Cucurbit Genetics Cooperative

Report No. 14

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

CGC Membership and Subscription Rates

Biennium	Member	Library
1991-92	$14.00 US*	$24.00 US

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

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

Table of Contents

III. Watermelon

Comments from the CGC Coordinating Committee

The Call for Papers for the 1992 Report (CGC Report No. 15) will be mailed in August 1991. Papers should be submitted to the respective Coordinating Committee members by 31 December 1991. The Report will be published by June 1992. As always, we are eager to hear from CGC members regarding our current activities and the future direction of CGC.

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

Comments from the CGC Gene List Committee

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

It is hoped that scientists will consult these lists as well as the rules of gene nomenclature for the Cucurbitaceae (see page 140) before selecting a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature were adopted in order to provide guidelines for the naming and symbolizing of genes previously reported and those while will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

Cucumber:	Todd C. Wehner
Muskmelon:	Michel Pitrat
Watermelon:	Warren R. Henderson
Cucurbita spp.:	Richard W. Robinson
Other Genera:	Richard W. Robinson

Comments from the CGC Gene Curators

CGC has appointed Curators for the four major cultivated groups: cucumber, muskmelon, watermelon and *Cucurbita* spp. A curator for the "Other Genera" category is needed; anyone wishing to take on this responsibility should contact the Chairman.

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

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

14th Annual CGC Business Meeting
6 November 1991, Tucson Arizona USA

The 14th Annual Business Meeting of the Cucurbit Genetics Cooperative was held on Tuesday, 6 November 1990, in Tucson, Arizona. The meeting was held in conjunction with the 87th Annual Meeting of the American Society for Horticultural Science (ASHS). Approximately 25 CGC members were in attendance.

Minutes from the 13th Annual Business Meeting (Tulsa, OK) and the CGC meeting at Cucurbitaceae '89 (Charleston, SC) were approved. Mention was made of two upcoming international cucurbit meetings, one in Israel in 1991 and the other in Poland in 1992. [Editor's note: the Israel meeting was subsequently canceled due to events in the Middle East.]

CGC membership figures and summary statistics for the first 13 CGC Reports were presented. CGC Report No. 13 (1990) was mailed on 26 June 1991 to (at that time) 240 members and subscribers. The Call for Papers for CGC Report No. 14 was scheduled to be mailed 31 August 1990, with submissions due by 31 December 1990.

A lively discussion ensued over whether the term "melon" should be used rather than "muskmelon" for cultigens in the species *Cucumis melo*. The rationale presented was that many *C. melo* cultigens (e.g. honeydew and casaba melons) lack the "musky" smell typical of some of the netted, orange-fleshed types, and that confusion arises when they are described as "muskmelons." At least one watermelon researcher expressed concern that using "melon" might cause some degree of confusion about whether watermelons were being discussed. However, most CGC members agreed that they rarely heard watermelons referred to (scientifically) as "melons" when they were the sole commodity being mentioned. After further discussion and a vote, it was decided that the term "melon" rather than "muskmelon" would be used by CGC in the future to describe cultigens of *C. melo*. Efforts would be made in the next year to get other scientific journals (e.g. the ASHS publications) and U.S. regulatory agencies to adopt the same practice.

A mention was made that few libraries subscribe to the CGC Report. Members were encouraged to request that their libraries subscribe and make the CGC Report available to students and researchers.

Because of discussions at the CGC meetings in 1989, the need for back-up gene curators was again brought up. There have been more volunteers, and currently there are two or more curators for each cucurbit category except for the "Other Genera" section.

According to the CGC By-Laws, CGC Coordinating Committee members serve 10-year terms, whereupon an election is held to select their replacement. Mark Hutton was nominated and elected to replace Dick Robinson as the Coordinating Committee member for the "Other Genera" section. Gratitude was expressed to Dick Robinson for his guidance to CGC over the years; Robinson was part of the organizing committee when CGC was initiated in 1977 and had served on the Coordinating Committee ever since. He was also instrumental in formulating the CGC By-Laws. He will be continuing on the CGC Gene List Committee and as a CGC Gene Curator.

A question of whether CGC should publish a listing of new cucurbit cultivars each year in the CGC Report was discussed; this was subsequently turned down because of the logistics of maintaining an accurate list.

Upcoming meetings of interest to CGC members:

Group	Date & Location	Contact Person
Cucurbit Genetics Cooperative	23 July 1991 11:15 AM - 12:15 PM Rm. 265 Willard Building Penn State University University Park, PA	Timothy J Ng 1122D Holzapfel Hall College Park, MD 20742 USA
Cucurbit Crop Advisory Committee	8 September 1991* 1:00 - 5:00 PM Rocky Ford, Colorado	J.D. McCreight USDA-ARS, 1636 E. Alisal St. Salinas, CA 93915 USA
National Melon Research Group	9 September 1991* 9:30 AM - 12:30 PM Rocky Ford, Colorado	Perry E. Nugent USDA, U.S. Vegetable Lab 2875 Savannah Highway Charleston, SC 29414 USA
Squash Breeders Group	9 September 1991* 1:30 - 5:00 PM Rocky Ford, Colorado	Henry Munger Cornell University 410 Bradford Hall Ithaca, NY 14853 USA
Pickling Cucumber Improvement Committee	16-18 October 1991 Louisville, Kentucky	Todd C. Wehner Dept. Horticultural Sci. North Carolina State Univ. Raleigh, NC 27695-7609 USA
Watermelon Research Group	February 1992 (time to be announced) Lexington, Kentucky	Gary W. Elmstrom Univ. Florida, IFAS, CFREC 5336 University Avenue Leesburg, FL 34748 USA
5th Eucarpia Cucurbitaceae Symposium	27-31 July 1992 Research Inst. Veg. Crops 22 Lipca 1/3 96-100 Skierniewice Warsaw, POLAND	Roch W. Doruchowski, or Katarzyna Niemirowicz-Szczytt tel. 41-93 telex: 886649 iwarz pl telefax: 31-86

* Note: The three September cucurbit meetings in Rocky Ford Colorado are being coordinated by Larry Hollar (Hollar Seeds, P.O. Box 106, Rocky Ford, Colorado 81067. Ph.: (719) 254-7411. FAX: (719) 254-3539.). The meetings will span the period of 7-10 September and will include visits to melon production areas, packing plants, and cultigen field trial locations.

A Brief History of the Development of Cucumber Cultivars in the U. S.

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Richard W. Robinson
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Cucumber (*Cucumis sativus* L.) has been grown and bred for centuries. It is mentioned in the Bible and in ancient herbals. Chakravarty (6) reported that cucumber has been cultivated in Iraq for more than 5,000 years, both as a vegetable and for medicinal purposes. The origin and domestication of the cucumber was probably not in the Middle East, however, nor in Africa as some have suggested, but rather in Asia (7). The ancestor of the cucumber was probably an Asian plant with 7 pairs of chromosomes and small, very bitter fruit, similar to that of *Cucumis sativus* var. *hardwickii* (R.) Alef., which is indigenous to the foothills of the Himalayan Mountains.

Cucumber may be the oldest crop grown in a controlled environment. Pliny informs us that cucumber was so relished by the ancient Romans that they grew it in coldframes so that that the emperor Tiberius could have them served on his table every day of the year (4). It has been grown since the early 1300s in England, where they were grown in the earliest greenhouses and known as "cowcumbers" (5).

Cucumber was introduced to the New World by Columbus who planted it in Haiti in 1494, and it was probably brought to the U. S. soon afterward (19). DeSoto reported finding a cucumber in Florida, which he considered superior to any he had seen in Spain. In 1779, General Sullivan destroyed cucumber fields grown by Indians (Native Americans) near Geneva, N. Y.

Some cultivars still available today from Amerian seedsmen were introduced to this country from abroad more than a century ago. 'Early Russian', for example, was described by Naudin in France in 1859 (15). 'Early Cluster' was introduced prior to 1800. Boswell in 1949 concluded that all of the distinct types of cucumber grown then were known at least 400 years before.

Perhaps the first important American-bred cucumber cultivar of the last century was 'Tailby's Hybrid', developed by Joseph Tailby of Massachusetts from a cross between American and English cultivars and introduced in 1872. The success of 'Tailby's Hybrid' encouraged different seedsmen to develop new cultivars, and in the following decade such important cultivars as 'Arlington White Spine', 'Boston Pickling', and 'Chicago Pickling' were introduced.

In 1937, Whitaker and Jagger (22) reported that all but one of the cucumber cultivars extensively grown then in the U. S. were developed by private seed companies. The one exception was 'National Pickling' (also called National or National Pickle). The National Pickle Packers Association commissioned George Starr of Michigan State University to breed a new cultivar with fruit type suitable for both small pickles and large dills, and he did so by breeding and introducing the cultivar 'National Pickling' in 1924. This was an important cultivar for many years, and its genes still live on. Munger (14) used it to breed 'Yorkstate Pickling' and other germplasm that is the source of CMV resistance for Ohio MR 17, Wisconsin SMR 18, MSU 713-5, and numerous other cultivars and breeding lines.

After the introduction of 'National Pickling', cucumber breeders continued to emphasize improvement of fruit shape and color (Table 1). Notable achievements were made by the Asgrow Seed Co. in the introduction of 'Marketer' in 1943, a slicer with good fruit type from the cross 'Longfellow' x 'Straight 8', and 'Model' in 1946, a pickling cultivar with white spines and dark

green color. Both 'Marketer' and 'Model' were popular for many years because of their good fruit type, but eventually were superseded by disease resistant cultivars.

Breeding cucumbers for disease resistance began in the late 1920s, when R. H. Porter brought cucumber mosaic virus resistant germplasm from China to this country (18). He bred the cultivar Shamrock in 1943 from the cross 'Chinese Long' x 'Davis Slicer' (1). H. M. Munger bred slicer cultivars with a higher level of CMV resistance and with good fruit type, 'Tablegreen' in 1961 and 'Marketmore' in 1968, which became important both as open pollinated cultivars and as parents of F_1 hybrid cultivars. 'Maine No. 2', released in 1939 by Bailey (2), was the first American cultivar resistant to scab. Its long, slender shape and late maturity limited its popularity, but it has been important as a parent of many other scab-resistant cultivars.

The next step in the improvement of cucumber cultivars was breeding for combined resistance to two or more diseases. J. C. Walker (21) combined resistance to scab and CMV in the cultivars Wisconsin SMR 9 and SMR 12, released in 1955, followed in 1958 by SMR 15 and SMR 18. Barnes (3) combined resistance to downy mildew (*Pseudoperonospora cubensis* (Berk. & Curt.) Rostow) and powdery mildew (*Sphaerotheca fuliginea* (Schl. ex Fr.) Poll.) in 'Palmetto' (1948) and 'Ashley' (1955), then added resistance to anthracnose (*Colletotrichum lagenarium* (Ross) Ellis & Halst.) for the 1961 release, 'Polaris', which is still popular today. Genes for resistance to additional diseases were identified and combined, culminating in the development of 'Sumter', with resistance to seven diseases, and Wisconsin 2757 (17), resistant to nine diseases--CMV, scab, anthracnose, downy mildew, powdery mildew, bacterial wilt (*Erwinia tracheiphila* (E.F. Smith) Holland), angular leaf spot (*Pseudomonas lachrymans* (E.F. Smith and Bryan) Carsner, target leaf spot (*Corynespora cassiicola* (Berk, & Curt.) Wei), and fusarium wilt (*Fusarium oxysporum* (Schlecht.) Snyd. & Hans f. sp. *cucumerinum* Owen. Actually, most cultivars with resistance to scab have resistance to fusarium wilt, since there appears to be linkage. However, the cultivars are usually not tested to verify fusarium wilt resistance, since that disease has not been important commercially.

Meanwhile, progress was made in the improvement of other traits, such as sex expression and plant habit. A. E. Hutchins pioneered the development of dwarf-determinate cultivars and introduced 'Midget' in 1940 and 'Minnesota Dwarf XII' in 1949 (10). Dwarf-determinate plant habit was not used extensively, however, until later with the introduction of 'Castlepik' (20). 'Geneva', the first American cultivar bred for parthenocarpic fruit set, was introduced by Hawthorne and Wellington in 1930 (9). It did not become important and is no longer available, but there has been increased interest in recent years in breeding parthenocarpic cucumbers. Research has been done on compact (super-dwarf) types for high density populations (11), but better dwarf genes and cultivars are needed. Multibranching types, such as little leaf lines (8) or derivatives of *C. sativus* var. *hardwickii* (12) may enhance simultaneous fruiting and improve the yield in once-over harvest systems.

Monoecious hybrid cultivars have been available since 1945, when Oved Shifriss developed 'Burpee Hybrid', but the high cost of hybrid seed limited their commercial use. Development of gynoecious cultivars reduced the cost of producing hybrid seed and improved earliness and adaptation to mechanical harvesting. Germplasm with the gynoecious gene was brought from Korea to this country by E. Meader and distributed by the U. S. D. A. Plant Introduction system as PI 220860. Peterson (16) backcrossed the gynoecious gene into 'Wisconsin SMR 18' to develop MSU 713-5, female parent of the first gynoecious hybrid cultivar, 'Spartan Dawn'.

The nonbitter gene, which provides resistance to cucumber beetles and prevents fruit bitterness, has been transferred from Dutch germplasm to American cultivars and breeding lines, including 'Marketmore 80' and Wisconsin 2757. Improvement in cucumber cultivars over the previous 7 decades in the U. S. is summarized in Table 1. Still needed are cultivars with resistance to diseases such as zucchini yellow mosaic virus, papaya ringspot virus, gummy stem

blight, and Rhizoctonia fruit rot. Arthropod (insect and arachnid) resistant cultivars are also needed, particularly for pickleworm, aphid, whitefly and spider mite. Further improvements are still needed in fruit quality, yield, earliness, and adaptation to the production environments of the U. S.

Table 1. Important steps in the genetic improvement of cucumbers in the U. S.

Cultigen	Source	Year	Trait(s) of interest[z]
Improvement of disease resistance			
Shamrock	Iowa Agr. Exp. Sta.	1937	CMV
Maine No. 2	Maine Agr. Exp. Sta.	1939	Scab
Palmetto	S. C. Agr. Exp. Sta	1948	DM PM
Wis. SMR 12	Univ. Wisconsin	1954	Scab CMV
Tablegreen	Cornell Univ.	1961	CMV PM
Polaris	S. C Agr. Exp. Sta	1961	DM PM Anth
Poinsett	S. C. Agr. Exp. Sta.	1966	DM PM Anth ALS
Marketmore	Cornell Univ.	1968	High CMV
Sumter	S. C. Agr. Exp. Sta.	1973	DM PM Anth ALS CMV Scab WMV
Wis. 2757	Univ. Wisconsin	1982	DM PM Anth ALS CMV Scab TLS BW FW
Improvement of other traits			
Nat. Pickling	Mich. Agr. Exp. Sta.	1924	Fruit type
Geneva	N. Y. Agr. Exp. Sta.	1930	Parthenocarpic
Midget	Minn. Agr. Exp. Sta.	1940	Dwarf-determinate
Marketer	Asgrow Seed Co.	1942	Fruit type
Burpee Hybrid	Burpee Seed Co.	1945	Mon-Hyb CMV DM
Model	Asgrow Seed Co.	1946	Fruit type
MSU 713-5	Mich. Agr. Exp. Sta.	1960	Gyn inbred
Spartan Dawn	Mich. Agr. Exp. Sta	1962	Gyn-Hyb CMV Scab
Ark. 79-75	Univ. Arkansas	1980	Little leaf habit
Castlepik	SunSeeds (ARCO-Castle)	1984	Dwarf-determinate CMV Scab DM PM

[z]ALS - angular leafspot resistance; Anth - anthracnose resistance; BW - bacterial wilt resistance; CMV - cucumber mosaic virus resistance; DM - downy mildew resistance; FW - fusarium wilt resistance; Gyn - gynoecious sex expression; Hyb - hybrid; Mon - monoecious sex expression; PM - powdery mildew resistance; Scab - scab resistance; TLS - target leafspot resistance; WMV - watermelon mosaic virus-2 resistance.
Table adapted from Miller and Wehner (13).

