i>Cucurbita melopepo</i> (summer squash)</b>							
Squash, Early Golden Summer Crookneck	Burpee	100%	20	15%	0%	0%	N/A
Summer Squash Zucchini	Michael Leonard	100%	21	29%	0%	0%	N/A
Zucchini, Dark Green	American Seed	71%	21	52%	5%	19%	N/A
Summer Squash, Crookneck	Michael Leonard	26%	19	68%	11%	21%	21%
Squash, Straightneck Early Yellow	American Seed	53%	19	68%	0%	0%	5%
<b><i>Cucurbita pepo</i> (winter squash)</b>							
Squash, Table Queen	American Seed	20%	20	95%	0%	5%	N/A
Winter Squash, Table Queen or Acorn	Michael Leonard	95%	20	10%	0%	0%	N/A

N/A = not assessed

leaves and shoots, presumably of adventitious origin since care was taken to remove the embryonic axis from the explants. Of the cultivars in which this feature was recorded, shoot growth was observed in all cultivars. The highest rates of shoot growth were recorded, shoot growth was observed in all cultivars. The highest rates of shoot growth were found in *Cucumis sativus*. 'Burpless Hybrid Cucumber' had the highest rate (50%) of any cultivar. However, the sample contained only six seeds. Of the samples with the full twenty seeds, 'Cucumber, Early Spring Burpless' had the highest rate of shoot growth (40%).

**Discussion.** *Cucumis sativus* had the highest rates of flower development, germination, root growth and adventitious shoot formation. *Cucurbita melopepo* also had high rates of flower development, germination, and root development. Members of these species should be good selections for studying the regeneration response of cucurbits. When initiating a project to study flowering, we suggest that the researcher screen other member of this genus to find specific cucurbits useful to their goals.

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# Seed Fill Occurs in Stored Fruit of *Cucurbita pepo* L. Harvested Prematurely

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**Introduction.** Pumpkin plants (*Cucurbita pepo* L.) often senesce prematurely in the fall because of disease pressures or low temperatures. Seeds from fruit of such plants are not well-filled, and it is a common practice among breeders to store prematurely harvested fruit with the assumption that additional seed fill will occur during storage. Information on seed fill during storage of prematurely harvested fruit is important for breeders and seed producers, but is especially vital when considering cultivars developed for snackseed production. In the present study we determined the extent of seed fill in prematurely harvested fruit in three hull-less seeded, snackseed cultigens.

**Materials and Methods.** Three cultigens were used: PI285611 (S<sub>3</sub>), NH29-13 (F<sub>9</sub>), and NH1003, the F<sub>1</sub> hybrid from these two inbreds. The field study was conducted in 1997 at the Woodman Horticultural Farm, Durham, NH, using a randomized complete block design with 6 replications. Hand pollinations were made between 22 July and 7 August. Fruit were harvested and initial seed weights were obtained at 5 day intervals between 35 and 50 days post-anthesis (PA). Seed fill was determined over 10 day intervals both for fruit left intact on plants and for fruit harvested from plants and stored in a greenhouse (ca. 25 to 30 C day and 18 to 21 C night). Because we had anticipated differences in fruit maturity among the cultigens, fruits for storage were not obtained for all cultigens at each sampling period.

**Results and Discussion.** Seed fill (calculated as embryo dry biomass) increased in 10-day stored fruit in all cul-

tigens and at all sampling periods (Table 1). However, the extent of seed fill in stored as compared to intact fruit differed among the genotypes. In PI285611, seed fill in stored fruit was comparable to that in fruit left on the plant (intact fruit) at both sampling periods (40 to 50 and 50 to 60 days PA). In the inbred line, NH29-13, seed fill in stored fruit was 12.7 to 20.5% lower than that of intact fruit. In the F<sub>1</sub> hybrid, NH1003, seed fill in stored fruit varied from 17.7 to 58.3% lower than that of intact fruit. The genotypic differences in capacity for seed fill in stored fruits probably relates directly to photosynthetic reserves in the fruits of different genotypes and at the different sampling dates. Because of their higher harvest index (higher fruit biomass to total biomass), plants of NH1003 were more stressed from powdery mildew than plants of PI285611. NH29-13 had the highest ratio of seed fresh weight to fruit fresh weight among the genotypes, but the fruit flesh had a higher percent dry matter than the other two genotypes. PI285611 had both a lower harvest index and thicker fleshed fruit than the other two genotypes used in this study.

The results of our preliminary research on seed fill in stored fruits indicate that (1) appreciable seed fill can occur in fruits abscised prematurely from plants, (2) this seed fill can occur in fruits differing widely in degree of fruit maturity, and (3) there may be genotypic differences in the capacity of abscised fruits to channel assimilates into seeds, and this may represent an important selection criterion for snackseed cultivars.

Table 1. Dry biomass accumulation in embryos of seeds from fruits harvested prematurely (35, 40, 45, and 50 days post-anthesis) and stored in a greenhouse for 10 days, as compared to seeds from fruits allowed to develop intact on plants for 10 days.

Genotypes	Treatments	Harvest intervals (days postanthesis)			
		35 to 45	40 to 50	45 to 55	50 to 60
		10-day dry biomass accumulation, mg/embryo <sup>z</sup>			
<b>NH29-13</b>	intact fruit	44.1	41.9		
	stored fruit	38.5 <sup>y</sup>	33.2		
<b>NH1003</b>	intact fruit	71.5	40.8	44.6	
	stored fruit	59.2	34.0 <sup>y</sup>	18.6 <sup>x</sup>	
<b>PI285611</b>	intact fruit		53.2		52.8
	stored fruit		55.6		54.6

<sup>z</sup>Values are means of 6 replications.

<sup>y</sup>Stored fruit harvested on day later (36 vs. 35 days post-anthesis) than control fruit.

<sup>x</sup>Stored fruit harvested two days later (47 vs. 45 days post-anthesis) than control fruit.

# Seed Development and Seed Fill in Hull-less Seeded Cultigens of *Cucurbita pepo* L.

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**Introduction.** A breeding program to develop hull-less seeded cultivars of pumpkin has been ongoing at the University of New Hampshire since the early 1980s (3). The initial goal of the program was to develop high seed yielding cultivars for use in the snackseed industry. More recently (1,2), we have focused on increasing seed size, and have evaluated some promising hybrids that combine high yields with large seed size. A limitation of our breeding program has been an absence of basic information on seed development and seed fill, and on the relationship of seed maturity to perceived fruit maturity. In the present study we obtained information on biomass accumulation among seed organs (seed coat, embryo and endosperm) over the course of seed development.

**Materials and Methods.** Three cultigens were used: PI285611 (S<sub>3</sub>) which has large fruit (3 to 5 kg) and seed (210 to 250 mg/seed); NH29-13 (F<sub>9</sub>) which has small fruit (0.5 to 1.0 kg) and medium seed (140 to 170 mg); and NH1003, the F<sub>1</sub> hybrid from the above lines, which has relatively large fruit (2 to 4 kg) and seed (180 to 220 mg). The field study was conducted in 1997 at the Woodman Horticultural Farm, Durham, NH, using a randomized complete block design with 6 blocks. Hand pollinations were made between 22 July and 7 August, and the resulting fruit were harvested on 13 sampling dates between 10 and 60 days post-anthesis (PA). Thirty-seed samples were removed from fruits collected 10 to 35 days PA, and 20 seeds per fruit were sampled thereafter. Seed coats, embryos and endosperm were separated under a dissecting scope, and data were collected on fresh and dry biomass of seed organs. These data were used to calculate percent dry matter (%DM) of seed organs and partitioning of dry matter within the whole seed.

**Results and Discussion.** Endosperm was detectable in all seeds between 10 and 35 days PA (Table 1); however, embryos were not large enough to efficiently dissect until 20 days PA. There was considerable variation in embryo size between 20 and 31 days PA, both among and within fruit. The bulk of the seed biomass (86 to 99.7%) was

comprised of seed coat tissues between 10 (99.7%) and 25 (86%) days PA. Seed coat dry matter peaked at 20 to 25 days PA and then progressively declined until 45 to 50 days PA, agreeing with an earlier study (4). Embryo biomass increased rapidly from between 1 and 8% of total seed dry weight at 25 days PA to between 51 and 71% total seed dry weight by 35 days PA. Embryo weight as function of total dry weight at 35 days PA was greatest in the F<sub>1</sub> hybrid, and lowest in the bush strain NH29-13. This was surprising in view of the fact that NH29-13 exhibited some orange color in fruit by 25 days PA, and apparent fruit maturity (skin totally orange) by 35 days PA. In contrast, F<sub>1</sub> hybrid fruits did not begin to change color until 40 days PA and were not totally orange/yellow until 55 days PA.

Total dry seed biomass continued to accumulate in all genotypes until 55 to 60 days post-anthesis. Although there was considerable sampling variation, a substantial proportion (11 to 30%) of seed fill (increases in embryo weight, primarily cotyledonary tissues) occurred during the latter stages of seed maturation (50 to 55 days PA in NH1003; 55 to 60 days in NH29-13 and PI285611) among all three genotypes. Again, in the case of NH29-13, this seed fill occurred much later than perceived fruit ripeness as evaluated by changes in skin color. There were minor differences in the duration of seed fill among genotypes, but there were large differences in seed size and therefore in rates of seed fill. In PI285611, the mean embryo dry weight was 204 mg/seed at 60 days PA, and embryo biomass accumulated at a rate of 5.72 mg per day between 25 and 60 days PA. The corresponding values for NH29-13 were 136 mg/seed at 60 days PA and mean increases in biomass of 3.14 mg/seed/day between 28 and 60 days PA. For NH1003, embryo weight was 182 mg/seed at 55 days, and rate of seed fill was 5.07 mg/seed/day between 25 to 60 days PA. Because seed fill in NH1003 was largely complete by 55 days PA, its rate of seed fill was actually comparable to that of PI285611.

The results of this study show that embryo enlargement and seed fill occur at relatively late stages in seed devel-

Table 1. Dry matter accumulation in seed organs between 10 days post-anthesis until seed maturity.