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Cucumber Cultivar Improvement in the People's Republic of China

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Cucumber (*Cucumis sativus* L.) is as important as tomato in vegetable production in China. Cucumber is grown widely throughout the country, especially in North China, where it is the number one summer vegetable. Cucumber is second in area after Chinese cabbage. China is the biggest cucumber producing country in the world, with a total production area of 230,000 ha and a total yield of approximately 300,000 Mg. The main provinces/cities for cucumber production are Shandong, Beijing, Tianjing, Liaoning, and Shaanxi. Shandong ranks first, with a total area of approximately 25,000 ha. Yields per hectare vary greatly (7 to 100 Mg) because of the differences of cultivars, growing season, growing system and cultivation techniques. The highest record is 328,112.5 kg/ha. Because of the favorable climate condition, most of the country's cucumber (80 to 90%) are grown during spring and early summer. A small part of cucumbers are produced during fall and winter in the greenhouse. The objective of this article was to review the work on genetic improvement of cucumber in China.

Cucumber improvement in China. China has a cucumber growing history of 2,000 years (4). Great genetic diversity has developed in the different parts of the country due to both natural and artificial selection. All of the cultigens (cultivars, gene mutant lines, breeding lines) can be classified into two groups, the North China group (so called 'Chinese Long') and the South China group. Each of the groups can be divided into four horticulture types: watering cucumber, staking cucumber, drought tolerant cucumber (usually rain fed) and unstaking cucumber. The former two types are commonly used, and the latter two types are scattered in small quantities. Most of the cucumbers in China are for fresh market, but cucumbers for pickling are also grown. We have no official estimate of the number of cucumber accessions maintained in China, but at least 1,000 are known. Investigations carried out by Shandong Academy of Agricultural Science indicated that there were more than 100 local races of cucumber in the country. However, much excellent germplasm has been unavailable because of lack of proper conservation.

Organized and scientific cultivar improvement was carried out only in the last 40 years, and cucumber breeding has been further improved during the last 20 years. Three important periods are summarized below according to the major work carried out.

Germplasm resource investigation (1949-1963). The vegetable research staff was organized and equipped in the early 1950s in the scientific units of many provinces and big cities of China. A national vegetable germplasm resource (including cucumber) investigation and collection was carried out in 1955. Some outstanding local cucumber cultivars, e.g. Ningyan Cigua, were found and collected during the national investigation. The collected local cultivars played an important role since they were utilized widely in commercial production. An extensive germplasm investigation of ten important vegetable crops, including cucumber, Chinese cabbage, etc., was organized in 1959. More germplasm resources were collected, and some efforts toward asexual gene transfer and F_1 hybrid breeding were made during these periods, but there is no successful work reported.

Breeding new cultivars (1964-1979). The program on sexual cross breeding initiated in Tianjing Academy of Agricultural Science in 1964 marked a new epoch of scientific cultivar improvement of cucumber in China. However, only a few researchers and breeders were focused on cucumber breeding in 1960s. In early 1970s research groups undertaking cucumber improvement were established in almost 20 universities and academies of agricultural science, including Northwestern Agricultural University, Shandong Academy of Agricultural Science, and Beijing Academy of Agricultural Science. Research in these periods was concentrated on breeding new

elite cultivars using genetic recombination. Gynoecious line and inbred line development were also included in breeding program (1). F_1 hybrid breeding was in consideration or in experiment. Attention was also paid to resistant and high yield cultivar selection because of the increasing severity of diseases.

F_1 hybrids with multiple disease resistance (1979-present). A national cucumber breeding group consisting of seven units (e. g. Chinese Academy of Agricultural Science, Northwestern Agricultural University) was organized in 1979. Cooperative programs and breeding objects were made at the annual meeting of the group. This group has been developed in the last eleven years. Up to now, the group covers 22 research institutes and universities or colleges. A National Cucumber Research Association was established in December, 1990 on the basis of the national cucumber breeding group. The association will be helpful for researchers and breeders exchanging their ideas and information, as well as germplasm. Breeding during this period was mainly on development of F_1 hybrids with multiple resistance to downy mildew (*Pseudoperonospora cubensis* Rostow), Phytophthora rot (*Phytophthora melonis* Katsura), anthracnose (*Colletotrichum orbiculare* (Pass.) Ell. & Halst.), and powdery mildew (*Sphaerotheca fuliginea* Pollacci) (6, 7). A number of desirable inbred lines, gynoecious lines, early F_1 hybrids and multiple resistant F_1 hybrids were developed. F_1 hybrids became popular, and presently occupy 40% of the production area.

Achievements. Many favorable local cucumber cultivars (Cunjingzi, Erzhaozi, TanshanQiougua, Beijing Daci, HanzhouQinpi, JinZaosheng, Dabacha, Xiaobacha, Wenshang, Yeershan, Heihantui, HanzhongQiougua, NinyangCigua and Yangxian) were found and collected during 1950s and early 1960s in different cucumber production areas with long growing histories. These local cultivars were used directly in commercial production, and also as breeding materials. There have been approximately 40 inbred and F_1 hybrid cultivars developed since the late 1960s. The most popular inbred cultivars are Ningqin, JingYan No. 2, JingYan No. 4, JingYan No. 6, JingYan No. 7, Xingong No. 58, Xiafeng No. 1, Shanghai Cucumber, and Yiyu No. 2. The F_1 hybrids grown extensively are Jingza No. 2, Zaofeng, Nongcheng No. 3, Zhongnong No. 5, 828, Xiaqing No. 1, Ningfeng No. 3, Xianhuan 881, Zhenhuan No. 2, Luhuan No. 1, and Bichong.

Screening systems of cucumber resistance to Fusarium wilt, downy mildew, Phytophthora rot, anthracnose, powdery mildew have been established in several research institutes and universities or colleges. Some screening programs were improved on the basis of those developed in foreign countries. Differentiation of downy mildew and Fusarium wilt fungus were extensively studied. The results obtained suggest that there is no race differentiation among downy mildew fungus isolates collected in different parts of China. All isolates of Fusarium wilt fungus collected in China show the same race reaction. The race of Fusarium wilt fungus in China is different from any of the races (1, 2 and 3) identified by Armstrong (1978). Race 4 was suggested for the Fusarium wilt fungus attacking cucumbers in China. Genetic study on cucumber is limited in China. Studies on quantitative inheritance and heterosis in cucumber carried out at Northwestern Agricultural University in recent years will make cultivar development more efficient (2, 5).

Future breeding objectives. The general objectives issued by the National Cucumber Research Association are to develop improved inbred and hybrid cultivars with resistance to multiple diseases for growing in different seasons and cultivation systems. There are as many as 15 diseases attacking cucumber in China. However, it is suggested that breeding work be focused on the most destructive diseases: downy mildew, Fusarium wilt, Phytophthora rot, anthracnose, powdery mildew and angular leaf spot (*Pseudomonas lachrymans*). The former three diseases led to the most serious problems for cucumber production in China. In addition, a more recently introduced disease from overseas, scab (*Cladosporium cucumerinum* Ellis & Arth.) is increasing in severity in Northeast China. It is expected this disease will be one of the most serious diseases unless great attention is paid right now. Early maturing inbred and hybrid

cultivars will be preferred by cucumber growers because of their higher value in the spring production season. Much emphasis is placed on early maturity. Cultivars have been developed with high resistance to downy and powdery mildew, and with tolerance to heat for fall production, but high-yielding cultivars with resistance to multiple diseases need to be developed.

The characters that Chinese breeders are interested in and in evaluating are resistance to downy mildew, Fusarium wilt, Phytophthora rot, anthracnose, powdery mildew and angular leaf spot, earliness and complex traits making cucumber favorable to fall production. Characters preferred by Chinese consumers include length/diameter > 8, dark-green skin, and thick, prominent spines.

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A Cucumber (*Cucumis sativus* L.) Mutant with Yellow Stem and Leaf Petioles

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A cucumber (*Cucumis sativus* L.) mutant with yellow stem and leaf petioles was obtained from a highly inbred line of 'Borszczagowski' after treatment of the seeds with the mutagen, ethyleneimine (1). It was one of frequent chlorophyll deficient mutations, restricted to cotyledons, stems, leaf petioles and veins.

Inheritance was studied after the mutant plants were self-pollinated several generations to make a stable, mutant inbred line (P_2). The mutant was crossed with an inbred line of 'Borszczagowski' (P_1) to obtain F_1, $F_1 \times P_1$, $F_1 \times P_2$ and F_2. All the observed traits of the mutant were compared with P_1.

Statistical measurements of 20 mutant plants were analyzed using Student's t-test. Inheritance studies were run in a greenhouse in the optimum spring to summer seasons, and data analyzed using chi-square.

It was possible to identify mutant plants initially in the cotyledon stage by the cotyledon color, which was yellow at the beginning and gradually becoming cream-like. In the course of growth, further characteristics appeared, such as cream colored stems, leaf petioles and leaf veins. The mutant hypocotyl was half the length of the standard (38 ± 6 to 92 ± 14 mm). Mutant first leaves were slightly narrower (30 ± 5 to 55 ± 10 mm) and more lobed. Mutant plants were considerably shorter (1567 ± 70 to 2660 ± 62 mm) with the same number of internodes (39) as normal. However, internodes were shorter in the mutant (39 ± 8 to 61 ± 7 mm), and it had fewer lateral shoots (14 ± 3 to 24 ± 3). Like the standard, the mutant was monoecious, with similar flowers and fruits. Pollen viability stain using 2% acetocarmine was nearly the same as the initial line (98 to 99%).

On the basis of the genetic analysis (Table 1), we concluded that the character of yellow stem and leaf petioles is determined a single recessive gene, designated *ys*. The gene also appears to cause the plants to be significantly shorter that normal.

Table 1. Inheritance of yellow stem and leaf petioles (*ys*).

	No. observed		No. expected		Tested		
Generation	Normal	Mutated	Normal	Mutated	ratio	X^2	P
Borszczagowski (P_1)	65	0	65	0	1:0	-	-
ys (P_2)	65	20	0	20	0:1	-	-
F_1	80	0	80	0	1:0	-	-
F_2	138	50	132	40	3:1	1.08	0.50
$F_1 \times P_1$	80	0	80	0	1:0	-	-
$F_1 \times P_2$	67	65	66	66	1:1	1.24	0.50

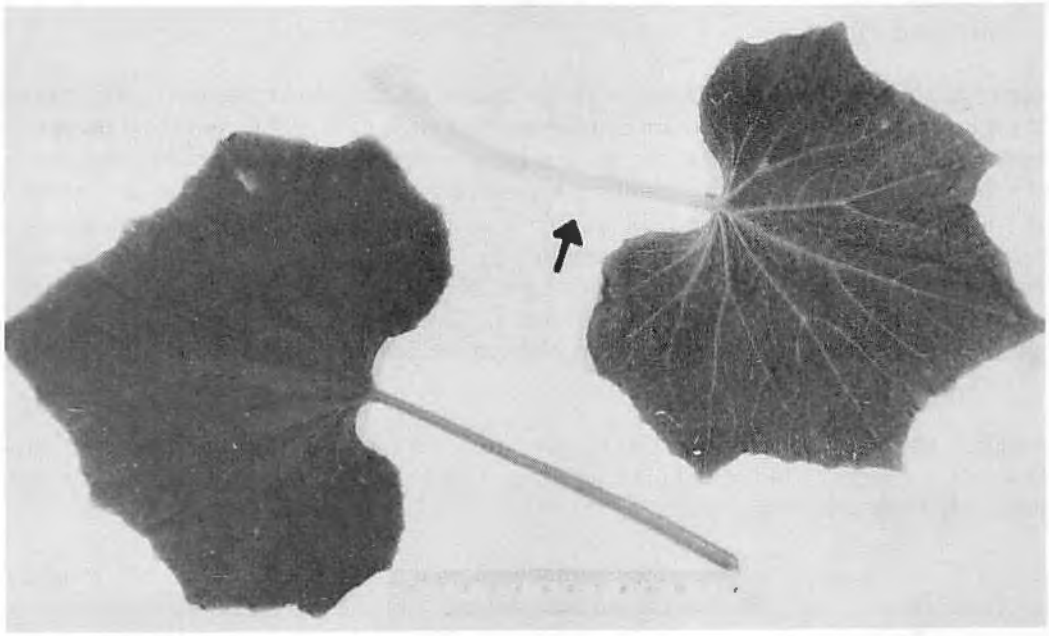

Figure 1. Comparison of mutant leaf (note cream-like petiole) and normal one (B-line).

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Some Relationships Among Fruit and Seed Properties in Seed-bearing Cucumbers of Different Types

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Seed yield in the field for new breeding lines is generally estimated by collecting random fruit samples from unit areas and measuring seed weight after processing. Fruit size and shape, and the proportion of normal versus cull (crooks and nubs) fruits can also give some indications about seed yield. We determined some relationships among fruit and seed characteristics of 5 pickling, 2 parthenocarpic pickling, 1 Beit Alpha and 2 field slicer types of cucumbers in 3 years of seed yield trials using multi-correlation analysis.

Though the years were different in fruit and seed yield, the correlations among fruit and seed properties showed good congruency, except for one parthenocarpic pickling line. For this reason data were summed over years.

The majority of the fruits were uniform in shape due to use regular irrigation. As a result, we obtained high correlations among fruit characteristics such as weight, length, diameter, and seed cell diameter (data not shown). Also, high correlations were measured between the seed weight and seed number per fruit. High or medium correlations occurred between seed wight per fruit and fruit weight or seed weight per fruit and fruit length in short-fruited lines, while in slicing types these relationship were loose or uncorrelated. This tendency was more pronounced in the comparison of seed number per fruit and fruit size. No plump seeds were found beyond 20 cm from the basal end of the longest (26 to 28 cm) fruits. This supports the theory of competition between pollen tube growth and initial fruit elongation during fertilization in long-fruited slicers (1).

Seed extraction ratio (the quotient of seed weight and fruit weight) was not a good predictor of seed yield itself, but its average is typical for each line. Good seed set improves this ratio, especially in lines with short fruit and thin skin.

On the basis of our results, no clear conclusion could be drawn for thousand-seed weight and fruit weight or fruit dimensions. Generally, no correlation was measured for maximum thousand-seed weight and highest seed numbers in a fruit. On the other hand some differences occurred among years in the size of the correlations.

The average line had relatively high coefficients of variation (40 to 50%) for seed weight and seed number per fruit, seed extraction ratio and average fruit weight, while low coefficients of variation (<20%) occurred for fruit length, fruit diameter, seed cell diameter and thousand-seed weight.

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Table 1. Correlation coefficients among fruit and seed characters of 10 cucumber lines over 3 years in seed production field trials.

Line	Mean fruit length (cm)	Seed wt./fruit vs. Fruit wt.	Seed wt./fruit vs. Fruit len.	Seed wt./fruit vs. Seed no.	Seed no./fruit vs. Fruit wt.	Seed no./fruit vs. Fruit len.	Seed extraction ratio vs. Seed wt.	Seed extraction ratio vs. Seed no.	Seed extraction ratio vs. Fruit wt.	Seed extraction ratio vs. Fruit len.	1000-seed weight vs. Fruit wt.	1000-seed weight vs. Fruit len.	1000-seed weight vs. Seed wt.	1000-seed weight vs. Seed no.
Monoecious pickling														
K. livme	15.0	0.93	0.83	0.95	0.83	0.78	0.34	0.45	0.03	-0.01	0.80	0.70	0.73	0.51
K 4599	14.6	0.90	0.78	0.92	0.73	0.63	0.54	0.68	0.15	0.05	0.61	0.59	0.48	0.14
Gynoecious pickling														
F 1040	13.2	0.70	0.70	0.94	0.54	0.59	0.70	0.77	0.02	0.15	0.44	0.36	0.19	-0.10
LV 19	14.5	0.84	0.73	0.99	0.71	0.62	0.66	0.72	0.22	0.15	0.38	0.31	0.13	-0.16
64 H	14.3	0.84	0.73	0.93	0.70	0.62	0.56	0.63	0.18	0.05	0.62	0.56	0.38	0.09
Parthenocarpic pickling														
76 C	11.8	0.66	0.55	0.98	0.62	0.51	0.73	0.72	0.10	0.04	0.08	-0.03	0.08	-0.09
KP 79	12.0	0.65	0.60	0.93	0.49	0.45	0.58	0.66	-0.11	-0.10	0.38	0.38	0.14	-0.15
Beit Alpha														
FWIW	15.3	0.57	0.46	0.95	0.38	0.30	0.65	0.73	-0.15	-0.15	0.44	0.42	0.29	0.02
Semi-long slicing														
VA 1717	22.1	0.58	0.48	0.96	0.43	0.34	0.66	0.76	-0.16	-0.15	0.49	0.52	0.12	-0.12
JSW	22.6	0.46	0.38	0.94	0.24	0.19	0.57	0.73	-0.40	-0.41	0.48	0.44	0.13	-0.16

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Evaluation of Seed Yield of Pickling and Slicing Cucumbers in the Field Affected by Year and Plant Density

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Experimental lines of cucumber are generally propagated in glasshouses, plastic houses or in net-covered cages. These circumstances rarely provide appropriate estimates of seed yield under field conditions.

During seed production of pickling and slicing cucumbers in the field, an average of 100 to 200 seeds per fruit is usually obtained. However, practical seed producers frequently conclude that environmental factors influence seed yield most, while genotype receives little attention.

Methods. Two small studies were designed to measure seed yielding capacity of F_5 to F_7 cucumber lines of different origin. Lines were chosen to represent different vine length, fruit type, sex type, and levels of parthenocarpy. Experiment 1 had normal (7.8 plant/m^2) density, and was run for four years (1987 and 1990) with 2 replications. Experiment 2 had normal density initially, but was thinned to 3.8 plant/m^2. It was run for two years (1988 and 1989) with 2 replications.

The lines tested were (average fruit length in parentheses): K. livmé (150 mm) and K. 4599 (146 mm) monoecious pickling type; F 1040 (132 mm), LV 19 (145 mm) and 64 H (143 mm) gynoecious pickling type; 6C (118 mm) and KP 79 (120 mm) parthenocarpic pickling type; FWIW (153 mm) gynoecious Beit Alpha type; JSW (226 mm) and VA 1717 (221 mm) semi-long, slightly parthenocarpic, gynoecious field slicers. Plots were sown between 15 and 20 May in succeeding years. The 5.8 m^2 randomized plots were irrigated by sprinklers. Normal cultural practices were observed. At seed maturity, a random sample of 15 fruits per plot was collected, and seeds were processed by hand.

Results. According to the analysis of variance, the highest variation can be attributed to year for fruit yield, seed yield and thousand seed weight (Tables 1 and 2). The fruit characteristics such as average fruit weight, fruit length, fruit diameter, fruit length/diameter ratio, fruit diameter, and seed cell diameter were measured for the genotypes at each extreme.

Number of seeds per fruit was influenced by genotype and year. Low plant density (3.8 plant/m^2) reduced the fruit and seed yield per m^2 at the majority of the lines. On the other hand, a slight increase in fruit size and thousand seed weight was observed. Larger influence of plant density was displayed only in fruit number (data not shown). Considering plant density, the normal (7.8 plant/m^2) spacing gave higher seeds yield.

The variation in seed number per fruit (Table 3.) was similar to the results of Wehner and Horton (2). In our trial we did not find more seeds in slicers than in short fruited types. On the other hand, small fruit size did limit seed yield in the case of parthenocarpic pickling types.

On the basis of our data, year was the most significant factor influencing fruit and seed yield, similar to the findings of Wehner and Swallow (1). The highest variation was found in both trials in the average of the lines over years in seed yield, followed by fruit yield, seed number per fruit, fruit number and thousand seed weight. It appears that vine growth and fruit bearing capacity of the genotypes are more important than seed number per fruit.

Table 3. The influence of years on fruit and seed yield of 10 cucumber lines.

Line	Year	Fruit wt. kg/m^2	Fruit no./m^2	Seeds per fruit	Seed yield g/m^2	Thousand seed wt.(g)
K. livmé	1987	2.80	12.1	171.1	41.3	19.86
	1988	6.60	21.1	201.8	89.7	21.19
	1989	4.13	16.6	157.0	42.9	16.75
	1990	5.22	18.7	185.6	76.0	21.63
K.4599	1987	4.05	15.8	224.7	63.6	17.98
	1988	6.41	24.8	181.5	85.8	18.99
	1989	3.98	15.9	174.8	46.7	16.62
	1990	6.22	19.5	228.3	93.2	20.88
F 1040	1987	2.40	10.9	123.9	29.7	22.10
	1988	5.19	19.2	171.1	75.7	23.40
	1989	3.00	15.0	112.1	31.4	18.68
	1990	4.74	16.6	173.1	69.3	24.23
64 H	1987	5.08	18.9	159.3	68.6	22.68
	1988	4.94	19.5	140.4	64.1	24.07
	1989	6.46	20.4	154.0	73.1	23.24
	1990	5.41	19.2	135.5	59.8	23.11
LV 19	1987	4.37	20.8	122.3	53.3	21.05
	1988	5.84	17.3	225.0	84.2	21.23
	1989	4.36	15.9	152.8	50.2	20.91
	1990	6.62	22.8	148.2	80.3	23.25
76 C	1987	2.30	22.1	81.7	38.4	21.06
	1988	1.08	9.9	117.6	22.7	22.69
	1989	1.74	16.7	79.0	19.6	19.56
	1990	2.84	23.3	75.0	22.8	22.78
KP 79	1987	2.95	23.6	83.5	39.1	19.95
	1988	3.13	21.3	131.7	66.9	24.05
	1989	1.55	15.0	105.0	27.0	17.68
	1990	2.46	20.8	74.2	32.5	21.12
FWIW	1987	3.41	14.8	150.1	45.4	20.70
	1988	6.41	19.9	157.6	73.7	23.55
	1989	3.06	13.6	78.8	19.7	18.34
	1990	5.27	14.5	130.1	50.4	25.62
JSW	1987	5.07	15.4	171.0	64.5	24.41
	1988	6.66	15.2	150.8	58.2	25.41
	1989	3.07	11.4	127.5	35.2	23.96
	1990	7.18	15.7	169.6	76.9	29.35
VA 1717	1987	5.35	14.5	104.1	36.8	24.33
	1988	10.40	20.5	177.6	82.7	22.75
	1989	5.35	14.0	182.2	55.4	21.88
	1990	7.46	17.1	135.7	65.1	28.37
LSD (5%)		2.10	6.1	52.8	29.4	2.86

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Table 1. Analysis of variance for fruit and seed yields for four years.

Source of variation	df	Fruit yield	Seed yield	Seed number per fruit	df	Thousand seed wt.
Total	79	4.23	522.8	2027.7	59	10.08
Replication	1	2.24	280.5	2.5	1	6.90
Treatment	39	7.44***	839.0***	3424.9***	29	18.31***
Lines	9	18.63***	1304.5***	9288.4***	9	27.63**
Years	3	21.26***	3786.8***	4095.8*	2	95.95***
L x Y	27	2.18*	356.3	1392.5*	18	5.02*
Error	39	1.09	212.7	682.0	29	1.97

*, **, *** = significant at 5, 1 and 0.1 % levels, respectively.

Table 2. Analysis of variance for fruit and seed yields for two years and at two plant densities.

Source of variation	df	Fruit yield	Seed yield	Seed number per fruit	Thousand seed wt.
Total	79	5.12	645.3	1.18	11.11
Replication	1	1.64	282.3	0.91	4.18
Treatment	39	8.74***	1118.5***	2.10***	20.22***
Lines (L)	9	21.58***	1550.4***	2.95***	37.81***
Years (Y)	1	70.70***	17207.6***	35.02***	278.59***
Density (D)	1	14.15**	2141.5**	1.30*	9.67*
L x Y	9	4.47*	828.9***	1.39***	12.58***
L x D	9	0.72	133.9	0.33	1.40
L x D	1	0.10	1.9	1.01	11.54
L x Y x D	9	1.65	150.1	0.28	2.51
Error	39	1.60	199.6	0.28	2.19

*, **, *** = significant at 5, 1 and 0.1 % levels, respectively.

Crop Loss to 14 Diseases in Cucumber in North Carolina for 1983 to 1988

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The North Carolina State University Department of Plant Pathology Disease loss committee issues annual state-wide reports (1, 2, 3, 4, 5, 6) on crop loss estimates due to plant diseases and nematodes. Data on cucumber was available for the years 1983 to 1988, but so far, has not been examined for trends or ranking of pathogens. The objective of this report was to review the data for trends and ranking of cucumber pathogens according to their severity in North Carolina.

Data from the reports reviewed here were *estimates*. Data were collected each year from collaborators with firsthand knowledge of each crop and its diseases. The data were also assigned a confidence rating indicating how the data was obtained and its reliability, but were not based on surveys or tests, only educated guesses.

The estimated incidence of most pathogens has remained constant for the years 1983 to 1988 (Table 1). The estimated incidence of downy mildew however, has increased by five fold for the last three years compared to the first three years. The estimated incidence of target spot and belly rot have both increased, but only slightly. There are strong decreases in the estimated incidence of both angular leaf spot and scab. Gummy stem blight was estimated to have the highest incidence of all pathogens for all six years followed by belly rot and anthracnose. Tomato ringspot virus had the lowest estimated incidence. It is important to note that most cultivars being grown in North Carolina have moderate to high resistance to anthracnose, angular leafspot, downy and powdery mildew, scab, and cucumber mosaic virus.

Table 1. Estimated incidence of 14 diseases on all field grown cucumbers (slicing and pickling cucumbers) in North Carolina from 1983 to 1988 (ranked by mean).

Rank	Disease	1983	1984	1985	1986	1987	1988	Mean
1	Gummy stem blight	80.0	80.0	80.0	80.0	80.0	80.0	80.0
2	Belly rot	70.0	70.0	70.0	70.0	75.0	75.0	71.7
3	Anthracnose	50.0	75.0	75.0	75.0	75.0	75.0	70.8
4	Air pollution	50.0	50.0	50.0	50.0	50.0	50.0	50.0
5	Cottony leak	50.0	50.0	50.0	50.0	50.0	50.0	50.0
6	Root knot	40.0	40.0	40.0	40.0	40.0	40.0	40.0
7	Target spot	25.0	33.4	42.4	42.3	42.2	42.1	37.9
8	Damping-off	30.0	30.0	30.0	30.0	30.0	30.0	30.0
9	Downy mildew	10.0	10.0	10.0	50.0	50.0	50.0	30.0
10	Angular leaf spot	30.0	30.0	30.0	30.0	5.0	5.0	21.7
11	Alternaria leaf spot	10.0	10.0	10.0	10.0	10.0	10.0	10.0
12	Powdery mildew	10.0	10.0	10.0	10.0	10.0	8.8	9.8
13	Scab	5.0	5.0	5.0	2.0	1.0	1.0	3.2
14	Tomato ringspot	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Mean	32.9	35.3	35.9	38.5	37.0	36.9	36.1

The estimated percentage loss in crop value caused by 9 of the 14 pathogens has remained fairly constant for the years 1983 to 1988 (Table 2). Downy mildew and target spot increased fivefold, and root knot decreased slightly the last two years reported. Root knot caused the greatest loss for all six years, accounting for nearly one third of all disease losses. Root knot was followed in importance by gummy stem blight, belly rot, and target spot.

The average incidence by year for all 14 pathogens indicated a slight increase in the estimated incidence of pathogens (R^2=0.46) (Tables 1 and 3). Estimated percentage loss of crop value by year also indicated a slight increase (R^2=0.51) (Tables 2 and 3). The increase appears related to the strong increases in downy mildew and target spot and, to a lessor degree, belly rot.

Table 2. Estimated percentage loss of value by 14 diseases on all field grown cucumbers (slicing and processing cucumbers) in North Carolina from 1983 to 1988 (ranked by mean)[z].

Rank	Disease	1983	1984	1985	1986	1987	1988	Mean
1	Root knot	12.0	12.0	12.0	12.0	8.0	8.0	10.7
2	Gummy stem blight	4.0	4.0	4.0	4.0	4.0	4.0	4.0
3	Belly rot	3.5	3.5	3.5	3.5	4.0	4.0	3.7
4	Target spot	1.0	3.3	3.8	3.8	5.0	5.0	3.6
5	Downy mildew	0.4	0.2	2.0	5.0	5.0	5.0	2.9
6	Anthracnose	2.0	3.0	3.0	3.0	3.0	3.0	2.8
7	Cottony leak	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8	Damping-off	1.5	1.5	1.5	1.5	1.5	1.5	1.5
9	Powdery mildew	0.4	0.4	0.5	0.5	0.5	0.5	0.5
10	Air pollution	1.0	0.3	0.3	0.3	0.3	0.3	0.4
11	Angular leaf spot	0.3	0.3	0.5	0.5	0.1	0.1	0.3
12	Scab	0.1	0.1	0.5	0.3	0.1	0.1	0.2
13	Alternaria leaf spot	0.1	0.1	0.1	0.1	0.1	0.1	0.1
14	Tomato ringspot	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Total	28.9	31.3	34.3	37.1	34.2	34.2	33.3

[z]Estimated percentage loss of value calculated as the percentage dollar loss due to yield and quality reduction.

Table 3. Coefficients of determination (R^2) for estimated incidence, estimated percentage loss of crop value, estimated cost, and year of estimate for field-grown slicing and pickling cucumbers due to 14 diseases in North Carolina from 1983 to 1988.

Variable	Year	Incidence[z]	Loss[y]	Cost[x]
Year	1.0000	0.4624	0.5105	0.7816
Incidence	0.4624	1.0000	0.9228	0.5274
Loss	0.5105	0.9228	1.0000	0.7310
Cost	0.7816	0.5274	0.7310	1.0000

[z]Estimated incidence calculated as the average incidence of 14 pathogens by year.
[y]Estimated percentage loss of value calculated as the total dollar loss due to yield and quality reduction by 14 pathogens by year.
[x]Estimated cost calculated as the total dollar loss due to yield and quality reduction and cost of prevention and control by 14 pathogens by year.

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Cucumber Disease Control in Wisconsin

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In 1989, Wisconsin ranked fifth in the U. S. for production of cucumbers for pickling, producing 642,700 tons (8% of U. S. production) valued at $9.4 million (1990 Agriculture Statistics, Wisconsin Dept. of Agriculture, Trade and Consumer protection, Madison, WI). In addition to acceptable yield and quality, cucumber growers and processors are concerned with protecting the crop from economically significantly damage by diseases and pests.

The primary diseases of cucumber in northern production regions are scab (*Cladosporium cucumerinum*), cucumber mosaic virus (CMV), and angular leaf spot (*Pseudomonas syringae*). Bacterial (*Erwinia tracheiphila*) and fungal (*Fusarium oxysporum* or *Verticillium* species) wilts are not commonly observed. Bacterial wilt is controlled effectively by eliminating spotted and striped cucumber beetles, and resistance to *Fusarium* wilt appears to be linked with scab resistance (M. Palmer, personal communication). Since scab resistance has been incorporated into most cultivars, both diseases have been controlled with genetic resistance.

Target leaf spot (*Corynespora cassiicola*), anthracnose (*Colletotrichum orbiculare*), and downy mildew (*Pseudoperonospora cubensis*) are diseases more commonly found in warmer production areas, so are generally not a problem in Wisconsin. Late season leaf diseases such as *Alternaria* occur sporadically on older leaves, but fungi isolated from lesions are only weakly pathogenic (Havey and Palmer, unpublished).