Genotypes	Days post-anthesis	Seed coat dry biomass (mg/seed)	Endosperm dry biomass (mg/seed)	Embryo dry biomass (mg/seed)	Total dry biomass (mg/seed)	% Dry biomass of embryos
<b>NH29-13</b>						
	10	20.47	0.03	0.00	20.50	0.00
	15	38.03	0.37	0.00	38.40	0.00
	20	51.17	1.93	0.07	53.17	0.13
	25	50.40	2.30	0.60	53.30	1.13
	28	47.97	2.13	5.33	55.43	9.62
	31	36.97	0.73	15.50	53.20	29.14
	35	31.77	0.17	33.87	65.80	51.47
	40	33.15	0.00	52.60	85.75	61.34
	45	23.00	0.00	77.95	100.95	77.22
	50	20.30	0.00	94.50	114.80	82.32
	55	17.65	0.00	74.90	92.55	80.93
	60	18.75	0.00	136.00	154.75	87.88
<b>NH1003</b>						
	10	23.10	0.07	0.00	23.17	0.00
	15	34.73	0.57	0.00	35.30	0.00
	20	50.67	2.57	0.13	53.37	0.25
	25	44.30	2.37	4.27	50.93	8.38
	28	42.50	1.53	18.67	62.70	29.77
	31	33.33	1.03	27.27	61.63	44.24
	35	23.50	0.03	59.87	83.40	71.78
	40	22.15	0.00	82.55	104.70	78.84
	45	24.45	0.00	131.40	155.85	84.31
	50	18.35	0.00	123.35	141.70	87.05
	55	17.50	0.00	176.00	193.50	90.96
	60	14.75	0.00	181.85	196.60	92.50
<b>PI285611</b>						
	10	20.33	0.07	0.00	20.40	0.00
	15	44.87	0.63	0.00	45.50	0.00
	20	67.83	3.60	0.20	71.63	0.28
	25	62.57	3.13	3.77	69.47	5.42
	28	57.23	2.50	17.00	76.73	22.15
	31	45.90	1.43	31.07	78.40	39.63
	35	37.20	0.57	52.17	89.93	58.01
	40	28.35	0.05	98.10	126.50	77.55
	45	31.50	0.00	154.30	185.80	83.05
	50	21.70	0.00	151.35	173.05	87.46
	55	21.65	0.00	171.65	193.30	88.80
	60	20.20	0.00	204.15	224.25	91.00

opment, and that substantial changes in seed fill can continue over a prolonged period of about 35 days. In some genotypes the period of seed fill extends well beyond the period of perceived fruit maturation as judged by changes in skin color from green to orange or yellow/orange.

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# Effectiveness of the Informal Seed Sector for Increasing Yield in Pumpkins Developed under Low Input Conditions

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**Introduction.** The “informal seed sector” describes the ability of farmers — rather than breeders — to maintain, develop, enhance, enrich and utilize pumpkin diversity. This sector has played an important role in seed management under low income conditions in developing countries (1,2). It would be of interest to identify some of the factors influencing the maintenance of pumpkin yields on low income farms using this model, particularly since seed farmers’ production is undergoing an economic crisis in Cuba.

**Materials and methods.** The Marucha and Fifi lines, selected from Cuban landraces, were sown according to two seed management systems. The *Formal Model*, frequently used by seed companies in Cuba, describes when seed of a variety is increased for several cycles in isolation (i.e., without any other pumpkin genotypes within 1000 m). In this experimental case, Marucha and Fifi lines were sown separately, avoiding any mix with extraneous pollen, for two cycles (winter and summer). The *Informal Model*, a common approach among farmers, describes when farmers grow pumpkins from other farms alongside their own pumpkins and save the seeds. In this experiment, Marucha and Fifi lines were grown among eight pumpkin landraces selected from those previously evaluated, and seeds were saved.

The seeds of Marucha and Fifi obtained by simulating the two models were compared by a random block design under San Jose de las Lajas low input growing conditions during the summer and winter.

**Results.** The isolation approach used by formal sector resulted in decreased yields for both Marucha and Fifi, with yield depressions ranging from 25 and 58% (Table 1). The higher depression of yields observed during the winter planting may have been due to increased water stress.

These results assume that inbreeding, and hence increased homozygosity, is occurring during seed multiplication in the *Formal Model*. On the contrary, in the *Informal Model* seeds from different sources are introduced by the farmers, and interpollination of Marucha

and Fifi with these sources may have increased heterozygosity, resulting in increased pumpkin yields under low input conditions.

Table 1: Yield depression and yield of two lines multiplied under summer and winter time.

Sowing period	Yield (t.ha <sup>-1</sup> )	
	Marucha	Fifi
Summer		
Formal Model	5.17	3.06
Informal Model	7.95	4.14
Yield depression <sup>2</sup>	35%	26%
Winter		
Formal Model	3.20	1.10
Informal Model	5.93	2.63
Yield depression <sup>2</sup>	46%	58%

As Cuba’s economic crisis continues, it is becoming essential that breeders involve the informal seed sector in pumpkin breeding. Also, breeders need to rethink the potential roles of farmers and breeders in breeding process.

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# Pumpkin Response under Different Environmental Conditions

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**Introduction.** Environment plays an important role on cucurbit production. In Cuba, prior to the current economic crisis, pumpkin (*Cucurbita moschata*) yields were highest with winter sowings (September-February), with moderate temperatures and artificial irrigation theoretically favoring fruit set. In contrast, spring and summer sowings (March-July) were usually characterized by high temperatures, and fruit set was lower despite the occurrence of rainfall during these months.

Recently, with the declining availability of chemical fertilizer, pesticides and irrigation in Cuba, some of the observed pumpkin yields have not corresponded to previous results. Thus, it has been essential to re-investigate the influence of the environment, particularly to determine what factors are limiting pumpkin production under low income conditions, and what breeding strategies should be used given the current limitations.

**Materials and Methods.** In order to classify different environmental responses, a data set consisting of yield, number fruit per plant, and weight of fruit of 10 genotypes (Ríos, 1997) grown in eight different environments (E1 through E8) of Havana province, was analysed by cluster multivariate technique. Six of the environments had a spring-summer growing season (E1, E2, E5, E6, E7, E8) and two had a winter growing season (E3, E4).

The selected environments for cluster analysis application did not use chemical fertilizers (organic fertilization according to Ríos, 1997) or pesticides. A light artificial irrigation was only used for germination and during flowering and female flowering times, regardless of whether rainfall had occurred during these periods.

**Result and Discussion.** In principle, the cluster analysis permitted classification of the environmental response into two large groups based upon yield (Table 1): moderate (2.90-7.29 t.ha<sup>-1</sup>) and poor (0.15-1.68 t.ha<sup>-1</sup>). The first group could be subdivided into two subgroups. Environments E1 and E2 were characterised by satisfactory rainfall during the shrub and male flowering periods, even though temperatures were over 25 C. E3 and E4 experienced less rainfall and yielded less, even though their temperatures were near the 22.2 C considered opti-

mum for pumpkins (Hernández, 1995). These results indicated that the amount of rainfall during the shrub and male flowering periods may be a limiting factor when moderate yield temperature conditions are experienced under low income regimens.

With regard to the poor yield responses experienced in environments E5, E6, E7 and E8, these were generally associated with high temperatures and drought stress conditions during shrub and male flowering.

Based upon these results, a breeding strategy for growing pumpkins under scarce water periods during spring and summer would have to be accompanied by practising intercropping and non-conventional irrigation, as well as introducing genes from other species of *Cucurbita*. In fact, it has become a real necessity to avoid the dangerous yield-reducing effects of drought stress and high temperatures when growing pumpkins under low income conditions.

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Table 1. Pumpkin yields under different environmental conditions.

Yield category	Environment	Yield (t.ha <sup>1</sup> )	Average temperature (°C)	Rainfall regime
Moderate	E1:spring-summer	7.29	25.9 ± 0.1	wet
	E2:spring-summer	4.89	25.1 ± 0.3	wet
	E3:winter	3.41	21.7 ± 0.6	dry
	E4:winter	2.90	22.2 ± 10.4	dry
Poor	E5:spring-summer	1.68	26.2 ± 0.6	dry
	E6:spring-summer	0.41	25.7 ± 0.4	dry
	E7:spring-summer	0.19	25.3 ± 0.4	dry
	E8:spring-summer	0.15	26.5 ± 0.4	dry

## Genotypes Selected by Farmers: An Interesting Option for Pumpkin Breeding

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**Introduction.** Specific genetic adaptation of pumpkin (*Cucurbita moschata*) is important in order to maximize agrobiodiversity and yields, and to satisfy the cultural and socio-economic demands of communities with low-input agriculture systems.

Hardon (1995) reported that the main results of plant breeding programs have been to produce varieties adapted to favorable agricultural environments. However, in marginal areas there are economic, technical and institutional constraints: *economic*, because farm households lack resources and cannot afford the risk involved in compensating for the high cost of external input; *technical* because plant breeding has not been very successful in adapting crops to more extreme and variable environments; and *institutional* because breeding is expensive and usually only justified for large geographic areas sharing a common environment.

In Cuba, considering its recent economic crisis and efforts to produce pumpkin genetic resources adapted to low income conditions (Ríos *et al.*, 1997), a breeding approach using farmers in the selection process may be advantageous as a complementary or supplemental method for pumpkin breeding. This would be important if Cuban farmers were able to efficiently select pumpkins for their own growing conditions.

**Materials, Methods and Results.** During 1989-1993, 16 lines from Cuban landraces were sown under site farming conditions at the "Juan Diaz" community of Batabanó municipality. Here a selection committee was created and 4 farmers chose 3 half sib families according to their own criteria (cooking quality, yield, perform and crookneck fruit shape and medium fruit size). The fruit

characteristics preferred by the farmers often differed from those of the commercial variety (RG) disseminated in the locality by the formal seed sector.

**Results and Discussion.** Almost immediately, the pumpkin populations resulting from the farmers' selection were adopted by different farms across the community, and the formal seed sector became involved in the seed multiplication process of these populations.

What were the breeders' functions in this effort?

- To select an experimental station (first screening).
- To supply germplasm potentially manageable by farmers.
- To contribute to the maintenance of varietal vigor.

In summary, the participation of Cuban farmers in pumpkin selection according their own socio-economic necessities may prove to be an interesting and valuable option for diversifying pumpkin varietal material. It would also contribute to the community welfare, and could serve as a resource for *in situ* pumpkin conservation.

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# Correlated Genetic Response in Pumpkin Genotypes Selected under Low Income Conditions

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**Introduction.** Centralized pumpkin (*Cucurbita moschata*) breeding programs (CPBP) have been highly effective in producing broadly adapted varieties or hybrids for high-input production environments. However, it is widely acknowledged that the varieties and hybrids developed from CPBP have not been extensively adopted by resource-poor farmers. As Cuba is currently undergoing a crisis for agricultural resources such as fertilizer, pesticides and irrigation, differences in environmental and socio-economic conditions have increased among localities during the last few years and CPBP has not been able to satisfy their requirements. The purpose of this study was to demonstrate the importance of genetic correlated responses as a justification for selecting for specific adaptation.

**Materials and Methods.** Genetic correlated responses (Calhoun *et al.*, 1994) of fruit yields for ten genotypes (Ríos *et al.*, 1998) were estimated for different stress conditions using the following four cases:

- *Case 1 (Indirect Selection).* Selecting under heat stress and testing under drought conditions.
- *Case 2 (Indirect Selection).* Selecting under drought stress and testing under heat conditions.
- *Case 3 (Direct Selection).* Selecting under heat stress and testing under the heat conditions.
- *Case 4 (Direct Selection).* Selecting under drought stress and testing under the drought conditions.