Wisconsin growers do not adhere to a weekly spray schedule to control diseases in their cucumber crop. Scab, CMV, and angular leaf spot are primarily controlled by genetic resistance. Resistance to scab and CMV appears to be effective. However, angular leaf spot may occur, even on cultivars listed as resistant. Cucumber fields are scouted and occasional sprays of cooper-based chemicals to control angular leaf spot may be recommended. In Illinois, belly rot (*Rhizoctonia solani*) may become prevalent late in the season as cooler nights allow heavy dew formation. Application of a fungicide (e.g. Bravo 720) may be needed. Overall, it appears that genetic resistance offers acceptable control of the diseases of northern-grown cucumbers and only occasional sprays are necessary.

Correlation of Shoot Weight with Root Galling in *Cucumis* spp. Inoculated with Root-knot Nematodes

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Root-knot nematodes (*Meloidogyne* spp.) are the most important pathogen of cucumbers (*Cucumis sativus* L.) in North Carolina. Root-knot nematodes destroy approximately 12% of the crop in the state annually (4). Root-knot nematodes reduce the fresh weight of plants (i.e. plant stunting) as well as reducing plant yields (i.e. fruit production).

Methods. A greenhouse study was conducted to determine whether shoot weight and root galling were correlated in six *Cucumis* cultigens (*C. sativus* L. cv. Sumter and *C. metuliferus* Naud. PI 482448, PI 482450, PI 482452, PI 482454 and PI 482461). Root galling was induced by inoculation of seedlings with one of four different species of root knot nematodes, *M. hapla*, *M. arenaria* race 1, *M. incognita* race 1, and *M. javanica*. The experiment was a factorial treatment arrangement in a randomized complete block design with four replications. Each treatment combination consisted of one *Cucumis* cultigen and one root knot nematode species in a 100-mm diameter (1450 cm^3 volume) pot. Pots contained sterilized sand and soil in a 1:1 ratio. Plants were grown from seed, with one plant per pot.

Inoculum was prepared using the technique developed by Hussey and Barker (3). Each pot was inoculated two weeks after planting with 5000 root-knot nematode eggs of the appropriate root-knot nematode species or race. Plants were rated 10 weeks after planting for root-knot nematode damage (0 to 100 % of roots galled), and shoot fresh weight.

Results. 'Sumter' had the lowest overall shoot weight and the highest percentage of galled roots, which indicated that it was the most susceptible cultigen tested (Table 1). However, 'Sumter' did not differ significantly from the other cultigens tested with respect to mean shoot weight. 'Sumter' was the most susceptible cultigen to *M. incognita* race 1, significantly different from the four plant introduction accessions of *C. metuliferus* (PI 482450, PI 482452, PI 482454 and PI 482461). With respect to mean percentage root galls, 'Sumter' was significantly different from the accessions of *C. metuliferus* tested. An accession of *C. metuliferus*, PI 482452, was the least susceptible cultigen tested, having a mean shoot weight of 514 g, and 35 % of the roots galled.

Table 1. Shoot fresh weight (ShWt) and percentage of roots galled (%G) of 'Sumter' (*C. sativus*) and 5 accessions of *C. metuliferus* inoculated with 4 species of root-knot nematodes[z].

	Mean		Mh		Ma1		Mi1		Mj	
Cultigen	ShWt	%G	ShWt	%G	ShWt	%G	ShWt	%G	ShWt	%G
PI 482452	514	35	529	6	448	36	484	50	596	46
PI 482454	499	42	552	6	588	40	420	60	434	60
PI 482450	469	43	552	6	367	60	448	66	510	41
PI 482461	478	50	546	16	493	56	417	80	456	46
PI 482448	413	56	398	20	445	50	381	86	428	66
Sumter	389	80	423	30	434	96	258	96	442	96
Mean	460	51	501	14	462	56	400	73	479	59
LSD (5 %) for row-column comparisons of means						135	22			

[z]Data are means of 4 replications of 1 plant each. Shoot weight is in grams. Mh = *M. hapla*, Ma1 = *M. arenaria* race 1, Mi1 = *M. incognita* race 1 and Mj = *M. javanica*.

Root galling was negatively correlated (-0.38) with shoot fresh weight (Table 2), as expected. Thus, susceptible plants with many root galls had a lower shoot weight than resistant ones. Correlations were largest for plants inoculated with *M. hapla* or *M. incognita* race 1, whereas plants inoculated with *M. arenaria* race 1 or *M. javanica* had only small negative correlations. The most pathogenic species tested was *M. incognita* race 1, indicated by the lowest shoot fresh weight and the highest percentage root galling (Table 1). The least pathogenic species tested was *M. hapla*.

Table 2. Correlations of fresh shoot weights and gall indices for each species of root-knot nematodes tested[z].

	Overall	Mh	Ma1	Mi1	Mj
Shoot fresh weight	-0.38	-0.64	-0.08	-0.58	-0.01

[z]Mh = *M. hapla*, Ma1 = *M. arenaria* race 1, Mi1 = *M. incognita* race 1 and Mj = *M. javanica*.

These results confirm previous work showing that species of *Cucumis* are resistant to *M. hapla* (5, 6), and 'Sumter' (*C. sativus*) is more susceptible to root-knot nematodes than are accessions of *C. metuliferus* (1, 2). In addition, shoot fresh weight might be a useful trait to aid in the selection for nematode resistance.

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Screening of the Cucumber Germplasm Collection for Tolerance to Clomazone Herbicide

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Weed competition is a major factor which limits yield in commercial cucumber production. Average estimated annual loss in value of the pickling cucumber crop in the United States due to weeds is $17,359,000 (1). Herbicides currently registered for cucumber production include bensulide, chloramben, ethalfluralin, DCPA, glyphosate, naptalam, sethoxydin and paraquat (2).

Clomazone (2-[2-chlorophenyl)methyl]-4, 4-dimethyl-3-isoxazolidinone) (2) herbicide is currently in the experimental stage for use in cucumber. It provides excellent weed control as a preemergence herbicide, but can cause injury to cucumber plants at or above the potential use rate depending on environmental conditions during germination. Since it shows potential for weed control in cucumber, we felt it appropriate to determine whether tolerance to this herbicide exists in the U. S. cucumber germplasm collection. If such resistance could be found and its genetic basis determined, then clomazone tolerant lines might be developed.

Seeds of each of 794 accessions in the U. S. germplasm collection were sown in a three replicate study. Replications (30 cm long) were arranged in a randomized complete block design at the University of Wisconsin experimental farm, Hancock, Wis. in 1990. Clomazone at 1.1 kg/ha was preplant incorporated in rows 153 cm apart, and 25 seeds per replication were planted approximately 25 mm apart within rows. The use rate for this soil (Typic Udipsamment; sandy, mixed, mesic) will probably be 0.28 kg/ha.

Phytotoxicity was rated visually to measure herbicide damage 2 and 3 weeks after planting (1 = green, no damage; 2 = marginal chlorosis, 25% damage; 3 = 50% of leaf surface chlorotic; 4 = 75% chlorotic; 5 = 100% chlorotic).

PI 165029 (Turkey), PI 212985 (India), PI 249562 (Thailand), PI 279466 (Japan), PI 390245 (Japan), and PI 504813 (Japan) were rated tolerant to clomazone injury (1.5 or below) in all replications. Reaction of all other germplasm accessions was either inconsistent, segregating or susceptible (>25% damage; Fig. 1; panel a). Two to 5 plants of each tolerant germplasm accession were selected, transplanted to a greenhouse, and self-pollinated.

An experiment was designed to reevaluate these field tolerant selections in a greenhouse herbicide test. Clomazone was incorporated at 0, 0.28, 0.55 and 1.1 kg/ha in soil media (unsterilized field soil used above) and deposited in 12 cm diameter pots which were arranged in a randomized complete block design with 3 replications. Five to 10 seeds of each selection and three susceptible cultigens (PI 390257, PI 391571, and WI 2870) were sown in each replicate and phytotoxicity ratings (as above) were taken at the cotyledon, and first and second true leaf stages.

The various clomazone concentrations elicited distinctive plant responses and cultigens displayed differing clomazone phytotoxicity (Table 1). A linear concentration dependent phytotoxic response to clomazone was recorded in all rating times. Although the cultigens responded differently at the cotyledon and first true leaf stage, no differences were detected at the third leaf stage. In the cotyledon stage PI 249562 and PI 279466 were less affected by clomazone treatment than controls (field susceptible). By the appearance of the second true leaf, response of these germplasm accessions was similar to controls. In contrast, PI 249561 (which was similar to the controls at the cotyledon stage) outperformed the controls at the second leaf stage. Tolerant plant responses recorded in the field were not observed in the greenhouse. Differing environmental conditions during growth and development may explain the lack of association between field and greenhouse response to clomazone.

Figure 1. Clomazone injury on cucumber plants 3 weeks after preplant incorporation under field conditions (top: 1.1 kg/ha, rating = 4.5) and in the greenhouse (bottom: from left to right 0, 0.28 and 1.1 kg/ha, rating = 1.25, and 4, respectively).

Table 1. Analysis of variance and means of phytotoxicity ratings of greenhouse grown plants subjected to clomazone herbicide (preplant incorporated) at 0, 0.28, 0.55 and 1.1 kg/ha.

Source of variation	df	Mean Square for stage of development		
		Cotyledon	1st Leaf	2nd Leaf
Cultigen (C)	8	1.70**	4.41**	0.52
Replication (R)	2	0.50	0.70	0.53
Error a	16	0.56	0.35	0.24
Treatment (T)	3	35.19**	38.56**	61.84**
C x T	24	0.50	0.75	0.16
Error b	6	0.23	0.32	0.36

Cultigen	Mean for stage of development		
	Cotyledon	1st Leaf	2nd Leaf
Field Tolerant			
PI 249561	2.58	1.79	2.75
PI 249562	1.94	3.18	3.33
PI 279466	1.85	3.33	3.46
PI 279467	2.35	2.38	3.17
PI 390245	2.35	2.48	3.17
PI 212985	2.37	1.42	2.94
Field Susceptible			
PI 390257	2.96	2.69	3.06
PI 391571	2.83	2.35	3.17
WI 2870	2.71	2.21	3.04
LSD (5%)	0.56	0.56	0.36

Treatment (kg/ha)	Mean for stage of development		
	Cotyledon	1st Leaf	2nd Leaf
0.0	1.0	1.0	1.0
0.28	2.13	2.04	3.09
0.55	2.99	2.85	4.01
1.10	3.63	3.81	4.38
LSD (5%)	0.35	0.35	0.22

**Indicates significant at 1% level.

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A Multivariate Re-evaluation of Biochemical Genetic Diversity in *Cucumis sativus* L.

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Genetic diversity in the U. S. *Cucumis sativus* var. *sativus* L. and var. *hardwickii* (R.) Alef. germplasm collection was assessed using 18 putative enzyme coding loci (2). These loci include *Gpi-1, Gpi-2, Gr-1, Gr-2, G2dh, Idh, Mdh-1, Mdh-2, Mdh-3, Pepla-2, Peppap-2, Per-4, Pgd-1, Pgd-2, Pgm-1, Pgm-3,* and *Skdh-2*. Three types of multivariate analyses were utilized to depict affinities and similarities among individual plant introductions (PIs) and PIs grouped by geographic region. Cluster analysis (compact linkage method) was used to group PIs by geographic region. PIs with similar isozyme phenotypes were placed in close proximity on the resulting dendrogram (Fig. 1). Principal component analysis (PCA) discriminated among individual PIs. A third procedure, classification and regression tree (CART) analysis identified enzyme loci (*Gr-1, Mpi-2, Pepla-2, Pgd-2, Pgm-1,* and *Skdh-2*) which were most discriminating in the analysis.

Inheritance and linkage studies (1) revealed that variation at *Gpi-2, Gr-1, Pgm-3,* and *Skdh-2* did not have predictable genetic bases and therefore this variation could not be classified as allozymic. This dictated that a multivariate re-evaluation omitting these 4 loci be conducted to more accurately describe the allozymic variation present in the U. S. germplasm collection.

The removal of data affected the classification of PIs which possessed putative rare alleles. The impact of rare alleles is important on the analyses and the removal of the putative rare (*Gpi-2, Gr-2, Pgm-3,* and *Skdh-2*) variants affected the results of principal component and cluster analysis partially by placing a greater weight on each of the remaining 14 loci in the re-evaluation.

PCA reduced the revised data set and identified 176 (a 77% reduction) different enzyme phenotypes as opposed to the 238 (a 68% reduction) in the initial analysis (2). This reflects a considerable decrease in the original amount of variability. PCA also grouped PIs according to their overall variability. Table 1 contains a partial listing of PIs according to the first principal component. PIs listed farther apart from one another. A complete listing of the entire U. S. *C. sativus* germplasm collection according to overall variability as determined by the first principal component, as well as the allozyme phenotypes for all PIs evaluated, can be found in the U. S. Germplasm Resources Information Network.

The CART analysis results remained unchanged with the exception of the data elimination. *Mpi-2, Pepla-2, Pgd-2,* and *Pgm-1* are the most discriminating loci. The ramifications of this result are that initial screening of additional germplasm might only include these loci in order to save time and expense.

Reanalysis of the data using cluster analysis resulted in changes in geographical relationships (Figure 2). Examples and explanations for some of these changes are:
1) In the original dendrogram Indonesian and Iraqi accessions were depicted to be more distinct from the rest of the collection. In the new dendrogram these distinctions disappear. Both of these countries possessed accessions with putative alleles which were eliminated after re-evaluation. Similar conformational changes occurred with Thai and Australian accessions.
2) Since *hardwickii* possessed alleles for *Per-4* and *Idh* which were not present in the remainder of the collection, an initial node separates it from the remaining *sativus* accessions. The removal of non-variable loci apparently put a greater weight on the remaining loci, allowing a more accurate description of relations. A similar event occurred with Polish accession in which

Figure 1. Cluster analysis (compact linkage method) of *Cucumis sativus* L. plant introductions grouped by country using 18 enzyme loci as framing criteria.

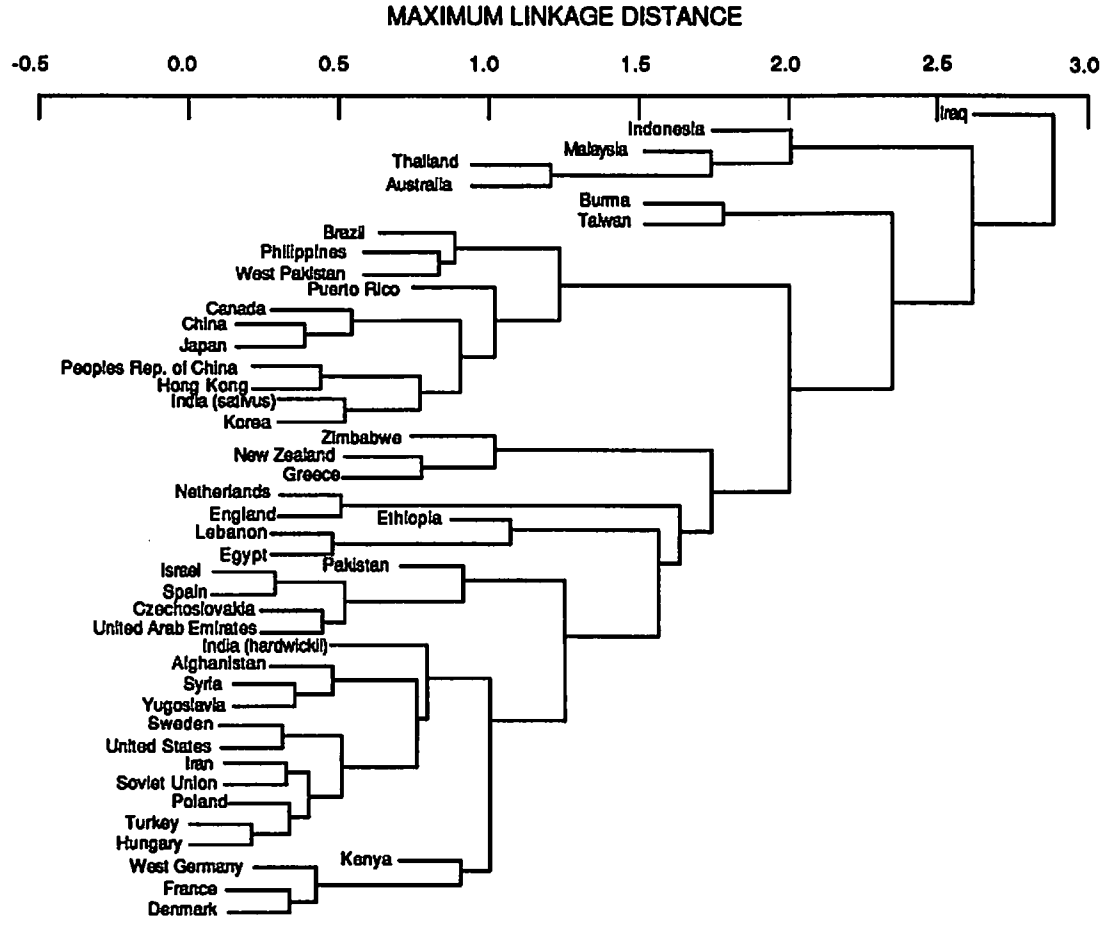
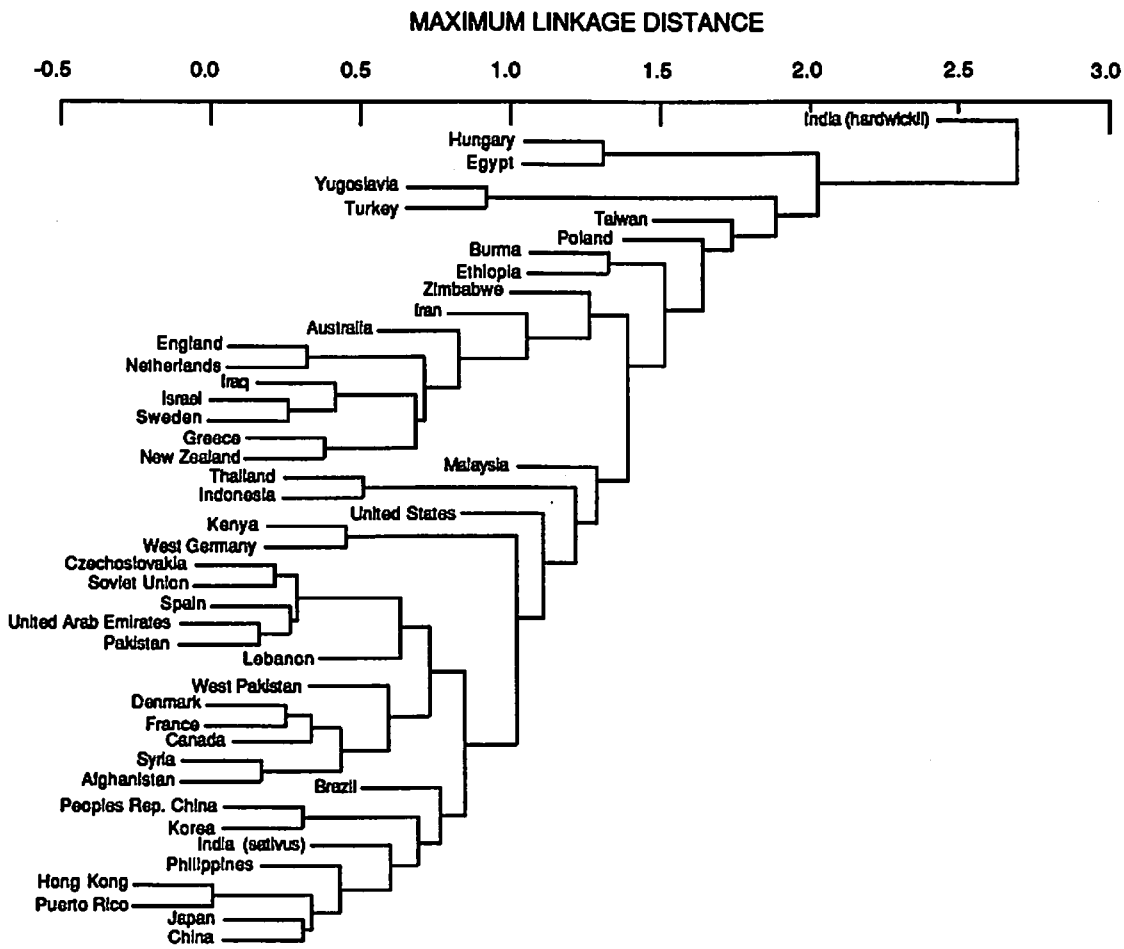


Figure 2. Cluster analysis (compact linkage method) of *Cucumis sativus* L. plant introductions grouped by country using 14 allozyme coding loci as framing criteria.



G2dh variation was unique. Certain countries, such as Egypt and Hungary, were partitioned into unique nodes due to the occurrence of multiple alleles that existed a low frequencies.

Due to the unknown ancestry of the collection and the sharing of germplasm among countries, geographic affinities described by clustering procedures require judicious appraisal. As this data set expands with the addition of polymorphic allozyme coding loci and their linkage relations are estimated, a more accurate depiction of relationships will be possible.

Table 1. A partial listing of *Cucumis sativus* plant introductions (PIs) listed by overall variability as determined by the first principal component (Prin1) from a principal component analysis of 14 biochemical loci.[z]

PI	Source	Prin1
432851	Peoples Republic of China	-10.1509
432854	Peoples Republic of China	-7.0065
432858	Peoples Republic of China	-6.2700
321007	Taiwan	-5.8088
390243	Japan	-5.8088
390246	Japan	-5.8088
390257	Japan	-5.8088
430585	Peoples Republic of China	-5.8088
432850	Peoples Republic of China	-5.8088
432853	Peoples Republic of China	-5.8088

458855	Soviet Union	2.2471
227013	Iran	2.2914
257286	Spain	2.3130
164734	India, sativus	2.3177
211984	Iran	2.3904
169353	Turkey	2.4774
292012	Israel	2.4774
344434	Iran	2.4774
344444	Iran	2.4774
458856	Soviet Union	2.4774

[z]Values for Prin1 indicate relative overall isozyme variability for a PI in ascending order. PIs with similar values for Prin1 ar similar with respect to isozyme phenotypes.

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Preliminary Studies on *Cucumis sativus* var. *xishuangbannanesis*

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Called "Da huang kua" (big cucumber) or "Shan huang kua" (mountain cucumber) by the local residents of the national minority in Yunnan, China, *Cucumis sativus* L. var. *xishuangbannanesis* Qi et Yuan, had not been brought to the horticulturist's attention until the Study Group of Vegetable Germplasm Resources investigated crop cultivars of the Yunnan province in 1979 and 1980 (1). This variety occurs in the mountain areas of Xi-shuang-ban-na Autonomous Region (southern Yunnan Province), where the fruits are eaten boiled or raw during different stages of maturity.

Cucumis sativus var. *xishuangbannanesis* (hereafter referred to as the Xishuangbanna gourd) is variable in fruit shape and rind color. The length : diameter ratio is between 1 and 4, a shorter ratio than that of the typical trellis cucumber, which ranges from 4 to 10. The fruits are round or square in shape, with ash or milky-white colored rind (Fig. 1). Cultivars of the Xishuangbanna gourd have been divided into six cultivar groups by Qi and Yuan (3). The diameter of the round-fruited cultivars reaches 46 mm. Fruits, which typically have three to five ventricles, are whitish-green when immature. The orange flesh and placenta of mature fruits darkens from the rind to the placenta with age. Weight of a single fruit averages 2 to 3 kg and contains upwards of 1000 seeds. Based on these characteristics, the Xishuangbanna gourd was originally thought to be variety of *C. melo* L. (1). However, the flavor of the flesh is similar to that of the common cultivated cucumber (*C. sativus*).

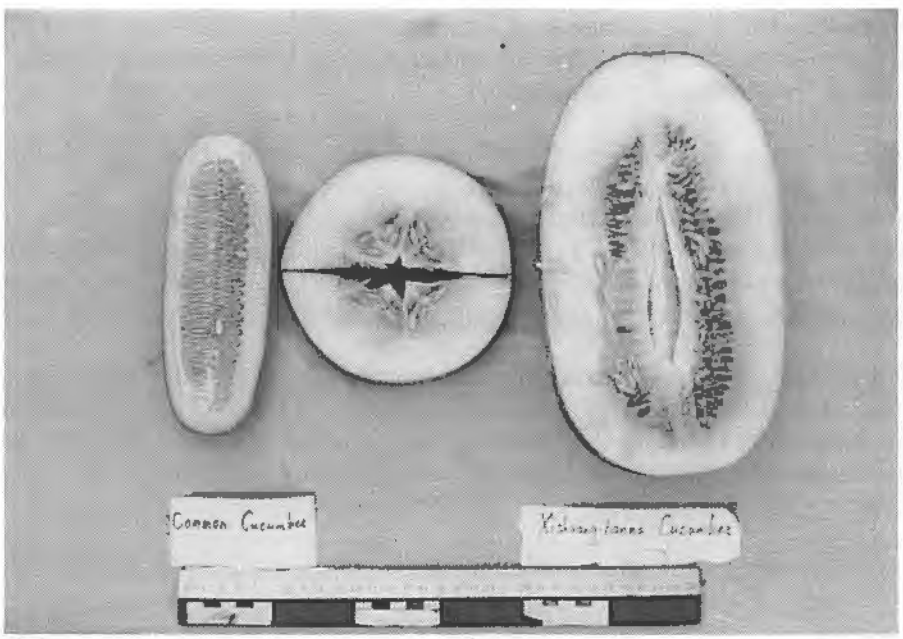

Fig. 1. Fruits of cucumber (L) and Xishuangbanna gourd (R) showing seedcell.

Xishuangbanna gourd is cultivated in mountain regions in south Yunnan, China at 800 to 1200 m elevation. Plants are usually intercropped with dry rice without trellis support. Seed germination to fruit harvest takes approximately six to seven months. Seeds are sown in April, and fruits harvested in September and October. Plants are vigorous and produce a large quantity of fruits. The raw flesh of the fruit has an excellent taste. Often, mature fruits are stored until the following February by the Hani and Dai people for the Chinese Spring Festival. The local people typically eat fruits raw. Xishuangbanna gourd is considered one of the most important off-season vegetables in the region.

Crosses between the Xishuangbanna gourd and the cultivars *C. melo* have never succeeded. However, crosses between the Xishuangbanna gourd and different Chinese cultivars of the cultivated cucumber have produced viable seed. The resulting plants of the F_1 generation from crosses of the Xishuangbanna gourd and cultivars of cucumber show some degree of heterosis in terms of growth habit. In addition, the frequency of pistillate flowers was reduced, and the length : diameter ratio was smaller than that of the typical cucumber. The orange-colored flesh, a distinctive character of the Xishuangbanna gourd, was transmitted to plants of the F_1 generation, suggesting the gene controlling flesh color is dominant. The recessive gene *yf*, reported as controlling orange flesh in the cucumber (2) appears not to be involved in controlling flesh color in the Xishuangbanna gourd.

Culture experiments in Beijing and Chong-ging indicate the Xishuangbanna gourd is difficult to bring into flower in temperate climates, and fruit set is low. These experiments suggest Xishuangbanna gourd requires a warm, humid environment for successful fruit set. The ecological requirements of the Xishuangbanna gourd are similar to that of *C. sativus* var. *sikkimensis* Hooker and *C. sativus* var. *hardwickii* Kitamura. These cultural requirements are believed to be primitive traits of the domesticated cucumber.

The patterns of peroxidase zymograms obtained from the Xishuangbanna gourd are similar to those of the common cultivars of cucumber and differ from those of *C. melo* (3). The chromosome number of the Xishuangbanna gourd is identical to that of other cucumber cultivars ($2n = 14$) and differs from *C. melo* ($2n = 24$). However, the karyotype of the Xishuangbanna gourd is different from the cultivated cucumber, with shorter chromosomes in the Xishuangbanna gourd. All seven pairs of chromosomes of both the Xishuangbanna gourd and cucumbers are metacentric, while *C. melo* chromosomes are mainly submetacentric. A satellite exists in the chromosomes of the cucumber, but is absent from Xishuangbanna gourd.

The primary gene center for the domestication of the cucumber is thought to be the southern Himalaya Mountains in the region of western India, Nepal, Bhutan and Burma (5). The Xishuang-ban-na Autonomous Region is located in the southern portion of Yunnan Province (latitude 20° 10' to 23° 40'; longitude 99° 55' to 101° 51'), and has a tropical (warm, humid) climate throughout the year. Thirty percent of vegetation of the region is tropical rain forest where *C. callosus* (Rottl.) Cogn. and *C. hystrix* Chakr (4; Yang, personal observation) can be found.

In conclusion, based on karyology, hybridization, and morphological studies, the Xishuangbanna gourd should be considered a variety of *C. sativus*. The center of origin of the cucumber should be expanded to include the Xishuangbanna region of China. Future studies are planned to examine the relationship between the Xishuangbanna gourd and *C. melo*.

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We would like to express our appreciation of Professor C. Z. Qi (Chinese Academy of Agricultural Science) for supplying germplasm for our studies.

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Mutants Induced by Pollen Irradiation in Cucumber

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Due to differentiation of male and female flowers and chimerism of mutated sectoring, seed irradiation is not an effective means to obtain induced mutants in cucumber. In order to solve that problem, we irradiated pollen to get uniform genotype within each seed embryo and were successful in obtaining 10 mutant lines (1, 2).

A cultivar "Nishiki-suyo" was self pollinated twice to confirm genetic stability and homozygosity before using in the experiment. Pollen (from staminate flowers) was irradiated by ^{137}Cs gamma-ray with 1, 2, 4, 6, 8 and 10 kR. Seeds from crossing using treated pollen were sown and each plant was self pollinated. In the progeny, we found interesting morphological mutants which are described below, along with a proposed gene symbol.

Wavy rimmed cotyledons (*wy*). Center of cotyledons occasionally white and rims green, later becoming wavy.

Albino cotyledons (*al*). Cotyledons white, hypocotyl light green.

Horn-like cotyledons (*hn*). Cotyledons warped like the horns of a bull, but transverse section of stem was circular.

Dwarf cotyledons-1 (*dwc-1*). Cotyledons small and hypocotyl short; leaves not expanding.

Dwarf cotyledons-2 (*dwc-2*). Same as *dwc-1*.

Light green cotyledons-1 (*lg-1*). Initially, cotyledons and young leaves light green, becoming normal green later.

Light green cotyledon-2 (*lg-2*). Initially, cotyledons and young leaves light green, becoming normal green earlier than *lg-1*.

Shrunken leaves (*shl*). First leaf shrunken, but later leaves progressively more normal.
Wilty leaves (*wi*). Rim of leaves wilted.

Heart shaped leaves (*hsl*). Leaves round-heart shaped; tendrils often branched.

Some mutants (*wy*, *lg-1*, *lg-2* and *hsl*) could be reproduced by self pollination, but others could be maintained only as heterozygotes because of lethality or poor viability of homozygote.

Segregation of self pollinated heterozygotes suggested that *wy*, *al*, *hn*, *dwc-1*, *lg-1*, *lg-2*, *shl*, and *hsl* indicated inheritance by a single recessive gene (Table 1). Therefore, we have proposed gene symbols for these mutants until genetic analyses to compare with comparable genes already reported. Two of the mutants, *dwc-2* and *wi*, deviated from the expected ratio of 3:1, but were recessive. Further genetic analysis is needed to clarify the reason for the deviation.