Genetic correlated responses (GCR) for Cases 1 and 2 (Indirect Selection) were 5.2 and 4.0% (Table 1). However, for Cases 3 and 4 (Direct Selection) GCR's were

approximately twice (10.1 and 11.4%), despite the fact that calculated heritabilities were similar to those for indirect selection. This illustrates the importance of evaluating genotypes under low income conditions, which are associated with adaptative value (Rios, 1998).

These results, with regard to the role of direct selection, are similar to those reported by Ceccarelli (1994) and Atlin (1997). They appear to be highly applicable to Cuban pumpkin response, since direct selection or local varietal selection would increase the selection efficiency for genetic advance with low heritability traits.

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Table 1: Genetic correlated response for different types of stress.

Type of selection		Selection environ.	Target environ.	Genetic correlation	Genetic response correlated
Indirect selection	Case 1	heat	drought	0.42	5.20
	Case 2	drought	heat	0.47	4.20
Direct selection	Case 3	heat	heat	0.88	10.10
	Case 4	drought	drought	1.80	11.40

## Evaluation of Two *Benincasa hispida* Genotypes for Fruit Yield, Vine Growth, Size and Shape Characteristics.

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**Introduction.** Winter melon (white or ash gourd, fuzzy gourd, don-kwa) is botanically known as *Benincasa hispida* (Thunb.) Cogn. and has a chromosome number of  $2n=24$  (2,3,6). Rubatzky and Yamaguchi (4) list four major cultivar groups recognizable by their size, shape, fuzziness, waxiness, and presence or absence of a dusty or ashy layer. There are reports that fruit weight ranges from 7.5 to 45 kg depending on genotype and the production system utilized (4,5). Furthermore, fruits of *B. hispida* are known to have medium (4) to large (5) size fruit, with oblong (5) to cylindrical or elongated (4) shapes. The objective of this study was to evaluate two genotypes of *B. hispida* for fruit yield, vine growth, size and shape characteristics when grown under northern Missouri conditions.

**Materials and Methods.** Seeds of a green, hairy winter melon with sparsely waxed surface (from Long Island, NY growers) and of a white/ashy gourd (Evergreen Enterprises) were planted and grown on inverted pyramid celled flats using Metro Mix 500 as soil media under greenhouse conditions during April-May 1997. On the last week of May 1997, the seedlings were transplanted in the field on a Sharpsburg silty clay soil (fine, montmorillonitic, mesic Typic Argiudolls) under black plastic mulch and drip irrigation. The transplants were established with 1.5 m distance between plants and 2.5 m between bed rows. The transplants were fertigated with a 10-10-10 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) liquid formulation. Fruits were harvested when reaching maximum growth, and were counted, weighed and measured for length and width until the first frost, 145 days after transplanting. Fruit shape index was calculated using L/W (1). At this time, plant vine length was also recorded. The collected data were statistically analyzed using a completely randomized experimental design.

**Results and Discussion.** The green winter melon genotype produced an average yield of 105.7 kg/plant while the fuzzy white gourd genotype produced an average yield of 27.3 lbs/plant (Table 1). The number of fruit per plant and average fruit weight were also greater with the

green winter melon genotype (Table 1). Thus, these two *B. hispida* genotypes can be categorized in two different groups based on their productivity and other genetic traits (3,4).

The U.S. market tends to have a lower degree of acceptance for extra-large fruit of *B. hispida*. In this study, the green winter melon genotype produced extra-large size fruit measuring 55.5 cm x 24.7 cm (LxW) compared to 48.9 cm x 22.1 cm for the fuzzy white gourd (Table 2). Both genotypes had cylindrical or elongated shape since their shape indices were greater than 1.6 (Table 2). The green winter melon genotype produced a longer vine, measuring an average of 460 cm/plant as compared to 310 cm/plant for the fuzzy white gourd genotype (Table 2). Field observations agree with these data, and clearly indicate that the greater vine length of the green winter melon increased the potential for a greater number of lateral branches, and hence a larger number of fruits. However, despite its higher productivity and larger melons, very few growers produce the green winter melon.

With regard to other observed characteristics, the fuzzy white gourd produced a slight bitter flavor as compared to the mild flavor of the green winter melon. When fruits were harvested late in the season, with temperatures ranging from 0-10 C, and stored for six months at 20 C, the green winter melon showed susceptibility to bacterial fruit soft rot compared to the very low susceptibility of the fuzzy white gourd. For breeding purposes, these two genotypes possess genetic traits that may bring together the best characteristics of both genotypes, with an acceptable fruit size, high yield, and resistance to bacterial fruit soft rot.

**Acknowledgement:** I am very grateful to Mr. Scott Walk and his crew for their fieldwork and assistance in the collection of data.

Table 1. The average fruit weight, average number of fruit/plant, and the average total yield plant from two *Benincasa hispida* genotypes.

Genotype	No. fruits/ plant	Fruit weight (kg)	Total yield per plant (kg)
Green winter melon	9.0**	11.7**	105.7**
Fuzzy white gourd	4.5	6.1	27.3

\*\*Highly significant (P = 0.01).

Table 2. Plant vine growth length, fruit size and shape characteristics of two *Benincasa hispida* genotypes.

Genotype	Fruit size LxW (cm)	Shape index (L/W)	Description	Plant vine length (cm)
Green winter melon	55.5 x 24.7	2.3	Cylindrical* or elongated	460
Fuzzy white gourd	48.9 x 22.1	2.2	Cylindrical or elongated	310

\*When shape index =>1.6

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# 1998 Gene List for Melon

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Gene lists of melon have been published previously (83, 13, 14, 69, 71). These lists included different types of genes: disease and pest resistance genes, isozymes, leaf, stem, flower, fruit and seed characters... The 1998 list includes a total number of 109 loci (Table 1).

Different types of molecular markers are used in studies on polymorphism, for instance RFLP (62, 50), RAPD (50, 44, 34), and SSR (44). Genetic maps using different types of markers have been published or are under construction:

- RAPD and RFLP (2)
- AFLP (93)
- RAPD (66)

These maps have been constructed using different melon genotypes as parents and some markers (RAPD, AFLP) cannot be transferred from one map to another. There is not yet a reference saturated map of melon.

One problem for the gene names is that there is no melon cultivar which is recognized as the reference or "wild type." This accession should be monoecious and susceptible to all diseases. Any proposition can be sent to the author.

Researchers are encouraged to send reports of new genes and seed samples to the melon gene curators. They should consult the lists and the rules of gene nomenclature for the *Cucurbitaceae* (84, 13) before proposing a gene name and symbol.

**Table 1. Gene list of melon.** 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.

Gene symbol		Character	L <sup>2</sup>	References
Preferred	Synonym			
<i>a</i>	<b><i>M</i></b>	<b><i>andromonoecious</i>. Mostly staminate, fewer perfect flowers; on <i>Aa</i> plants, pistillate flowers have no stamens; epistatic to <i>g</i>.</b>	4	78, 85, 92
<i>ab</i>	-	<i>abrachiate</i> . Lacking lateral branches. Interacts with <i>a</i> and <i>g</i> (e.g. <i>abab aa</i> <i>G_</i> plants produce only staminate flowers).		29
<i>Ac</i>	-	<i>Alternaria cucumerina</i> resistance (in MR-1).		89
<i>Af</i>	-	<i>Aulacophora foveicollis</i> resistance. Resistance to the red pumpkin beetle.		90
<i>Ag</i>	-	<i>Aphis gossypii</i> tolerance. Freedom of leaf curling following aphid infestation (in PI 414723).		5

Gene symbol		Character	L <sup>z</sup>	References
Preferred	Synonym			
<i>Ala</i>	-	<i>Acute leaf apex</i> . Dominant over obtuse apex, linked with <i>Lobed</i> leaf. ( <i>Ala</i> in Maine Rock, <i>ala</i> in PV Green).		33
<i>Al-1</i>	<i>Al<sub>1</sub></i>	<i>Abscission layer-1</i> . One of two dominant genes for abscission layer formation. See <i>Al-2</i> . ( <i>Al-1 Al-2</i> in C68, <i>al-1 al-2</i> in Pearl).		87
<i>Al-2</i>	<i>Al<sub>2</sub></i>	<i>Abscission layer-2</i> . One of two dominant genes for abscission layer formation. See <i>Al-1</i> .		87
<i>Ap-1<sup>1</sup></i>	<i>APS-1<sup>1</sup></i>	<i>Acid phosphatase-1<sup>1</sup></i> . One of two codominant alleles, each regulating one band. The heterozygote has two bands. See <i>Ap-1<sup>2</sup></i> .		27
<i>Ap-1<sup>2</sup></i>	<i>APS-1<sup>2</sup></i>	<i>Acid phosphatase-1<sup>2</sup></i> . One of two codominant alleles, each regulating one band. The heterozygote has two bands. See <i>Ap-1<sup>1</sup></i> .		27
<i>bd</i>	-	<i>brittle dwarf</i> . Rosette growth with thick leaf. Male fertile, female sterile (in TAM-Perlita45).		16
<i>Bi</i>	-	<b><i>Bitter</i>. Bitter seedling (common in honeydew or in Charentais type while most American cantaloupes are <i>bi</i>).</b>		54
<i>Bif-1</i>	<i>Bif</i>	<i>Bitter fruit-1</i> . Bitterness of tender fruit in wild melon. Relations with <i>Bi</i> are unknown.		68
<i>Bif-2</i>	-	<i>Bitter fruit-2</i> . One of two complementary independent genes for bitter taste in young fruit: <i>Bif-2Bif-2 Bif-3Bif-3</i> are bitter. (Relations with <i>Bi</i> and <i>Bif-1</i> are unknown).		57
<i>Bif-3</i>	-	<i>Bitter fruit-3</i> . One of two complementary independent genes for bitter taste in young fruit: <i>Bif-2Bif-2 Bif-3Bif-3</i> are bitter. (Relations with <i>Bi</i> and <i>Bif-1</i> are unknown).		57
<i>cab-1</i>	-	<b><i>cucurbit aphid borne yellows virus resistance-1</i>. One of two complementary independent genes for resistance to this virus: <i>cab-1cab-1 cab-2cab-2</i> plants are resistant. (in PI 124112)</b>		21
<i>cab-2</i>	-	<b><i>cucurbit aphid borne yellows virus resistance-2</i>. One of two complementary independent genes for resistance to this virus: <i>cab-1cab-1 cab-2cab-2</i> plants are resistant. (in PI 124112)</b>		21
<i>cb</i>	<i>cb<sub>1</sub></i>	<i>cucumber beetle</i> resistance. Interacts with <i>Bi</i> , the nonbitter <i>bibi cbcb</i> being the more resistant (in C922-174-B).		64
<i>cf</i>	-	<b><i>cochleare folium</i>. Spoon-shaped leaf with upward curling of the leaf margins (spontaneous mutant in Galia)</b>		52
<i>cl</i>	-	<i>curled leaf</i> . Elongated leaves that curl upward and inward. Usually male and female sterile.		16
<i>dc-1</i>	-	<i>Dacus cucurbitae-1</i> resistance. One of two complementary recessive genes for resistance to the melon fruitfly. See <i>dc-2</i> .		86



Gene symbol		Character	L <sup>z</sup>	References
Preferred	Synonym			
<i>dc-2</i>	-	<i>Dacus cucurbitae-2</i> resistance. One of two complementary recessive genes for resistance to the melon fruitfly. See <i>dc-1</i> .		86
<i>dl</i>	-	<i>dissected leaf</i> (in URSS 4). Highly indented leaves.	10	22
<i>dl'</i>	<i>cl</i>	<i>dissected leaf Velich</i> . First described as <i>cut leaf</i> in Cantaloupe de Bellegarde. Allelic to <i>dl</i> .	10	91
<i>dl-2</i>	-	<i>dissected leaf-2</i> . First described as 'hojas hendidas'.		26
<i>dlet</i>	<i>dl</i>	<i>delayed lethal</i> . Reduced growth, necrotic lesions on leaves and premature death.		98
<i>f</i>	-	<i>flava</i> . Chlorophyll deficient mutant. Growth rate reduced (in K 2005).	8	72
<i>fas</i>	-	<i>fasciated stem</i> (in Vilmorin 104).		30
<i>fe</i>	-	<i>fe</i> (iron) inefficient mutant. Chlorotic leaves with green veins. Turns green when adding Fe in the nutrient solution.		63
<i>Fn</i>	-	<i>Flaccida necrosis</i> . Semi-dominant gene for wilting and necrosis with F pathotype of Zucchini Yellow Mosaic Virus ( <i>Fn</i> in Doublon, <i>fn</i> in Védreantais).	2	82
<i>Fom-1</i>	<i>Fom<sub>1</sub></i>	<i>Fusarium oxysporum melonis</i> resistance. Resistance to races 0 and 2 and susceptibility to races 1 and 1,2 of Fusarium wilt ( <i>Fom-1</i> in Doublon, <i>fom-1</i> in Charentais T).	5	81
<i>Fom-2</i>	<i>Fom<sub>1,2</sub></i>	<i>Fusarium oxysporum melonis</i> resistance. Resistance to races 0 and 1 and susceptibility to races 2 and 1,2 of Fusarium wilt. ( <i>Fom-2</i> in CM 17187, <i>fom-2</i> in Charentais T).	6	81
<i>Fom-3</i>	-	<i>Fusarium oxysporum melonis</i> resistance. Same phenotype as <i>Fom-1</i> but segregates independently from <i>Fom-1</i> . ( <i>Fom-3</i> in Perlita FR, <i>fom-3</i> in Charentais T).		99
<i>g</i>	-	<i>gynomonoecious</i> . Mostly pistillate, fewer perfect flowers. Epistatic to <i>a</i> : <i>A_ G_</i> monoecious; <i>A_ gg</i> gynomonoecious; <i>aa G_</i> andromonoecious; <i>aa gg</i> hermaphrodite.		78
<i>gf</i>	-	<i>green flesh</i> color. Recessive to salmon. ( <i>gf</i> in honeydew, <i>Gf</i> in Smiths' Perfect cantaloupe).		40
<i>gl</i>	-	<i>glabrous</i> . Trichomes lacking (in Arizona glA).	3	28
<i>gp</i>	-	<i>green petals</i> . Corolla leaf like in color and venation.		61
<i>Gs</i>	-	<i>Gelatinous sheath</i> around the seeds. Dominant to absence of gelatinous sheath.		31
<i>gyc</i>	-	<i>greenish yellow corolla</i> .		97

Gene symbol		Character	L <sup>2</sup>	References
Prefered	Synonym			
<i>gy</i>	<i>n, M</i>	<b>gynoecious. Interacts with <i>a</i> and <i>g</i> to produce stable gynoecious plants (<i>A_gg gygy</i>) (in WI 998).</b>		45, 47
<i>h</i>	-	<b><i>halo</i> cotyledons. Yellow halo on the cotyledons, later turning green.</b>	4	65
<i>jf</i>	-	<b><i>juicy flesh</i>. Segregates discretely in a monogenic ratio in segregating generations.</b>		9
<i>L</i>	-	<b><i>Lobed</i> leaf. Dominant on non lobed, linked with <i>Acute leaf apex</i>. (<i>L</i> in Maine Rock, <i>l</i> in P.V. Green).</b>		33
<i>lmi</i>	-	<b><i>long mainstem internode</i>. Affects internode length of the main stem but not of the lateral ones (in 48764).</b>	8	58
<i>Mc</i>	-	<b><i>Mycosphaerella citrullina</i> resistance. High degree of resistance to gummy stem blight (in PI 140471).</b>		79
<i>Mc-2</i>	<i>Mci</i>	<b><i>Mycosphaerella citrullina</i> resistance. Moderate degree of resistance to gummy stem blight (in C-1 and C-8)</b>		79
<i>Mca</i>	-	<b><i>Macrocalyx</i>. Large, leaf like structure of the sepals in staminate and hermaphrodite flowers (<i>Mca</i> in makuwa, <i>mca</i> in Annamalai).</b>		32
<i>Me</i>	-	<b><i>Mealy</i> flesh texture. Dominant to crisp flesh. (<i>Me</i> in <i>C. callosus</i>, <i>me</i> in makuwa).</b>		31
<i>ms-1</i>	<i>ms<sup>1</sup></i>	<b><i>male sterile-1</i>. Inehiscent anthers with empty pollen walls in tetrad stage.</b>	3	7
<i>ms-2</i>	<i>ms<sup>2</sup></i>	<b><i>male sterile-2</i>. Anthers inehiscent, containing mostly empty pollen walls, growth rate reduced.</b>	6	6
<i>ms-3</i>	<i>ms-L</i>	<b><i>male sterile-3</i>. Waxy and translucent inehiscent anthers, containing two types of empty pollen sacs.</b>	12	60
<i>ms-4</i>	-	<b><i>male sterile-4</i>. Small inehiscent anthers. First male flowers abort at bud stage (in Bulgaria 7).</b>	9	55
<i>ms-5</i>	-	<b><i>male sterile-5</i>. Small inehiscent anthers. Empty pollen (in Jivaro, Fox).</b>	13	53
<i>Mt</i>	-	<b><i>Mottled</i> rind pattern. Dominant to uniform color. Epistatic with <i>Y</i> (not expressed in <i>Y_</i>) and <i>st</i> (<i>Mt_sst</i> and <i>Mt_StSt</i> mottled; <i>mtmt_sst</i> striped, <i>mtmt_sst</i> uniform). (<i>Mt</i> in Annamalai, <i>mt</i> in makuwa).</b>		31
<i>Mu</i>	-	<b><i>Musky</i> flavour (olfactory). Dominant on mild flavour (<i>Mu</i> in <i>C. melo callosus</i>, <i>mu</i> in makuwa or Annamalai).</b>		31
<i>n</i>	-	<b><i>nectarless</i>. Nectaries lacking in all flowers (in 40099).</b>		3
<i>Nm</i>	-	<b><i>Necrosis with Morocco</i> strains of Watermelon Mosaic Virus (<i>Nm</i> in Védrantais, <i>nm</i> in Ouzbèque).</b>		80

Gene symbol		Character	L <sup>z</sup>	References
Preferred	Synonym			
<i>nsv</i>	-	Melon <i>necrotic spot virus</i> resistance (in Gulfstream, Planters Jumbo).	7	15
<i>O</i>	-	<i>Oval</i> fruit shape. Dominant to round; associated with <i>a</i> .	4	92
<i>p</i>	-	<i>pentamerous</i> . Five carpels and stamens; recessive to trimerous (in Casaba).	7	85
<i>Pa</i>	-	<i>Pale green</i> foliage. <i>PaPa</i> plants are white (lethal); <i>Papa</i> are yellow (in 30567).	3	59
<i>Pc-1</i>	-	<i>Pseudoperonospora cubensis</i> resistance. One of two complementary incompletely dominant genes for downy mildew resistance (in PI 124111). See <i>Pc-2</i> .		12, 88
<i>Pc-2</i>	-	<i>Pseudoperonospora cubensis</i> resistance. One of two complementary incompletely dominant genes for downy mildew resistance (in PI 124111). See <i>Pc-1</i> .		12, 88
<i>Pc-3</i>	-	<i>Pseudoperonospora cubensis</i> resistance. Partial resistance to downy mildew (in PI 414723).		24
<i>Pc-4</i>	-	<i>Pseudoperonospora cubensis</i> resistance. One of two complementary genes for downy mildew resistance in PI 124112. Interacts with <i>Pc-1</i> or <i>Pc-2</i> .		48
<i>Pgd-1<sup>1</sup></i>	<i>6-PGDH-2<sup>1</sup></i> <i>Pgd-2<sup>1</sup></i>	<i>Phosphoglucosehydrogenase-1<sup>1</sup></i> . One of two codominant alleles that regulates 6-phospho-glucosehydrogenase, each regulates one band. The heterozygote has one intermediate band. See <i>Pgd-1<sup>2</sup></i> .		27
<i>Pgd-1<sup>2</sup></i>	<i>6-PGDH-2<sup>2</sup></i> <i>Pgd-2<sup>2</sup></i>	<i>Phosphoglucosehydrogenase-1<sup>2</sup></i> . One of two codominant alleles that regulates 6-phospho-glucosehydrogenase, each regulates one band. The heterozygote has one intermediate band. See <i>Pgd-1<sup>1</sup></i> .		27
<i>Pgi-1<sup>1</sup></i>	<i>PGI-1<sup>1</sup></i>	<i>Phosphoglucoisomerase-1<sup>1</sup></i> . One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See <i>Pgi-1<sup>2</sup></i> .		27
<i>Pgi-1<sup>2</sup></i>	<i>PGI-1<sup>2</sup></i>	<i>Phosphoglucoisomerase-1<sup>2</sup></i> . One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See <i>Pgi-1<sup>1</sup></i> .		27
<i>Pgi-2<sup>1</sup></i>	<i>PGI-2<sup>1</sup></i>	<i>Phosphoglucoisomerase-2<sup>1</sup></i> . One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See <i>Pgi-2<sup>2</sup></i> .		27
<i>Pgi-2<sup>2</sup></i>	<i>PGI-2<sup>2</sup></i>	<i>Phosphoglucoisomerase-2<sup>2</sup></i> . One of two dominant alleles, each regulating two bands. The heterozygote has three bands. See <i>Pgi-2<sup>1</sup></i> .		27
<i>Pgm-1<sup>1</sup></i>	<i>PGM-2<sup>1</sup></i> <i>Pgm-2<sup>1</sup></i>	<i>Phosphoglucomutase-1<sup>1</sup></i> . One of two codominant alleles, each regulating two bands. The heterozygotes has three bands. See <i>Pgm-1<sup>2</sup></i> .		27
<i>Pgm-1<sup>2</sup></i>	<i>PGM-2<sup>2</sup></i> <i>Pgm-2<sup>2</sup></i>	<i>Phosphoglucomutase-1<sup>2</sup></i> . One of two codominant alleles, each regulating two bands. The heterozygotes has three bands. See <i>Pgm-1<sup>1</sup></i> .		27