Table 1. Segregation of cucumber mutants obtained by pollen irradiation.

Mutant line	Irradiation dose (kR)	No. of plants		X^2 (3:1)	Prob.	Tentative gene symbol
		Normal	Mutant			
wavy rimmed cot.	2	49	17	0.20	P>0.70	*wy*
albino cot.	2	46	10	1.52	P>0.20	*al*
horn-like cot.	2	40	17	0.71	P>0.70	*hn*
dwarf cot.-1	2	36	15	0.53	P>0.70	*dwc-1*
dwarf cot.-2	2	45	4	7.41	P>0.01	*dwc-2*
light green cot.-1	4	46	11	0.99	P>0.80	*lg-1*
light green cot.-2	8	33	11	0.00	P>0.99	*lg-2*
shrunken leaves	4	40	10	0.67	P>0.80	*shl*
wilty leaves	2	67	12	4.05	P>0.05	*wi*
heart shaped leaves	6	33	15	1.00	P.0.80	*hsl*

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Cucumber (*Cucumis sativus* L.) Variants Resistant to Metribuzin or Linuron are not Viable

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In vitro culture techniques can be employed to improve cucumber (5), including selection of mutants for herbicide resistance. That would be desirable due to high cucumber sensitivity to those substances and because to date there is only one case of cucumber resistance to herbicides (10).

This study was aimed at obtaining metribuzin or linuron resistant cucumber mutants by means of mass selection in suspension cultures.

Methods. The experiments were carried out on a callus and cell suspension of two cucumber inbred lines: Borszczagowski (B) and GY3 (G). They were obtained by a method described previously (5). The plating density was $5 \cdot 10^5$ cells·ml^{-1}, and 2 ml of cell suspension employed in the selection was exposed to the action of 1 µM N-nitroso-N-ethyl-urea. The mutagen was applied in the last phase of cell suspension culture (before plating) along with an adequate amount of solution into a conical flask. After 12 hours, the culture was filtered, centrifuged and plated. The application of mutagen decreased in inoculation efficiency by approximately 50 %.

Lethal concentrations of herbicides for the callus were established by placing 100 mg of callus tissue on the medium with different concentrations of pure active substance: 150, 80, 40 and 20 mg·l^{-1} of metribuzin (M) and 100, 50, 25 and 12.5 mg·l^{-1} of linuron (L). Five calli were spread over the surface of approximately 25 ml of the medium in 80 mm Petri plates. After 3 weeks of culture in diffuse light (approximately 800 lux) at day temperatures of 27° ± 1°C under a 16 hour photoperiod and at night temperatures of 22 ± 1°C the calli were weighed (to ± 3 mg).

Two concentrations (higher and lower) of each growth regulator were used for the selection of resistant colonies (Table 1). Seven days after inoculation the medium was supplemented with agarose and then, after 14 days in culture-growing colonies, resistant variants were observed. Individual resistant colonies were transferred onto agar medium with adequate herbicide active substance added. Linuron-resistant variants were placed on fresh medium supplemented with 40 mg·l^{-1} linuron, while metribuzin-resistant variants were transferred onto a medium supplemented with 170 mg·l^{-1} metribuzin. After 2 weeks in culture, the herbicides were reduced to 20 mg·l^{-1} linuron and 85 mg·l^{-1} metribuzin.

The callus of resistant variants was used in further experiments to determine the resistance level and the presence of cross resistance. The resistance level was examined by comparing an increase in fresh weight of the callus after 3 and 6 days in culture on the medium supplemented with 150 and 300 mg·l^{-1} M or 50 and 80 mg·l^{-1} L. Shoot regeneration ability was examined on the media containing 20 or 85 mg·l^{-1} of active substances L or M respectively, according to the methods described for cucumber (5, 6).

Root regeneration ability was studied on media with the following hormone concentrations (mg·l^{-1}: IAA-0.5; BAP-0.05; IAA-5, BAP-0.5; 2,4-D - 1.2, BAP-0.3; NAA-3, BAP-0.05). Five to seven small calli of 200 to 250 mg were tested.

In the selected lines, resistance stability was investigated by comparing the intensity of callus growth obtained from regenerated shoot leaves of the resistant line with the intensity of callus growth of this line before regeneration took place.

In all experiments on callus growth intensity, the result was the average of 4 to 7 independent measurements (callus samples, depending on the experiment, amounted to 60 or 100 mg.).

Various methods were applied to induce roots: in vitro cultures, in perlite, peat, or perlite-peat mixtures. Mineral salts and pH were identical to those applied in the cultivation of seed-derived cucumbers. Shoot grafting of resistant lines was carried out on *Cucurbita ficifolia* or young cucumber plants.

Results. The effect of linuron and metribuzin on fresh weight growth in B and G callus cultures was examined to establish adequate selection concentrations. The application of 150 $mg \cdot l^{-1}$ M, and 50 $mg \cdot l^{-1}$ L brought about a 90% growth reduction after 3 weeks in culture (Table 1), whereas no growth was found after 6 weeks (data not shown). On the basis of the above results, the following concentrations of herbicide active substances for the selection of resistant lines in the suspension were chosen: 120 $mg \cdot l^{-1}$ and 150 $mg \cdot l^{-1}$ for metribuzin, 60 $mg \cdot l^{-1}$ and 40 $mg \cdot l^{-1}$ for linuron. Plating efficiency (PE) of the control on herbicide-free medium after 3 weeks amounted to 1.8% and approximately 50% reduction of PE was noted when the mutagen was added to the medium.

A week after plating, each Petri dish was supplemented with cell suspension medium having 2 % agarose (low gelling - 0.5 ml per plate). After the next 2 weeks, the number of initially resistant variants was established. Callus pieces (at least 2 mm in diameter) were treated as variants (Table 2). Resistant variants were obtained only after mutagen treatment. Their number was not affected by the concentrations of herbicide active substances during resistance selection to L. Resistant selection of M, however, showed a slightly higher number of variants at the lower concentration, and differences between G and B lines were noted. An average of 3.05 and 2.15 variants per plate was isolated in the B line, and 1.6 and 1.5 in the G line at the concentrations of 120 and 150 $mg \cdot dm^{-3}$ respectively. On the whole, 17 M resistant variants and 15 L resistant variants were isolated in the B line, 12 and 9 respectively in the G line.

An analysis of resistance to a given herbicide was carried out in a selected group of variants only in the B line. Two active substance concentrations - the same as in the selection and two (M) or 0.6 times (L) higher, were employed. The results obtained (Table 3) show that individual variants exhibited a very differentiated resistance level. At the lower active substance concentration, some variants were developing as in the herbicide-free medium. Most variants, however, showed a certain growth inhibition. At higher concentrations the differences were subject to the kind of herbicide used. All metribuzin-resistant variants exhibited poor growth whereas the reduction level was different in individual lines. In some L-resistant variants, however, callus growth was identical at both concentrations. The measurements taken after 6 weeks in culture more clearly show line reactions than those after 3 weeks.

Shoot regeneration and the ability to form somatic embryos from callus were markedly reduced in most variants. Above all, this affected M-resistant variants (Table 4). Thirty percent of them failed to develop embryos at all, whereas 20% formed only sporadically. All L-resistant variants formed somatic embryos and only one of them failed to develop shoots.

The embryos of some variants were not capable of further growth, and formed neither shoots nor plants. None of the shoots obtained were phenotypically similar to the control (achieved without herbicide selection). Variant shoots were very compact with a large number of small, dark-green leaves and easy branching. They usually formed no roots, but some had a few which failed to develop when transferred to perlite or peat. Flower buds were often noted which, when removed, resulted in shoot death.

Significant phenotypic differences among the lines can easily be seen in the regeneration characteristics of some of them: M1 had thickened, fasciated petioles, cabbage-like growth;

branching average compared to line 3; plants aged rapidly; lower leaves became yellow and, when removed, the next leaves grew and took the same color. M2 had small, not fasciated plantlets of average growth formed with characteristic almost triangular shape of hairy leaves; plants developed a relatively good root system. M16 had shoots, with thick fasciated petioles and very small leaves; aged rapidly. L33 regenerated best on the media used; developed most embryos and grew best; bud formation in leaf axils was so intense that, in 10 days, an individual plant formed a multi-shoot rosette. L7 was very compact with hairy leaves and poor branching.

Since rooting was impossible to achieve, grafting on *C. ficifolia* and young cucumber plants was attempted. This, however, proved unsuccessful, mostly because the shoot and the stock failed to grow together. In the callus of resistant variants, root formation ability was selected for, but none formed a root system. The callus, however, which did not undergo selection, was found to form roots very easily on the media used.

Shoots regenerating from resistant variants were not capable of regular growth, which made it impossible to carry out investigations on inheritance of resistance to L and M. Attempts, however, were made to establish stability of this characteristic. They were of two types. In type 1, the callus of resistant variants was maintained on L- and M-free medium for 6 weeks and transferred to a medium added with the herbicides. The growth intensity was then compared (retesting). In type 2, the callus was obtained from shoot leaves of some variants (R_1 generation) and its growth intensity was established on a medium supplemented with an adequate herbicide (Table 5). The investigations of cross resistance showed that all M-resistant variants were also, to a greater or smaller degree, resistant to L and vice versa (Table 6).

Discussion. Different resistance levels of herbicide resistant variants were described in other plant species, for example 2,4-D resistant *Lotus corniculatus* (Swanson and Tomes, 1980), paraquat resistant *Ceratopteris richardii* (3) and in 2,4-D resistant *Citrus sinensis* (9).

Cross resistance was observed in the cucumber variants since some linuron resistant variants were metribuzin resistant and vice versa. The cross resistance level was differentiated: from very high (M2, M4 and L2, L5, L7) to medium or low (M12, M20, M13 and L6). No close correlation between metribuzin and linuron resistance was noted. Some variants exhibited high (M1, L2) or low (M12, M22) resistance whereas others, despite the high herbicide resistance level in the selection, had low cross resistance (M113, M14, L6). The cross resistance characteristic manifested itself differently in the callus obtained from plant leaves of R_1 plants. In the case of L3 line it remained almost unchanged but it was significantly lower in M16 line.

Cross resistance is generally recognized in the case of structural similarity of chemicals. That was true in paraquat resistant tomatoes where the resistance was found to be correlated with diquat resistance (4). Similarly, Chaleff and Ray (1) noted cross resistance of tobacco mutants resistant to chlorsuphuron and methylsuphometuron two structurally similar urea herbicides. Cross resistance to herbicides belonging to different groups is known to react on the same metabolic pathway, e.g., photosynthesis, respiration (2). Linuron and metribuzin belong to different chemical groups. They, however, inhibit photosynthesis and other processes in cells of sensitive plants.

Regenerants from individual lines were significantly phenotypically different. They were, however, much more uniform within the line. A few lines did not regenerate at all or growing embryos failed to develop into plantlets. Only a few lines (7 or 18) were capable of forming poorly rooted plantlets. None of them, however, were able to grow under normal in vivo conditions. They were dwarf, and many had fasciated petioles and modified leaves. Most of them were characterized by rich branching but failed to grow achieving an average of 4 to 5 strongly twisted internodes. Growth incapability under normal in vivo conditions could not be overcome through

grafting, changing environmental conditions (from phytotron to greenhouse) or the application of chemical substances (rooting substances or gibberelins).

The variants obtained seem to be incapable of root organogenesis and development outside culture. Similarly, an attempt to form roots from R1 resistant callus was unsuccessful. No roots developed on any used media which stimulate root regeneration in unselected cucumber cultures. The failure to induce roots in shoots of the variants resistant to growth regulators has been reported in literature. Muller et al. (7) described NAA resistant mutants of *Nicotiana tabacum* incapable of inducing roots. Mutation was conditioned by one dominant nucleus gene. No other abnormality except incapability for inducing roots and modified leaf morphology was noticed in this study.

Some *Citrus sinensis* lines lost their ability to regenerate (9) and some *N. tabacum* lines were characterized by reduced variability and fertility, shortened nodules and rich branching (4).

The resistant variants obtained in this study were not always capable of regeneration, all the shoots possessed a strongly modified phenotype and failed to grow outside culture. Such responses may be explained in various ways: some authors report that reduced regeneration abilities of selected resistant cells are generally attributed to prolonged culture and the application of mutagens (2, 9). Both factors may have affected our variants. It should be stressed, however, that no variants were obtained without mutagen treatment. The last result and callus resistance obtained from R1 shoots suggested genetic background of selected resistance. The improvement in regeneration and in the quality of the obtained cucumber shoots might be achieved in the following ways: 1) selection of young calli or the application of in vivo - in vitro methods (at least in the case of M); 2) use of shorter culture duration on herbicide-containing media; 3) lower dose of mutagen; 4) use of a two step selection procedure as for the selection of Fusarium wilt-resistant cucumber (6). Another cause of reduced mutant regeneration abilities may be of genetic nature, such as a pleiotropic effect or correlation with unfavorable mutations (7). It does not seem probable, however, that in such a great number of independent variants, all of them have suffered the same damage.

Summary. The selection of metribuzin or linuron resistant mutants was carried out in suspension cultures of two cucumber lines (B and G) exposed to the mutagenic action of N-nitroso-N-ethyl-urea. Numerous variants of different resistance levels and shoot regeneration abilities were obtained, but only from line B. Generally, the shoots failed to take root or formed very weak rhizoids and exhibited inability to develop outside in vitro conditions. The shoots of all the variants were markedly modified but phenotypically differed among the variants. Generally, they were markedly shorter, diminished and formed rosettes of dark-green leaves. In most variants, cross resistance was exhibited.

Table 1. The growth of fresh callus of Borszczagowski and GY3 cucumber lines after 3 weeks of culture on media supplemented with various concentrations of active substances of metribuzin or linuron. The values were expressed as percentage of control.

Line	Herbicide active substance	Concentration ($mg \cdot l^{-1}$)	% of control callus growth
Borszczagowski	Metribuzin	150	23.80
GY3			36.59
Borszczagowski		80	51.84
GY3			53.77
Borszczagowski		40	61.00
GY3			74.63
Borszczagowski		20	91.28
GY3			78.33
Borszczagowski	Linuron	100	12.55
GY3			12.68
Borszczagowski		50	21.71
GY3			23.27
Borszczagowski		25	57.31
GY3			50.72
Borszczagowski		12.5	71.78
GY3			77.04

Table 2. Number of linuron and metribuzin resistant cucumber variants isolated 3 weeks after plating of cells on media containing herbicide active substances.

Line	Herbicide	Conc. of herbicide active substance ($mg \cdot dm^{-3}$)	No. of Petri dishes	Number of variants
Borszczagowski	Metribuzin	120	5	5,2
"	"	120	2	2
"	"	150	4(1*)	3
"	"	150	3	3
"	Linuron	40	4	4
"	"	60	5(1*)	4
"	"	60	3	3
GY3	Metribuzin	120	3	0
"	"	120	4	4
"	"	150	5	5
"	"	150	3	3
"	Linuron	40	3(1*)	2
"	"	60	4	4
"	"	60	3	3
Borszczagowski	Control	-	5	0
"	"	-	5	0
"	Linuron	40	4	4
"	Metribuzin	120	4	4
GY3	Control	-	4	0
"	"	-	5	0

*Number of infected dishes.