Gene symbol		Character	L <sup>z</sup>	References
Preferred	Synonym			
<i>Pm-1</i>	<i>Pm</i> <sup>1</sup> <i>Pm-A?</i>	<i>Powdery mildew</i> resistance-1. Resistance to race 1 of <i>Sphaerotheca fuliginea</i> (in PMR 45).		42
<i>Pm-2</i>	<i>Pm</i> <sup>2</sup> <i>Pm-C?</i>	<i>Powdery mildew</i> resistance-2. Interacts with <i>Pm-1</i> . Resistance to race 2 of <i>Sphaerotheca fuliginea</i> (in PMR 5 with <i>Pm-1</i> ).		8
<i>Pm-3</i>	<i>Pm</i> <sup>3</sup>	<i>Powdery mildew</i> resistance-3. Resistance to race 1 of <i>Sphaerotheca fuliginea</i> (in PI 124111).	7	37, 38
<i>Pm-4</i>	<i>Pm</i> <sup>4</sup>	<i>Powdery mildew</i> resistance-4. Resistance to <i>Sphaerotheca fuliginea</i> (in PI 124112).		37, 38
<i>Pm-5</i>	<i>Pm</i> <sup>5</sup>	<i>Powdery mildew</i> resistance-5. Resistance to <i>Sphaerotheca fuliginea</i> (in PI 124112).		37, 38
<i>Pm-6</i>	-	<i>Powdery mildew</i> resistance-6. Resistance to <i>Sphaerotheca fuliginea</i> race 2 (in PI 124111).		46
<i>Pm-E</i>	-	<i>Powdery mildew</i> resistance-E. Interacts with <i>Pm-C</i> in PMR5 for <i>Erysiphe cichoracearum</i> resistance.		25
<i>Pm-F</i>	-	<i>Powdery mildew</i> resistance-F. Interacts with <i>Pm-G</i> in PI 124112 for <i>Erysiphe cichoracearum</i> resistance.		25
<i>Pm-G</i>	-	<i>Powdery mildew</i> resistance-G. Interacts with <i>Pm-F</i> in PI 124112 for <i>Erysiphe cichoracearum</i> resistance.		25
<i>Pm-H</i>	-	<i>Powdery mildew</i> resistance-H. Resistance to <i>Erysiphe cichoracearum</i> and susceptibility to <i>Sphaerotheca fuliginea</i> (in 'Nantais oblong').		25
<i>Pm-w</i>	<i>Pm-B?</i>	<i>Powdery mildew</i> resistance in WMR 29. Resistance to <i>Sphaerotheca fuliginea</i> race 2	2	70
<i>Pm-x</i>	-	<i>Powdery mildew</i> resistance in PI 414723. Resistance to <i>Sphaerotheca fuliginea</i> .	4	70
<i>Prv</i> <sup>1</sup>	<i>Wmv</i>	<i>Papaya Ringspot Virus</i> 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 <i>Prv</i> <sup>2</sup> .	5	74, 94
<i>Prv</i> <sup>2</sup>	-	<i>Papaya Ringspot Virus</i> resistance. Allele at the same locus as <i>Prv</i> <sup>1</sup> but different reaction with some strains of the virus (in 72-025 derived from PI 180283). Recessive to <i>Prv</i> <sup>1</sup> .	5	43, 74
<i>Px-11</i>	<i>PRX-11</i>	<i>Peroxidase-11</i> . One of two codominant alleles, each regulating a cluster of four adjacent peroxidase bands. The heterozygote has five bands. See <i>Px-12</i> .		27
<i>Px-12</i>	<i>PRX-12</i>	<i>Peroxidase-12</i> . One of two codominant alleles, each regulating a cluster of four adjacent peroxidase bands. The heterozygote has five bands. See <i>Px-11</i> .		27

Gene symbol		Character	L <sup>z</sup>	References
Preferred	Synonym			
<i>Px-2<sup>1</sup></i>	<i>Px2A</i> <i>Prx2</i>	<i>Peroxidase-2<sup>1</sup></i> . One of two codominant alleles, each regulating a cluster of three adjacent peroxidase bands. The heterozygote <i>Px-2<sup>1</sup> Px-2<sup>2</sup></i> has 4 bands. See <i>Px-2<sup>2</sup></i> .		10, 18
<i>Px-2<sup>2</sup></i>	<i>Px2B</i> <i>Prx2</i>	<i>Peroxidase-2<sup>2</sup></i> . One of two codominant alleles, each regulating a cluster of three adjacent peroxidase bands. See <i>Px-2<sup>1</sup></i> .		10, 18
<i>r</i>	-	<b>red stem. Red pigment under epidermis of stems, especially at nodes; tan seed color (in PI 157083).</b>	3	4, 59
<i>ri</i>	-	<i>ridge</i> . Ridged fruit surface, recessive to ridgeless. ( <i>Ri</i> in Pearl, <i>ri</i> in C68).		87
<i>s</i>	-	<i>sutures</i> . Presence of vein tracts on the fruit ("sutures"); recessive to ribless.		1
<i>Sfl</i>	<i>S</i>	<i>Subtended floral leaf</i> . The floral leaf bearing the hermaphrodite flowers is sessile, small and encloses the flower. ( <i>Sfl</i> in makuwa, <i>sfl</i> in Annamalai).		32
<i>si-1</i>	<i>b</i>	<b>short internode-1. Extremely compact plant habit (bush type) (in UC Topmark bush).</b>	1	20
<i>si-2</i>	-	<b>short internode-2. Short internodes from 'birdnest' melon (in Persia 202).</b>		67
<i>si-3</i>	-	<b>short internode-3. Short internodes in 'Maindwarf' melon.</b>		49
<i>Skdh-1<sup>1</sup></i>	-	<i>Shikimate dehydrogenase-1</i> . One of two codominant alleles, each regulating one band (see <i>Skdh-1<sup>2</sup></i> ).		10
<i>Skdh-1<sup>2</sup></i>	-	<i>Shikimate dehydrogenase-1</i> . One of two codominant alleles, each regulating one band (see <i>Skdh-1<sup>1</sup></i> ).		10
<i>So</i>	-	<i>Sour</i> taste. Dominant to sweet.		51
<i>sp</i>	-	<i>spherical</i> fruit shape. Recessive to obtuse; dominance incomplete.		1, 56
<i>st</i>	-	<i>striped</i> epicarp. Recessive to non-striped.		36
<i>v</i>	-	<b>virescent. Pale cream cotyledons and hypocotyls; yellow green foliage (mainly young leaves).</b>	11	39
<i>v-2</i>	-	<b>virescent-2.</b>		23
<i>v-3</i>	-	<b>virescent-3. White cotyledons which turn green, light green young leaves which are normal when they are older</b>		76
<i>Vat</i>	-	<b>Virus aphid transmission resistance. Resistance to the transmission of viruses by <i>Aphis gossypii</i> (in PI 161375).</b>	2	73
<i>w</i>	-	<b>white</b> color of mature fruit. Recessive to dark green fruit skin. ( <i>w</i> in honeydew, <i>W</i> in Smiths' Perfect cantaloupe).		40

Gene symbol		Character	L <sup>2</sup>	References
Preferred	Synonym			
<i>wf</i>	-	<i>white flesh</i> . Recessive to salmon. <i>Wf</i> epistatic to <i>Gfgf</i> .		11, 41
<i>Wi</i>	-	White color of <i>immature</i> fruit. Dominant to green.		51
<i>Wmr</i>	-	<i>Watermelon Mosaic Virus 2</i> resistance (in PI 414723)		35
<i>Wt</i>	-	<i>White testa</i> . Dominant to yellow or tan seed coat color.		36
<i>Y</i>	-	<i>Yellow epicarp</i> . Dominant to white fruit skin.		36
<i>yg</i>	-	<i>yellow green</i> leaves. Reduced chlorophyll content.	6	95
<i>yg<sup>w</sup></i>	<i>lg</i>	<i>yellow green Westlaco</i> . First described as <i>light green</i> in a cross Dulce x TAM-Uvalde. Allelic to <i>yg</i> .		17
<i>yv</i>	-	<i>yellow virescence</i> . Pale cotyledons; yellow green young leaves and tendrils; bright and yellow petals and yellow stigma; etiolated; older leaves becoming green.	1	96
<i>yv-2</i>	-	<i>yellow virescence-2</i> . Young leaves yellow green, old leaves normal green		77
<i>Zym-1</i>	<i>Zym</i>	<i>Zucchini Yellow Mosaic Virus</i> resistance. Resistance to pathotype 0 of this virus (in PI 414723).	4	75
<i>Zym-2</i>	-	<i>Zucchini Yellow Mosaic Virus</i> resistance. One of three complementary genes (see <i>Zym-1</i> and <i>Zym-3</i> ) for resistance to this virus (in PI 414723)		19
<i>Zym-3</i>	-	<i>Zucchini Yellow Mosaic Virus</i> resistance. One of three complementary genes (see <i>Zym-1</i> and <i>Zym-2</i> ) for resistance to this virus (in PI 414723)		19

<sup>2</sup> Linkage group to which this gene belongs.

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## Gene Nomenclature for the Cucurbitaceae

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. All letters of the symbol and name are in lower case if the mutant gene is recessive, with the first letter of the symbol capitalized for the dominant or normal allele. (Note: For CGC *research articles*, the normal allele of a mutant gene may be represented by the symbol "+", or the symbol of the mutant gene followed by the superscript "+", if greater clarity is achieved for the manuscript.)
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 re-occurrences 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.
10. 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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- Brown, Rebecca** Dept. Hort., Oregon St. Univ., Ag Life Sci Bldg 4017, Corvallis, OR, 97331. Ph.: (541) 737-5462; Email: [brownr@bcc.orst.edu](mailto:brownr@bcc.orst.edu). Virus resistance, *Cucurbita* germplasm, squash breeding.
- Burkett, Al** Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219. Pickling cucumber breeding.
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- Carey, Edward E.** International Potato Center (CIP), P.O. Box 25171, Nairobi, Kenya. Ph.: 254-2-632054; Fax: 254-2-631499/630005. Email: [t.carey@cgnnet.com](mailto:t.carey@cgnnet.com). Breeder with interest in cucurbits.
- Carle, R. Bruce** UF/IFAS, Central Florida R&E Ctr, 5336 University Ave., Leesburg, FL, 34748-8232. Ph.: (352) 360-6686; Fax: (352) 360-6691. Email: [rbcwm@gnv.ifas.ufl.edu](mailto:rbcwm@gnv.ifas.ufl.edu). Watermelon and squash breeding.
- Chen, Fure-Chyi** Dept. Plant Industry, Natl. Pingtung Univ. Sci. & Techn., Neipu, Pingtung 91207, Taiwan, Rep. China. Ph.: 886-8-774-0267; Fax: 886-8- 774-0371. Email: [fure-chen@mail.npust.edu.tw](mailto:fure-chen@mail.npust.edu.tw). Gene transfer, breeding, tissue culture and isozymes.
- Ching, Alejandro "Alex"** Alternative Crops Res Ctr, NW MO St U, 106 Valk, 800 Univ Dr Maryville, MO, 64468. Ph.: (660) 562-1126; Fax: (660) 562-

1621. E mail: [alching@mail.nwmissouri.edu](mailto:alching@mail.nwmissouri.edu). Breeding & introduction of new cucurbits. Production & nutritional quality.
- Chung, Paul** Seminis Vegetable Seeds, Inc., 37437 State Highway 16, Woodland, CA. 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219.
- Coffey, Robyn** Willhite Seed, Inc., P.O. Box 23, Poolville, TX, 76487. Ph.: (817) 599-8656; Fax: (817) 599-5843. Email: [robyn@willhiteseed.com](mailto:robyn@willhiteseed.com).
- Cohen, Ron** Neve Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 30095, Israel. Ph.: 972-4-953-9516; Fax: 972-4-983-6936. Plant pathology; root and foliar diseases of cucurbits.
- Cohen, Yigal** Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52 100, Israel. Ph.: + 9723-5318251; Fax: + 9723-6771088. Melon.
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- Corella, Pilar** Asgrow Spain S.L., Paraje San Nicolas s/n, 04547 La Mojenera, Almeria, Spain. Ph.: 34-51-580012; Fax: 34-51-581162.
- Coyne, Dermot P.** Department of Horticulture, University of Nebraska, Lincoln, NE. 68583-0724. Ph.: (402) 472-1126; Fax: (402) 472-8650. Email: [dcoyne@unlinfo.unl.edu](mailto:dcoyne@unlinfo.unl.edu). Breeding and genetics of squash.
- Cramer, Chris** Dept. Agron. & Hort., NMSU, P.O. Box 30003, Dept. 3Q, Las Cruces, NM, 88003-8003. Ph.: (505) 646-3405. Email: [chris\\_cramer@nmsu.edu](mailto:chris_cramer@nmsu.edu). Cucumber yield, yield components, combining ability, heterosis and recurrent selection.
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- Decker-Walters, Deena** The Cucurbit Network, 11901 Old Cutler Road, Miami, FL, 33156-4242. Ph.: (305) 667-3800; Fax: (305) 661-5984. Email: [walters@servax.fiu.edu](mailto:walters@servax.fiu.edu). Communication via The Cucurbit Network: the whole family Cucurbitaceae.
- Della Vecchia, Paulo T.** Agroflora S/A, Caixa Postal 427, 12.900-000 Braganca, Paulista - SP, Brazil. Ph.: (011) 7871-0855; Fax: (011) 7843-6572. Breeding & genetics, seed production and disease resistance of melon and squash.
- Denlinger, Phil** Mt. Olive Pickle Co., Inc., P.O. Box 609, Mount Olive. NC. 28365. Ph.: (919) 658-2535; Fax: (919) 658-6090. Email: [cn1713@coastal-net.com](mailto:cn1713@coastal-net.com).
- Dhaliwal, Major Singh** Dept. of Vegetable Crops, L.S.&F. Punjab Agriculture Univers Ludhiana-141004, Punjab, India.
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- Funakushi, Hisashi** Mikado Seed Growers Co., Ltd., 1203 Hoshikuki, Chuo-ku, Chiba City 260, Japan. Ph.: 81-43-265-4847; Fax: 81-43-266-6444.
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- Gabert, August C.** Sunseeds, 8850 59th Ave. NE, Brooks, OR, 97305-9625. Ph.: (503) 393-3243; Fax: (503) 390-0982. Email: *agabert%sunseeds@mci-mail.com*. Cucumber breeding and genetics.
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- Gómez Paniagua, Humberto** Semillas Fito, S.A., Calle Selva de Mar, 111, 08019 Barcelona, Spain. Ph.: (34) 93 307 6212; Fax: (34) 93 307 0364. Email: *humberto@abonados.cplus.es*. Disease resistance and quality of melons (esp. Spanish) & cucumber; breeding schemes & genetic markers.
- Gómez-Guillamón, M. Luisa** Estacion Experimental "La Mayora", 29750 Algarrobo- Costa, Malaga, Spain. Ph.: (952) 51 10 00; Fax: (952) 51 12 52. Email: *guillamon@mayora.csic.es*.
- Green, C. Ed** Seminis Vegetable Seeds, Inc., 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219.
- Groff, David** Asgrow Seed Company, Rt. 1, Box 1907, Omega TyTy Road, Tifton, GA, 31794. Ph.: (912) 386-8701; Fax: (912) 386-8805. Breeding of squash, cucumber, melon and watermelon.
- Grumet, Rebecca** Dept. Hort., Plant & Soils Building, Michigan State University East Lansing, MI, 48824-1325. Ph.: (517) 353-5568; Fax: (517) 353-0890. Email: *grumet@pilot.msu.edu*. Disease resistance, gene flow, tissue culture and genetic engineering.
- Gupta, Satish C.** Reitzel India Ltd., 220 Agil Campus, Whitefield Post, Bangalore 560066, India. Ph.: 91-080-8452415; Fax: 91-080-8453063.
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- Hirabayashi, Tetsuo** Nihon Horticultural Production Inst., 207 Kamishiki, Matsudo Chiba-ken 270-2221, Japan. Ph.: 0473-87-3827; Fax: 0473-86-1455. Varietal improvement of cucurbit crops, especially melon, cucumber and pumpkin.
- Hollar, Larry A.** Hollar & Co., Inc., P.O. Box 106, Rocky Ford, CO, 81067. Ph.: (719) 254-7411; Fax: (719) 254-3539. Email: [lahollar@iguana.rural-net.net](mailto:lahollar@iguana.rural-net.net). Cucurbit breeding and seed production.
- Holle, Miguel** CALCE 2, # 183 Urb. El Rancho, Miraflores - Lima 18, Peru. Ph.: 51-14-383749; Fax: 51-14-351570. Email: [m.holle@cgnet.com](mailto:m.holle@cgnet.com). Plant genetic resources.
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- Iida, Akira** Minowa Noen, 63-1 Ichieda-cho, Yamato-Kohriyama City, Nara Pref., Japan, T639-11.
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- Jiang, Jiping** Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219. Developing disease screens for fungal diseases of cucurbits.
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- Kamimura, Shoji** 421-19 Furuichi-machi, Maebashi City, Gunma-ken 371, Japan.
- Kampmann, Hans Henrik** Breeding Station Dane-feld, Odensevej 82, 5290 Marslev, Denmark. Ph.: 65 95 17 00; Fax: 65 95 12 93.
- Karchi, Zvi** 74 Hashkedim St., Kiryat-Tivon 36501, Israel. Ph.: 04-9830107; Fax: 972-4-9836936. Cucurbit breeding, cucurbit physiology.
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- Love, Stephen Loyd** Aberdeen R&E Center, P.O. Box AA, Aberdeen, ID, 83210. Ph.: (208) 397-4181; Fax: (208) 397-4311. Email: *slove@uidaho.edu*. Small scale private watermelon breeding with emphasis on adaptation to cold climates.
- Lower, Richard L.** Coll. Agriculture, Univ. Wisconsin, 1450 Linden Drive, Room Madison, WI, 53706. Ph.: (608) 262-2349; Fax: (608) 265-6434. Email: *richard.lower@ccmail.adp.wisc.edu*. Effects of plant type genes on yield, sex-expression, growth parameters, pest resistance & adaptability.
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- Lydon, Lewis R.B.** Arthur Yates & Co. Pty. Limited, Research Farm, Burroway Roa Narromine, N.S.W. 2821, Australia. Ph.: (068) 89-1144.
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- Markiewicz-Ladd, Krystyna** Polonica International, P.O. Box 2305, Gilroy, CA, 95021. Ph.: (408) 675-0103; Fax: (408) 842-1022. Email: *polonica@aol.com*. Melons - breeding, new germplasm, postharvest physiology, biotechnology, cultural practices, new diseases.
- Martyn, Ray D.** Dept. Botany & Plant Pathology, 1155 Lilly Hall, Purdue Univ West Lafayette, IN, 47907-1155. Ph.: (765) 494-4615; Fax: (765) 494-0363. Email: *Martyn@bmy.purdue.edu*. Soilborne diseases of watermelon and melon, particularly the *Fusarium* wilts and vine declines.
- Matsuura, Seiji** Kiyohara Breeding Sta., Tohoku Seed Co., 1625 Nishihara, Him Utsunomiya, Japan. Ph.: 0286-34-5428; Fax: 0286-35-6544.
- Maynard, Donald N.** University of Florida, 5007 60th Street East, Bradenton, FL, 34203. Ph.: (941) 751-7636; Fax: (941) 751-7639. Email: *bra@gnv.ifas.ufl.edu*. Tropical *moschata* improvement; watermelon variety evaluation and production practices.
- Mazereeuw, J.P.** SETO A.S., Cebecoy Caddesi, Akasya Apt. 45/1, 07100 Antalya, Turkey.
- McClurg, Charles A.** University of Maryland, Dept. Natural Resource Sci., College Park, MD, 20742-