Table 3. Callus fresh weight (mg) of line B variants resistant to linuron or metribuzin after 3 and 6 weeks of culture on a medium with or without herbicides.[z]

Variant	Herbicide conc. ($mg \cdot dm^{-3}$)	Fresh weight (mg) after 3 weeks	Fresh weight (mg) after 6 weeks
Metribuzin			
M1	150	279	710
	300	195	249
M2	150	128	228
	300	104	132
M4	150	221	433
	300	202	342
M6	150	279	601
	300	236	365
M8	150	246	383
	300	184	272
M12	150	248	350
	300	160	176
M13	150	243	478
	300	154	188
M14	150	278	667
	300	123	107
M16	150	326	517
	300	256	321
M20	150	200	502
	300	121	156
M21	150	161	327
	300	127	152
M22	150	149	258
	300	141	218
Linuron			
L1	50	187	359
	80	137	148
L2	50	308	586
	80	309	621
L3	50	260	302
	80	131	111
L5	50	354	507
	80	171	578
L6	50	334	435
	80	243	390
L7	50	145	276
	80	163	218
Control	**0**	**306**	**840**

[z]Initial size of inoculum was 100 mg. Data are means of 5 replications.

Table 4. The number of embryoids (after 10 weeks) and shoots (after 15 weeks) obtained from the resistant variants of line B.

Variant	No. embryoids	No. shoots
M1	14	18[s]
M2	19	7
M4	14	15[s]
M6	18	15[s]
M8	1	0
M12	0	0
M13	6	0
M14	0	0
M16	19	9
M20	0	0
M21	0	0
M22	1	0
L1	8	6[s]
L2	7	0
L3	77	53[s]
L5	46	6
L6	6	3
L7	16	5[s]

[s]Grafting was made.

Table 5. The growth of fresh callus on media with L or M. Callus was initiated from leaf explants of some L- or M-resistant variants. Initial callus mas - 100 mg, 3 weeks of culture.

Variant	Herbicide active substance	Fresh weight (mg)
M 1	M 159	250
M 16	M 150	360
L 1	L 50	140
L 3	L 50	220

Table 6. The ability of M-variants to grow on the media supplemented with L and vice versa. The callus fresh weight of variants after 6 weeks of culture.[z]

Variant	Concentration of herbicide	Weight	Concentration of herbicide	Weight
M1	M 150	710	L 50	334
	M 300	249	L 80	318
M2	M 150	228	L 50	374
	M 300	132	L 80	350
M4	M 150	433	L 50	++++
	M 300	342	L 80	++++
M6	M 150	601	L 50	+++
	M 300	365	L 80	+++
M8	M 150	383	L 50	++++
	M 300	272	L 80	+++
M12	M 150	350	L 50	+++
	M 300	176	L 80	+++
M13	M 150	478	L 50	+++
	M 300	188	L 80	++
M14	M 150	667	L 50	+++
	M 300	107	L 80	++
M16	M 150	517	L 50	++++
	M 300	321	L 80	++
M20	M 150	502	L 50	+++
	M 300	156	L 80	++
M21	M 150	327	L 50	+++
	M 300	153	L 80	+
M22	M 150	258	L 50	+++
	M 300	218	L 80	+
L1	L 50	359	M 150	387
	L 80	148	M 300	154
L2	L 50	586	M 150	762
	L 80	621	M 300	160
L3	L 50	302	M 150	+++
	L 80	111	M 300	++
L5	L 50	507	M 150	451
	L 80	578	M 300	88
L6	L 50	435	M 150	+++
	L 80	390	M 300	+++
L7	L 50	276	M 150	++++
	L 80	218	M 300	++++

[z]Initial size of inoculum was 100 mg, each value is the mean of 5 replicates. ++++ - very strong growth, +++ - strong growth, ++ - weak growth, - no growth.

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Nomenclature of *Cucumis melo* L.

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The Common Name of *Cucumis melo* L.

The question has arisen recently as to whether the common name of the species should be "melon" or "muskmelon". Advocates of the latter say that "melon" may be misconstrued as referring to watermelon. On the other hand, nearly all precedent in the literature and in general usage favors "melon" as the common name. Bailey's Cyclopedia of Horticulture (1914) and Manual of Cultivated Plants (1949) give "melon" as the common name as does Hortus III published by the Bailey Hortorium. Nearly all textbooks give "melon" as the first or only common name for the species, as do Smith and Welch (8). Whitaker and Davis (10) give "musk-melon" as the common name, but on the same page they write about "winter melon", "pickling melon", "snake melon", etc., implying that "melon" is really the general name for the species.

As to confusion with watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, is called "watermelon" in numerous publications, and we find no instance of its being shortened to "melon" in discussions about it. This distinction between the species is made on menus, supermarket signs, and in the produce trade.

In view of these considerations, the National Muskmelon Research Group voted in November 1990 to change its name to National Melon Research Group and endorsed the statement that "The preferred and usual common name for the species *Cucumis melo* is "melon", while muskmelons or cantaloupes are a group within the species".

Groups Within *Cucumis melo*

Many monographs and texts follow the groupings or "tribes" of Naudin (5). These have usually been called "botanical varieties", but Smith and Welch (8) called them "groups", stating that the former term applies only to plants in the wild. Hortus III likewise calls them "groups". There are some truly wild melons, but most are known only in cultivation. Still others apparently grow as wild melons after escaping from cultivation, e.g. *C. melo chito* and *C. melo dudaim* in the southern United States. The question of which to call groups and which to call botanical varieties can be avoided by writing trinomials as zoologists do and Pangalo did (6).

One problem with Naudin's classification, as he himself recognized, is that intermediate forms are produced from the ready hybridization between groups. This is seen today in melons coming from crosses between *reticulatus* and *cantalupensis*. Another problem is that some melons grown widely in Asia do not fit any of Naudin's categories. Others which do fit have been given different Latin names, especially in India. The ten tribes of Naudin (5) were reduced to seven groups by Whitaker and Davis (10). We propose to simplify the grouping further by combining *cantalupensis* with *reticulatus* and *chito* with *dudaim*. We need to add a group, for which *momordica* seems to be the accepted name, to include the widely-grown snap melon of India. Our attempt at a revision of groups within the species is as follows:

1. C. melo agrestis Naud. Wild types with slender vines and small, inedible fruit. Probably synonymous with C. melo callosus and C. melo trigonus.
2. C. melon cantalupensis Naud. Cantaloupe or muskmelon. Medium size fruits with netted, warty, or scaly surface, flesh usually orange but sometimes green, flavor aromatic or musky. Fruit dehiscent at maturity. Usually andromonoecious.

This group includes Naudin's *C. melo reticulatus* which differed from *C. melo cantalupensis* mainly in having a netted surface. Combining these groups solves

the problem of naming intermediate types. It has been difficult to decide which name to give the combined group. The majority of melons in it were probably in the *reticulatus* group. On the other hand, these netted melons are usually called cantaloupes by U.S. growers, shippers, and consumers. After resisting this inaccurate terminology for several decades, we concede defeat and suggest *cantalupensis* for the combined group. Naudin's *C. melo saccharinus*, not usually mentioned in recent publications, appears to be similar enough for inclusion in this group.

3. C. melo inodorus Naud. Winter melons. Smooth or wrinkled surface with flesh usually white or green and lacking musky odor. Usually larger, later in maturity, and longer-keeping than cantalupensis, and not dehiscent at maturity. Usually andromonoecious.

4. C. melo flexuousus Naud. Snake melon. Synonym of snake cucumber, a common name causing confusion and therefore to be avoided. Fruit long and slender, used when immature as an alternative to cucumber. Monoecious. C. melo utillisimus or long melon described in literature from India is considered by some to be synonymous with flexuousus (2,3), but the original description by Roxburgh (7) was more like conomon.

5. C. melo conomon Mak. Pickling melon, sweet melon. Small fruit with smooth skin, white flesh, early maturity, and usually with little sweetness or odor. However, some melons in this group have high sugar content when mature and are eaten like apples, rind included. Vines of both types have similar appearance and have in common resistance to cucumber mosaic. Andromonoecious.

6. C. melo chito and C. melo dudaim Naud. Mango melon, vine peach and other similar names for the former; pomegranate melon, Queen Anne's Pocket melon for the latter. Distinction between these two groups is not clear from published descriptions. The dudaim melon collected by Wall (6) and used at Cornell as a source of monoecious flowering, does not have the fragrance attributed to this group. Bailey (1) mentions that certain accessions of dudaim lack fragrance, eliminating thereby the main distinction between it and C. melo chito. Further study may reveal some clearer differences between the groups or alternatively that they should be combined.

7. C. melo momordica, 'Phut' (2) or snap melon (2). Grown in India and other Asian countries and distinct from any other group. Flesh is white or pale orange, low in sugar, and mealy. The smooth surface of the fruit cracks as maturity approaches and the fruit disintegrates when barely ripe. PI 371795 and 414723 belong to this group and have provided important resistance, such as to aphids, zucchini yellow mosaic, and watermelon mosaic. We do not know whether the extreme susceptibility to cucumber mosaic in these PIs is characteristic of the entire group. Roxburgh's description of Cucumis momordica corresponds to this group (7). Most melons in this group are monoecious.

Naudin (5) listed *C. melo acidulus* with a description in some respects like *mordica* (i.e. breaking into pieces when ripening) and in some respects like *flexuousus* (fruits similar to cucumber). Recent publications have omitted this name when listing Naudin's grouping. Clarification is needed.

This tentative revision of melon groups is presented with the hope that it will stimulate discussion and thought. We urgently request comments on it, particularly on the name for group 2, the questions about *C. melo utillisimus* and *acidulus*, and distinctions or lack thereof between *chito* and *dudaim*.

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Two Virescent Mutants in Melon (*Cucumis melo*)

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In the course of breeding programs at the Vegetable Breeding Station of Avignon-Montfavet, two natural occurring virescent mutants have been observed.

The first one was a yellow virescent mutant. Young leaves were yellow green and they turned to normal dark green when becoming older. The growth was reduced specially on young plants and there can be some mortality just after germination if conditions (light intensity, temperature) were not optimum. When they were transplanted in greenhouse for pollination and seed production, growth was better and the male and female fertility were good. The F_1 hybrid was normal green and the F_2 segregated 3 normal *vs* 1 yellow virescent (448 *vs* 136, Chi-square = 0.913, Prob. = 34%) indicating a monogenic recessive control.

In the second mutant young leaves were more light green than yellow green with old leaves turning normal green. This mutant looks like the virescent mutant described by Hoffman and Nugent (2). Growth was not very reduced (less than the yellow virescent mutant) and the fertility was normal. The F_1 hybrid with a normal plant was indicating a recessive control of the character. F_2 progenies have not yet been studied.

Allelism tests must be done in order to know if these two mutants are reoccurrences of *v (virescent)* (2), *v-2 (virescent-2)* (1) and *yv (yellow virescence)* (3) before new names and symbols can be assigned.

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A Rapid and Sensitive Method for Evaluation of Melon Resistance to *Sphaerotheca fuliginea*

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When studying the inheritance of the resistance against *Sphaerotheca fuliginea*, the causal agent of powdery mildew in melon (*Cucumis melo* L.), one of the difficulties is the lack of a satisfactory, quantitative, objective and at the same time rapid method, to test different levels of resistance when the possibility of some type of quantitative inheritance of the resistance is to be investigated.

Many rating systems for the host-parasite interaction have been proposed, usually based on visual scales, but those are not mathematically linear nor even continuous (5).

We could mention the percentage scale, which notice the symptoms between 0% to 100%.

Several variations of this method have been used:

- The 1 to 5 scale proposed by Markarian and Harwod (4).
- Some other authors (5) considered only three classes:
  a) Susceptible reaction, characterized by abundant sporulating powdery mildew colonies.
  b) Resistant reaction, characterized by none or few colonies.
  c) Moderately resistant, by mild infection.
- Temmen et al. (6) also grouped the genotypes in three classes, based in the percentage of vacuolization of the epidermal tissue.

These modifications of the percentage scale, simplify the characterization of the cultivars, but classified them a priori into discrete classes which is not suitable for quantitative analysis.

Possibly a good method is the evaluation of the ADPC (area under the disease progress curve) (3), but it needs to spend a lot of time monitoring the infection rates.

A preliminary study was carried out in 1986, with some local cultivars of melon (Table 1), finding intermediate and different levels of resistance/susceptibility to powdery mildew (1).

Because of these results, the necessity of finding a good rapid and easy method for testing those intermediate degrees of resistance appeared.

Table 1. Mean values of ADPC for different muskmelon cultivars grown in the field.

Cultivar	ADPC
PMR45	0.0000
PMR5	0.0000
Negro	0.0000
Roteño	0.0000
Moscatel Grande	0.0005
Amarillo	0.0005
Agostizo	0.1033
Invernizo	0.0555
Doublon	0.2225
Piel de Sapo	0.2016

Blancard et al. (2) working with *Pseudoperonospora cubensis* proposed a method which is related with the amount of pathogen present in host tissues. Based on this, we tried to assess if the number of *S. fuliginea* conidia is related with the degree of resistance measured as the percentage of diseased tissue.

This percentage was visually determined between 0% and 100%.

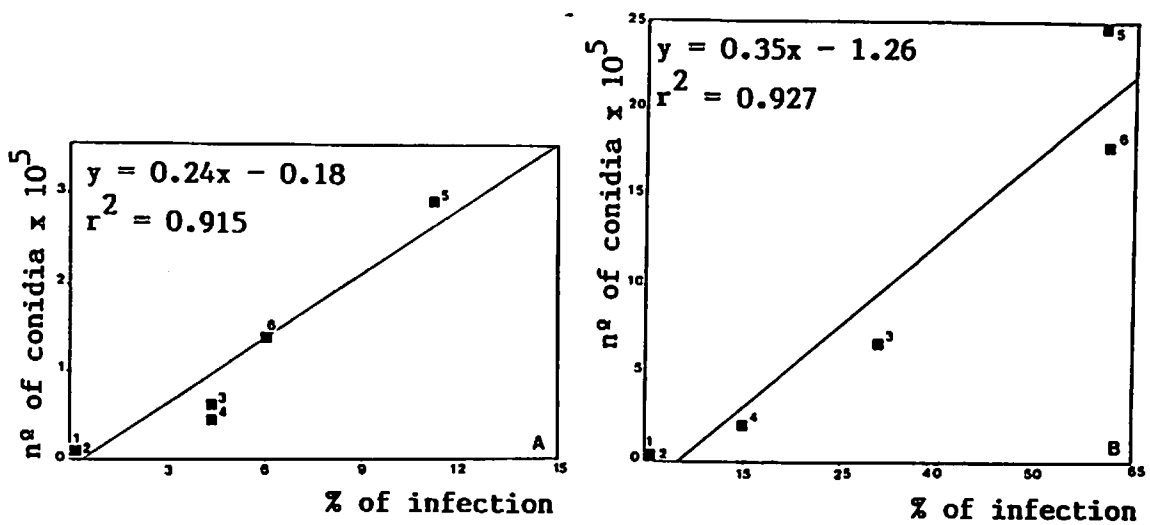
Plants of six genotypes of *C. melo* L. (PMR5, PMR45, Roteño, Invernizo, Piel de Sapo and Rochet) were grown in pots in a growth chamber, with 24°C constant temperature, 15 hours of light, and 9 of darkness. Two plants per pot, and ten plants for each genotype were used.

At second true-leaf stage, plants were inoculated by spraying on them a suspension of 40,000 conidia/ml of *S. fuliginea* race 1, in a watery medium with 1 ppm of Triton X100.

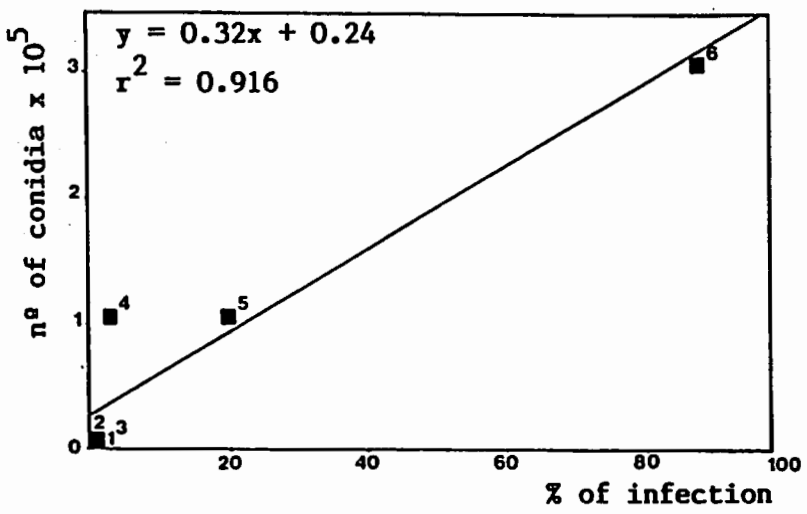
Six and ten days post inoculation both number of conidia and visual symptoms were recorded on inoculated plants.