4452. Ph.: (301) 405-4342; Fax: (301) 314-9308. Email: *cm19@umail.umd.edu*. Production and culture of cucurbit crops.
- McCreight, J.D.** USDA-ARS, 1636 E. Alisal St., Salinas, CA, 93905. Ph.: (831) 755-2864; Fax: (831) 755-2814. E-mail: *jmccreig@asrr.arsusda.gov*. Melon breeding and genetics.
- McGrath, Desmond John** Dept. Primary Ind., Hort. Res. Sta., P.O. Box 538, Bowen, Queensland 4805, Australia. Ph.: + 61-7-4785 2255; Fax: + 61-7-4785 2427. E-mail: *mcgratdj@prose.dpi.qld.gov.au*. Disease resistance in *Cucumis melo*, particularly gummy stem blight.
- Meadows, Mike** Rogers Seed Co., 10290 Greenway Road, Naples, FL, 34114. Ph.: (941) 775-4090; Fax: (941) 774-6852. E-mail: *Mike.Meadows@GWA.Sandoz.com*. Vegetable diseases.
- Meléndez, Roberto Compeán** Heriberto Valdez 647 PTE., C.P. 81200, Los Mochis, Sinaloa, Mexico. Ph.: (68) 18-37-22.
- Merrick, Laura C.** Dept. Agron., Iowa St. Univ, @101 Agronomy Hall, Ames, IA, 50011-1010. Ph.: (515) 294-7636; Fax: (515) 294-3163. E-mail: *lmerri@iastate.edu*. *Cucurbita* evolution; cucurbit germplasm evaluation and conservation; ethnobotany and evolution.
- Milerue, Sompong** Peto Thailand, P.O. Box 171, 99 Moo 2, Wiang-Y, Mae Gorn, A Muang Chiang Rai 57000, Thailand.
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- Mohamed, El Tahir Ibrahim** PGR unit/Horticulture, Agr Res Corp, P.O. Box 126, Wad Medani, Sudan.
- Mohamed, Yousif Fadlalla** Dept Plant Pathol, Fac Agric Sci, University of Gezira, Wad Medani, P.O. Box 20, Sudan.
- Moraghan, Brian Joseph** Asgrow Seed Co., P.O. Box 667, Arvin, CA, 93203. Ph.: (805) 854-2390; Fax: (805) 854-4379. E-mail: *brian.moraghan@svseeds.com*. Melon and watermelon breeding and disease resistance.
- Morelock, Ted** Dept. Horticulture & Forestry, University of Arkansas, Fayetteville, AR, 72701. Ph.: (501) 575-2603; Fax: (501) 575-8619. E-mail: *morelock@comp.uark.edu*. Cucumber breeding.
- Munger, H.M.** Cornell University, 252 Emerson Hall, Ithaca, NY, 14853. Ph.: (607) 255-7820; Fax: (607) 255-6683. E-mail: *hmm11@cornell.edu*. Cucurbit breeding and disease resistance.
- Navazio, John P.** Chriseed, P.O. Box 1788, Mount Vernon, WA, 98273. Ph.: (360) 336-9727; Fax: (360) 424-9520. Breeding for increased pigments in cucurbits, carrots and beets.
- Nea, Larry** Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219. Cucumbers, melons, squash, watermelon.
- Nechama, Shulamit** Breeding Department, Mivhor Farm, Post Sde Gat 79570, Israel.
- Ng, Timothy J** Dept. Natural Resource Sci., University of Maryland, College Park, MD, 20742-4452. Ph.: (301) 405-4345; Fax: (301) 314-9308. E-mail: *tm5@umail.umd.edu*. Melon breeding and genetics; postharvest physiology; seed germination.
- Niemirowicz-Szczytt, Katarzyna** Warsaw Ag Univ, Dept Gen & Plt Brdng, ul. Nowoursynowska 166 02-766 Warsaw, Poland. Ph.: 43 09 82; Fax: (48-22) 471562. Cucumber, melon, winter and summer squash, watermelon - genetics, breeding, tissue culture, biotechnology.
- Norton, Joseph D.** Dept. Horticulture, 101 Funchess Hall, Auburn Univ., Auburn, AL, 36849. Ph.: (205) 844-3031; Fax: (205) 844-3131. Breeding and genetics of melon and watermelon.
- Nuez, Fernando** Cat.de Genetica, ETS Ingen. Agron., Univ. Politecnica, Camin 46020 Valencia, Spain. Ph.: 34 (6) 387-74-21; Fax: 34 (6) 387-74-29. E-mail: *fnuez@btc.upv.es*. Genetics and plant breeding.
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- 60511-110-Fortaleza-Ceara, Brazil. Ph.: (085) 299.18.01; Fax: (085) 299.18.03. Email: [Walde@cnpat.embrapa.br](mailto:Walde@cnpat.embrapa.br). Research with cucurbit species, especially *Cucumis*, and particularly *Cucumis melo*.
- Om, Young-Hyun** Natl Horticultural Res Inst, 475 Imok-Dong, Suwon 440-310, Republic of Korea. Ph.: 82-0331-290-6171; Fax: 82-0331-295-9548. Email: [omyh@nhri.go.kr](mailto:omyh@nhri.go.kr). Breeding of cucurbit vegetables.
- Omara, Sadig Khidir** Dept. Horticulture, Fac. Agric. Sci., University of Gezira, Wad Medani, P.O. Box 20, Sudan.
- Ortega, Sergio Garza** Univ. Sonora, Dept. Agric. y Ganad., A.P. Postal 305, Hermosillo, Sonora, Mexico. Breeding of *Cucurbita* spp.; testing of new muskmelon lines.
- Ouyang, Wei** Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219. Squash & cucumber breeding.
- Owens, Ken** Seminis Vegetable Seeds, Inc., 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219. Cucumber breeding.
- Palmer, Louis** Pioneer Vegetable Genetics, 4331 Cockroach Bay Road, Ruskin, FL, 33570-2612. Ph.: (813) 645-3946; Fax: (813) 645-4900. Breeding and genetics.
- Palomares, Gloria** Dept Biotecnologia, Univ Politecnica, Camino de Vera, s/n., E-46022 Valencia, Spain. Ph.: 34(6)387-421/7426; Fax: 34(6)387-429. Email: [gpaloma@btc.upv.es](mailto:gpaloma@btc.upv.es). Genetic improvement in horticultural plants.
- Paris, Harry** Dept. Vegetable Crops, A.R.O., Newe Ya'ar Research Ctr, PO B Ramat Yishay 30-095, Israel. Ph.: 972-4-9894516; Fax: 972-4-9836936. Email: [hsparis@netvision.net.il](mailto:hsparis@netvision.net.il). Breeding and genetics of squash and pumpkin.
- Peiro Abril, Jose Luis** Apartado de Correos no. 2, 04720 Aguadulce, Almeria, Spain. Fax: 34 50 34 34 01.
- Perl-Treves, Rafael** Dept. Life Science, Bar-Ilan University, Ramat-Gan, Israel 52900. Email: [perl@brosh.cc.biu.ac.il](mailto:perl@brosh.cc.biu.ac.il).
- Peter, K.V.** Natl. Research Ctr for Spices, ICAR, Post Bag No. 1701, Mari Calicut - 673 012, Kerala, India. Ph.: 011-91-495-258457.
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- Poulos, Jean M.** Asgrow Italia, Veg. Seeds Srl, Pontinia Research Station, C.P. 110-04014 Pontinia, Italy. Ph.: 39(0)773 848549; Fax: 39(0)773 848548.
- Price, E. Glen** American Sunmelon Research Center, P.O. Box 153, Hinton, OK, 73047. Ph.: (405) 542-3456; Fax: (405) 542-3457. Seedless watermelon; polyploidy, genetics, breeding, cytogenetics.
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- Reuling, G.T.M.** Nunhems Zaden B.V., P.O. Box 4005, 6080 AA Haelen, The Netherlands. Ph.: 0475-599222; Fax: 0475-599223. Email: [bre@nunhems.nl](mailto:bre@nunhems.nl). Cucumber breeding.
- Rhodes, Bill B.** Clemson Univ./Horticulture, Poole Agricultural Center, Clemson, SC, 29634-0375. Ph.: (864) 656-0410; Fax: (864) 656-4960. Email: [BRhodes@clemson.edu](mailto:BRhodes@clemson.edu). Watermelon genetics, breeding, micropropagation, disease resistance, male sterility, triploids.
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- Rizzo, Adriana A. do Nascimento** FCAV-UNESP-Campus de Jaboticabal, Departamento de Horticultura, Rod. Carlos Tonnan, Km5, Jaboticabal-SP-Brazil Cep-1870-000. Email: [drarizzo@fcav.unesp.br](mailto:drarizzo@fcav.unesp.br).
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- Robledo, Claude** Seminis - Recherch France, Mas de Rouzel - Chemin des Canaux 30900 Nimes, France. Ph.: 33(0)4.66.38.79.80; Fax: 33(0)4.66.38.79.81. Melon breeding.
- Roig, Luis A.** Departamental Biotechnology, ETS Ingen. Politec., Camino de 46022 - Valencia, Spain. Ph.: 34(6) 3877424; Fax: 34(6) 3877429.
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- Sarfatti, Matti** Hazera Ltd., Research Dept., Mivhor, M.P., Lakhish Daram 79354, Israel.
- Schipper, Bets.** Novartis Seeds B.V., Westeinde 62 / P.O. Box 2, 1600 AA Enkhuizen, The Netherlands. Ph.: (0228) 36 62 79; Fax: (0228) 36 63 48. Email: [bets.schipper@seeds.novartis.com](mailto:bets.schipper@seeds.novartis.com).
- Schroeder, Robert Harold** Harris Moran Seed Co., 9241 Mace Blvd., Davis, CA, 95616. Ph.: (530) 756-1382; Fax: (530) 756-1016. Incorporating disease resistance into useful commercial cultivars.
- Schultheis, Jonathan R.** Dept. Horticulture, 264 Kilgore Hall, North Carolina St. Uni Raleigh, NC, 27695-7609. Ph.: (919) 515-3131; Fax: (919) 515-7747. Email: [jonathan\\_schultheis@ncsu.edu](mailto:jonathan_schultheis@ncsu.edu). Cultural management of cucurbits; plant spacing, establishment, nutrition, pollination & cultivar evaluation.
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- Shiga, Toshio** Plant Biotech. Ctr., Sakata Seed Corp., 358 Uchikoshi, Sodeg Chiba, 299-02, Japan. Ph.: 0438-75-2369; Fax: 0438-75-2594. Cell biology.
- Simon, Philipp W.** USDA/ARS-Veg Crops, Dept. Hort., Univ. Wisconsin, 1575 Linde Madison, WI, 53706. Ph.: (608) 262-1248; Fax: (608) 262-4743. Email: [psimon@facstaff.wisc.edu](mailto:psimon@facstaff.wisc.edu). Breeding and genetics.
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- Email: [skirvin@uxl.cso.uiuc.edu](mailto:skirvin@uxl.cso.uiuc.edu). Micropropagation; somaclonal variation.
- Snyder, James W.** 1231 Kirkwood Drive, Vineland, NJ, 08360. Ph.: (609) 794- 3880; Fax: (609) 794-3881.
- Staub, Jack E.** USDA-ARS, Dept. Horticulture, Univ. Wisconsin, Madison, WI, 53706-1590. Ph.: (608) 262-0028; Fax: (608) 262-4743. Email: [jestaub@facstaff.wisc.edu](mailto:jestaub@facstaff.wisc.edu). Cucumber breeding & genetics, physiology, biochemical genetic markers, evolution, environmental stress.
- Stephenson, Andrew G.** 208 Mueller Lab, Penn State University, University Park, PA, 16802. Ph.: (814) 863-1553; Fax: (814) 865-9131. Email: [as4@psu.edu](mailto:as4@psu.edu).
- Stevens, M. Allen** Seminis Vegetable Seeds, Inc., 37437 State Highway 16, Woodland, CA, 95695. Ph.: (916) 666-0931; Fax: (916) 668-0219. Direction of research.
- Stravato, Vittorio M.** c/o Peto Italiana S.R.L., Via Canneto di Rodi, 04010 Borgo Sabotino (LT), Italy. Ph.: 773-643336; Fax: 773-643722. Email: [Vstravato@svseeds.nl](mailto:Vstravato@svseeds.nl).
- Suh, Hyoung** c/o Nath Sluis Ltd., Nath House, Nath Road, Aurangabad, 431005, India.
- Summers, William L.** Iowa State University, Dept. Horticulture, Rm. 251, Ames, IA, 50011-1100. Ph.: (515) 294-1978; Fax: (515) 294-0730. Email: [summers@iastate.edu](mailto:summers@iastate.edu). Genetic improvement of watermelon
- Susic, Zoran** Inst. "Srbija" - Ctr Vegetable Crops, Karadjordjeva 71, 11420 Smederevska Palanka, F.R. Yugoslavia. Ph.: + 381-26-314 170; Fax: + 381-26-314 786. Email: [djelovac@eunet.yu](mailto:djelovac@eunet.yu). Genetics and breeding of cucurbita species.
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- Taurick, Gary** Harris Moran Seed Co., P.O. Box 392, Sun Prairie, WI, 53590. Ph.: (608) 837-6574. Fax: (608) 837-3758. Development of commercial hybrids of pickle, slicer and Beit Alpha cucumbers.
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- Thomas, Claude E.** USDA-ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC, 29414. Ph.: (803) 556-0840; Fax: (803) 763-7013. Email: [cthomas@awod.com](mailto:cthomas@awod.com). Disease resistance in cucurbits.
- Thompson, Gary** Dept. Plant Sciences, University of Arizona, Tucson, AZ, 85721. Email: [garyt@u.arizona.edu](mailto:garyt@u.arizona.edu).
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- van Eijk, Manuel** East West Seed Co. Philippines, P.O. Box 2384 MCPO, 1263 Makati MM, Philippines. Ph.: 63-2-8238345; Fax: 63-2-8238346. Email: [ewseedph@webquest.com](mailto:ewseedph@webquest.com). Breeding of bitter gourd, squash, cucumber, melon, watermelon, sponge gourd, and bottle gourd.
- van Kooten, Henk** Bruinsma Seeds B.V., P.O. Box 93, 2675 ZH Honselersdijk, The Netherlands. Ph.: + 31-174-61 50 15; Fax: + 31-174-61 50 20.
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- Warid, Warid A.** 11 Cairo University Street. Apartment # 4, Giza - 12211, Egypt. Breeding of cucurbits.
- Wasilwa, Lusike** Rutgers Blueberry/Cranberry Res Ctr, 125a Lake Oswego Rd., Chatsworth, NJ, 08019. Ph.: (609) 726-1590; Fax: (609) 726-1593. Email: *wasilwa@aesop.rutgers.edu*. Disease screening, fungal genetics, evaluation of fungal diversity of *Colletotrichum* spp.
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- Wu, Mingzhu** Hort Inst, Xinjiang Acad Agric Sci, Nanchang Road NO. 38, Urumqi, Xinjiang, People's Rep. China. Ph.: 0991-4840311-2094.
- Wu, Wendy Y.** Known-You Seed Co., Ltd., 330, Kao Tan Village, Jen Wu Hsing Kaohsiung, 814, Taiwan, R.O.C. Ph.: 886-7-3719725; Fax: 886-7-3718510. Breeding and growing cucurbits (all).
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- Yorty, Paul** Qualiveg Seed Production, 3033 E., 3400 N., Twin Falls, ID, 83301. Ph.: (208) 733-0077; Fax: (208) 733-0077. Cucurbit breeding.
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Joseph H. Kirkbride, Jr.  
Charles A. McClurg  
Timothy J Ng

### Michigan

Rebecca Grumet

### Missouri

Alejandro "Alex" Ching

### Nebraska

Dermot P. Coyne

### New Hampshire

J. Brent Loy

### New Jersey

Oved Shiffriss  
James W. Snyder  
Gang Wang  
Lusike Wasilwa

### New Mexico

Chris Cramer

### New York

Thomas C. Andres  
Molly Kyle Jahn  
H.M. Munger  
Rosario Providenti  
R.W. Robinson  
Thomas A. Zitter

### North Carolina

Phil Denlinger  
Jonathan R. Schultheis  
Nischit Shetty  
Todd C. Wehner

### Oklahoma

E. Glen Price

### Oregon

Rebecca Brown  
Louis Victor Di Nitto  
August C. Gabert  
Mark Hutton  
Joel Reiten

### Pennsylvania

Andrew G. Stephenson

### Puerto Rico

Linda Wessel-Beaver

### South Carolina

Bill B. Rhodes  
Claude E. Thomas

### Texas

Robyn Coffey  
Joseph O. Kuti  
Gene Lester

### Virginia

Greg Welbaum

### Washington

John P. Navazio

### Wisconsin

Michael J. Havey  
Richard L. Lower  
Philipp W. Simon  
Jack E. Staub  
Gary Taurick



## International CGC Members

### Argentina

Fernando López Anido

### Australia

Lewis R.B. Lydon  
Desmond John McGrath  
Anthony E. Rumsey

### Austria

Tamas Lelley  
Herwig Teppner  
Johanna Winkler

### Bangladesh

Gertjan Feitsma

### Brazil

Paulo T. Della Vecchia  
Wilson Roberto Maluf  
Waldelice Oliveira de Paiva  
Adriana A. do Nascimento Rizzo

### Canada

Zamir K. Punja

### China, P.R.

Haiqing Bao  
Hongwen Cui  
Jiabin Ji  
Depei Lin  
Ming Wang  
Mingzhu Wu  
Jiannong Zhang

### Cuba

Humberto Ríos Labrada

### Czech Republic

Eva Kristková

### Denmark

Hans Henrik Kampmann

### Egypt

Ahmed Abdel-Moneim Hassan  
Warid A. Warid

### England

Iraj Poostchi

### France

Sylvie Baudracco-Arnas  
C. Dogimont  
Graines Gautier  
Frédéric Ignart  
Michel Lecouviour  
Florence Picard  
Michel Pitrat  
Claude Robledo  
Bruno Sipeyre

### Germany

Hubert Kuhlmann  
Turan Tatlioglu

### Greece

A.S. Tsafaris  
Demetrios J. Vakalounakis

### Guadeloupe (F.W.I.)

Nathalie Boissot

### India

Major Singh Dhaliwal  
Satish C. Gupta  
Vasanth Kumar  
K.V. Peter  
Hyoung Suh

### Indonesia

Doretta Akkermans

### Israel

Ron Cohen  
Yigal Cohen  
Yael Danin-Poleg  
Victor Gaba  
Davidi Haim  
Ran Herman  
Zvi Karchi  
Nurit Katzir  
Shulamit Nechama  
Harry Paris  
Rafael Perl-Treves  
Matti Sarfatti  
Eyal Vardi

### Italy

Erik de Groot  
Gianni Gatto  
Jean M. Poulos  
Vittorio M. Stravato

### Japan

Hiroshi Ezura  
Hisashi Funakushi  
Toshitsugu Hagihara  
Tetsuo Hirabayashi  
Akira Iida  
Kimio Ito  
Shoji Kamimura  
Kenji Kato  
Sugiyama Keita  
Yoshihiro Konno  
Yasuhisa Kuginuki  
Seiji Matsuura  
Tatsuya Mochizuki  
Takeo Saito  
Toshio Shiga  
Hisako Yamanaka

### Kenya

Edward E. Carey

### Korea, Rep. of

Chang-Soon Ahn  
Sang Joo Han  
Young-Seok Kwon  
Soo Nyeon Kwack

Cheol-Sang Kwon

Hak-Tae Lim  
Young-Hyun Om

### Mexico

Roberto Compeán Meléndez  
Sergio Garza Ortega

### Namibia

Gillian Maggs

### Netherlands, The

A.G.B. Beekman  
P.A. Boorsma  
Monique Bosma  
A.C. de Ruiter  
K. Hertogh  
Paul Heuvelmans  
Ad Klapwijk  
G.T.M. Reuling  
Bets Schipper  
Henk van Kooten

### Peru

Miguel Holle

### Philippines

Manuel van Eijk

### Poland

Katarzyna Niemirowicz-Szczytt

### Spain

Ma Cruz Ayuso  
Pilar Corella  
Humberto Gómez Paniagua  
M. Luisa Gómez-Guillamón  
Peter Kraakman  
Fernando Nuez  
Gloria Palomares  
Jose Luis Peiro Abril  
Luis A. Roig

### Sudan

Ali Elamin El Jack  
El Tahir Ibrahim Mohamed  
Yousif Fadlalla Mohamed  
Sadig Khidir Omara

### Sweden

Louis Carl Lehmann

### Taiwan, R.O.C.

Fure-Chyi Chen  
Wendy Y. Wu

### Thailand

Usa Duangsong  
Sompong Milerue

### Turkey

Gülal Çağlar  
J.P. Mazereeuw

### Yugoslavia, F.R.

Zoran Susic

## **Covenant and By-Laws of the Cucurbit Genetics Cooperative**

### **ARTICLE I. Organization and Purposes**

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

### **ARTICLE II. Membership and Dues**

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

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

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

### **ARTICLE III. Committees**

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

### **ARTICLE IV. Election and Appointment of Committees**

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

their discretion. The nominations shall be announced and election held by open ballot at the Annual Meeting of the CGC. The nominee receiving the highest number of votes shall be declared elected. The newly elected member shall take office immediately.

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

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

## **ARTICLE V. Publications**

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

## **ARTICLE VI. Meetings**

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

## **ARTICLE VII. Fiscal Year**

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

## **ARTICLE VIII. Amendments**

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

## **ARTICLE IX. General Prohibitions**

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

1. The CGC shall be organized and operated exclusively for scientific and educational purposes.
2. No part of the net earnings of the CGC shall or may under any circumstances inure to the benefit of any individual.

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

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

#### **ARTICLE X. Distribution on Dissolution**

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

**Cucurbit Genetics Cooperative  
Financial Statement  
31 December 1997**

**Balance (31 December 1996) \$3,408.61**

**Receipts:**

Dues & CGC back issue orders	\$3,242.00
Proc. Cucurbitaceae '96	\$777.00
Interest on savings	\$90.06
<b>Total Receipts</b>	<b>\$4,019.00</b>

**Expenditures:**

CGC Report No. 20 (1997)	
Printing	\$1,716.14
Mailing	\$639.07
Proc. Cucurbitaceae '96	\$862.50
Call for papers (Report No. 21)	\$162.00
Renewal notices	\$84.00
Bank fees & adjustment charges	\$69.00
Miscellaneous (envelopes, postage)	\$466.33
<b>Total Expenditures</b>	<b>\$3,999.04</b>

**Balance (31 December 1997) \$3,428.57**