For counting the conidia, one leaf disk of 1 cm of diameter was introduced and shook into a glass tube with 1 ml of the same Triton X100 medium used for spraying, and the conidia were counted with the aid of a cytometer.

Results are shown in figure 1.



Another assay carried out in the field, monitoring the symptoms in the same way, 35 days post infection results are shown in figure 2.



A good correlation between the two rating systems was observed, it remains constant along the disease progress, and it is independent of the growing conditions.

The method reported here may also be adapted easily to other studies of resistance against different powdery mildews in other crops.

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Resistance to *Acremonium* sp. in Spanish Landraces of Melon

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Melon dieback is a new disease that becomes dominant all along the Spanish Mediterranean coast (1). The early symptoms begin to show when fruiting starts: it is observed a interveinal yellowing on the older leaves. Later these leaves turn flaccid and the end of the shoots wilt. A few days later the plant dies. The causal agent of melon dieback is a fungus which hurts the rootlets since the first stages of plant development. This fungus belongs to genus *Acremonium* and performs the Koch's postulates (2).

A technique of artificial inoculation was designed to carry out a fast screening of resistant genotypes to melon dieback: seeds were sown into containers filled with vermiculite and germinated in a growth chamber. When the seedlings had a true leaf they were removed from the vermiculite and immediately placed in continuously-aerated nutrient culture solution. Previously it was added a triturated PDA plate where *Acremonium* had grown at 28°C for 15-20 days. Tanks were kept under laboratory conditions. The susceptible genotypes showed root rot within 144-240 hr after inoculation and the resistant ones display undamaged roots. 46 Spanish landraces were evaluated by this means (Table 1).

Table 1. Resistance to *Acremonium* sp. in Spanish landraces of melon.

	Replicate	
Landrace code	1	2
Pat 6	R/S*	R
Pat 13	R/S	S
Pat 22	R	-
Pat 29	R/S	R
Pat 34	R/S	R/S
Pat 35	R	S
Pat 36	R	-
Pat 38	R	R/S
Pat 39	R	-
Pat 42	R/S	-
Pat 46	R/S	-
Remaining landraces	S	-

*R: No plant showed root rot. S: All of the plants showed root rot.
R/S: Doubtful reaction.

A similar disease to melon dieback existed in Spain in the thirties (3 and 4). In any case melon dieback has been known for 20 years although it has begun to be a serious problem in the eighties. The increase of this disease in the last years may have been owed to a rise in inoculum in the soil as a result of a continuous practice of melon cultivation, and to the massive substitution of landraces by improved cultivars which are susceptibles to melon dieback. The results of the present work seem to suggest that some of old landraces could be resistant.

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Potential Sources of Resistance to Lettuce Infectious Yellows in Melon

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Lettuce infectious yellows (LIY) is a sweetpotato whitefly, *Bemesia tabaci* (Genn.), transmitted virus disease that occurs in the lower deserts of California, Arizona, and Mexico (1). Foliar symptoms appear approximately eight weeks after inoculation and are characterized by interveinal yellowing beginning with the older leaves. LIY also reduces yield. The objective in the present study was to compare LIYV susceptible 'Topmark' with cultivars and germplasm that have resistance to other virus diseases, insects, or that are from regions where the sweet potato whitefly is endemic throughout the year.

Twenty-three melon cultivars, varieties, breeding lines, and plant introductions (Table 1) were tested for resistance to LIYV in the field at Brawley, California in 1988. The wild species *C. prophetarum* L. was also included. Seeds were planted into dry soil on 17 August and irrigated by furrow on 18 August. Symptoms were evaluated on 19 October using a 1 (symptomless) to 9 (dead) scale. At that time plant size was evaluated using a 1 (runt) to 9 (large, completely covering the bed) scale and numbers of fruit were counted. Plots were 8.4 m in length on 2.4 m centers and consisted of 10 hills spaced 0.7 m apart; four seeds were planted per hill. The design was a completely randomized block with two replications. Means separation was done using Duncan's Multiple Range test ($\alpha = 0.05$). LIYV infection was confirmed by ELISA (1).

The 24 entries differed greatly for plant size, number of fruit per plant and LIYV symptom severity (Table 1). The entries varied greatly also for horticultural qualities such as earliness; fruit shape, size and skin color; and flesh color and texture (data not shown). LIY symptom severity was not correlated with plant size ($r = -0.06$) or number of fruit per plant ($r = -0.16$).

Sudden wilt-like symptoms may have confounded LIYV symptoms to some extent, but probably did not greatly affect the relative ranking for LIYV symptoms among the entries because interveinal yellowing is not part of the sudden wilt syndrome (data not shown). Wild Melon could not be evaluated for LIYV because the plants had senesced, but the entry was prolific and had set an average of 7.2 fruit per plant (Table 1). The correlation between LIYV and sudden wilt symptoms was small ($r = 0.09$, $P_{0.05}$).

Esteva et al. (3) reported that PI 161375 was heterogeneous for resistance to a greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) transmitted yellows virus. In this test, PI 161375 was rated 4.5 for LIYV symptom severity and was uniform in its response. Esteva et al. (2) and Soria et al. (4) reported resistance to the greenhouse whitefly-transmitted yellows virus in wild relatives of melon. In this test, *Cucumis prophetarum* expressed mild LIYV symptoms.

Snake Melon, a local variety from Saudi Arabia had the mildest LIYV symptoms and large vines. PI 124111, 92577 (a different increase of PI 124111), and MR

1, a downy mildew, *Pseudoperonopsora cubensis* (Berk. & Curt.) (5), resistant inbred of PI 124111 also had relatively mild LIYV symptoms.

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Table 1. Mean LIYV symptoms, fruit per plant and plant size.

Entry	LIYV	Fruit	Plant
Snake Melon	2.0 a	1.7 b	8.5 ab
92577	3.0 ab	1.8 b	6.5 b-e
C. prophetarum	3.0 ab	8.3 a	3.0 g
PI 124111	3.0 ab	2.1 b	5.5 c-g
MR 1	3.5 ab	1.8 b	6.5 b-e
PMR 45	3.5 ab	1.5 b	5.5 c-g
Quantif	3.5 ab	1.3 b	9.5 a
WMR 29	4.0 a-c	1.8 b	5.5 c-g
FC (WMR 29 x FC)	4.5 a-d	1.8 b	6.0 b-g
PI 161375	4.5 a-d	1.9 b	6.0 c-g
18236[z]	5.0 b-e	2.0 b	5.0 d-g
PMR 6	5.0 b-e	1.6 b	6.5 b-e
AR Hale's Best Jumbo	5.5 b-e	2.2 b	7.0 a-d
AR Topmark	5.5 b-e	2.1 b	7.0 a-d
Juane Canari	5.5 b-e	1.4 b	6.5 b-e
Quascem	6.5 c-f	1.8 b	8.0 a-c
Topmark	6.5 c-f	2.0 b	6.5 b-e
PMR Honeydew	7.0 de	1.4 b	5.5 c-g
Local Melon	7.5 f	1.6 b	9.5 a
92516	8.5	1.8 b	7.0 a-d
Freeman Cucumber	8.5	1.5 b	3.5 fg
PI 124112	8.5	1.5 b	5.5 b-f
AR 5[z]	9.0	2.1 b	4.0 e-g
Wild Melon	-	7.2 a	4.0 e-g

[z]Only 1 replication.

Progress in Breeding Melons for Watermelon Mosaic Resistance

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When melon breeders and pathologists met in 1986 and 1987, there was general agreement that, although some watermelon mosaic resistance (WMR) in melons had been suggested by the work of Moyer et al. (2), high priority should be given to locating better sources. At that time the causal virus was usually called WMV2 but now is simply WMV. In November 1989 we began screening a few seed lots already on hand, melons for which we had either experience or reports of resistance to some virus disease. We then obtained from the Plant Introduction Station at Ames, Iowa, 50 introductions from India and 50 from Afghanistan for a greenhouse planting in March 1988.

The November test consisted of 30-40 plants per entry, but space limited the March test to 12 per entry. No entry in either test appeared to be uniformly resistant, but the introductions from Afghanistan were judged to be uniformly susceptible to WMV. No other conclusions can be made about the frequency of resistance because of disparity of numbers between the two plantings. Resistance might have been found in some discarded accessions if larger numbers had been grown. The following summary is based on the November and March tests jointly.

Although none of the germplasm screened was immune to WMV, a small proportion of plants had the ability to grow and produce fruit, while the standard varieties did neither. The best plants were found in 4 distinct types of melons:

1. 'Freeman Cucumber', *C. melo conomon*, described by Enzie as resistant to cucumber mosaic (1), and subsequently used and maintained at Ithaca NY.

2. PI 371795 from India, PI 414723 derived from it, and PI 414723-4 selected for resistance to ZYMV by Dr. R. Provvidenti. Probably *C. melo momordica* (3).

3. PI 182938 from India, a small hard melon with no edible flesh, probably *C. melo agrestis* (3).

4. *C. melo dudaim*, a monoecious melon collected in Louisiana by Wall (5).

With the exception of 'Freeman Cucumber' (FC) female flowers were scarce in this greenhouse planting, and pollinations were necessarily made to some extent on the basis of expediency. They included selfs, crosses to susceptible melons, and intercrosses of resistant plants.

A field planting made in June 1988 included all seeds available from the greenhouse tests and repeats of the more promising introductions. It was inoculated with WMV in the seedling stage. A number of entries with supposed WMR were badly stunted or died without producing fruit, probably because of natural CMV infection. This was especially evident with PI 371795 and selections from it.

At the other extreme, the outstanding rows were crosses between FC selected for WMR and either PI 414723 or 414723-4, likewise selected for WMR. These rows were pollinated with several susceptible varieties or breeding lines. The resulting hybrids were the starting point for most of the backcross progenies on which our subsequent effort has concentrated. We thought that the superiority of these parents might be due to the complementary effect of genes for WMV from the very different parents and that it might be difficult to retain such resistance through several backcrosses. This assumption appears to have been erroneous. The partially dominant cucumber mosaic resistance of the FC parent may have been largely or wholly responsible for the superiority of the hybrids.

The FC x 414723 F_1 was crossed with 'UC Topmark FR', 'TAM Uvalde', and CPM339, a Cornell monoecious PMR line with moderate cucumber mosaic resistance (CMR). Three successive backcrosses to each parent were

made in two greenhouse generations and one intervening in the field, selecting each time for WMR. The resulting BC3 seed was used for the 1990 field planting. All segregating generations were inoculated at the first true leaf stage, but as Pitrat had reported (4), differences in resistance were not apparent for several weeks. Approximately 40 plants from backcrosses to each recurrent parent were transplanted to the field with only a little elimination of segregates thought to be clearly susceptible. For each recurrent parent four or five plants selected for WMV in BC2 were represented.

At flowering, about half the plants in most BC3 progenies were larger than their recurrent parents and had milder leaf mottling. These were used to make BC4. At that time the CPM329 parent row had distinctly better vines than 'Topmark' or 'TAM Uvalde', suggesting the CMV had spread naturally in the field. It was the only recurrent parent to set much fruit. Then as fruit approached half size in early September, all the recurrent parents and part of the plants in the BC3 progenies suddenly wilted and died, the CPM339 parent as much as the others. Most of the BC3 plants selected as resistant matured fruit and seed, but the backcrosses to CPM339 had much better fruit size and quality and vines remained green longer. It was clear that there had been natural CMV infection and that melons with resistance to both it and WMV were far superior to those with resistance to either one singly.

Backcrosses to 'Honeydew' were comparable in behavior to the other two CMV susceptible backcrosses but their background was different. They included two BC3 progenies in which the original cross was to a WMR selected plant in *C. melo dudaim* and three BC1 progenies in which resistance came from the same F1 used to start the other WMR backcrosses. All had the same proportion and performance of resistant plants in the field even though progenies with *dudaim* parentage had seemed less resistant in the greenhouse generation.

These 1990 results indicate that it may be possible to transfer a usable level of WMV rapidly and with relatively small populations and that similar resistance may be derived from different sources. They also suggest that this resistance may be more important than we previously thought. Progenies selected CMR have frequently looked resistant during most of the season but died suddenly as maturity approached. It now seems that this may not have been due to an inadequate CMR level as much as to natural spread of WMV.

Much further study of watermelon mosaic resistance is needed in melons. We do not know whether the resistance in our backcross progenies comes from 'Freeman Cucumber', from PI 414723, from the same gene in both, or from complementary genes in the two parents. Studies on the value of resistance from various sources as well as its inheritance are now underway.

Acknowledgments: Prof. Lin Depai from Zinjiang August 1st Agricultural College, China, was an active participant in the initial phases of this work from November 1987 to August 1988. Mr. Yiping Zhang likewise devoted much effort to the project during all of 1990. Their contributions were indispensable and are gratefully acknowledged.

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Field screening of melon cultigens for multiple race resistance to *Fusarium oxysporum* f. sp. *melonis*

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Fusarium wilt is one of the primary diseases limiting melon production in the mid-Atlantic regions of the U.S. Although multiple races of fusarium wilt have traditionally existed in Europe, only race 2 had been present in North America until recently. Race 1 was first reported in the U.S. (in Maryland) in 1985 (1). Subsequently, race 0 was reported in Texas (3) and in Maryland (2).

Fungicides and soil fumigants do not provide adequate control of fusarium wilt. The most desirable means of control is through the development of melon cultigens with multiple resistances to the pathogen. Since 1987, screening for fusarium wilt resistance has been underway in a field at the University of Maryland Lower Eastern Shore Research and Education Center in Salisbury, Maryland. This field has been in continuous melon cultivation for over 30 years and is heavily infested with both race 1 and race 2 of *F. oxysporum*. Results of the screening for the years 1987 through 1989 were published recently (4).

In 1990, a total of 56 entries were evaluated for their relative susceptibility to the wilt pathogen. As before, the field was fumigated and covered with clear plastic mulch. Four replicates of each cultigen were transplanted through the mulch in a randomized complete block design with eight plants per replicate. Normal cultivation practices were observed during the growing season and wilt resistance was assessed in mid-August by counting the number of surviving plants.

Although the majority of the entries suffered greater than 50% mortality in the test, eight new cultigens exhibited better than 88% survival. These were: PSR 15789, PSR 15889, PSR 17689, PSR 21789, PSR 21889 and PSR 22089 (Petoseed Co. Inc.); ACX-88815 (Abbott & Cobb); and Primo (Northrup-King). Many cultivars known to be resistant to race 2 (e.g. 'MaryGold' and 'Superstar') succumbed to the combined race situation. The 1991 field screening is currently underway to verify the multiple resistances of the new entries.

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Screening of Domestic and Wild *Cucumis melo* Germplasm for Resistance to the Yellow-Stunting Disorder in the United Arab Emirates

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Cucurbits are the most important group of vegetables grown in the U.A.E. Their production has been seriously threatened, however, by a yellow-stunting disorder (YSD) caused by a new virus of the closterovirus-like group similar to lettuce infectious yellows virus (LIYV)(2). The tobacco whitefly, *Bemisia tabaci* Genn. is thought to be the vector of this virus which was observed in 15 out of 16 affected samples examined by electron microscopy as long flexuous filamentous particles (3).

All attempts made locally to control the disorder through the control of whiteflies failed. The use of spunponded polysters and polypropylene plant covers for about 35 days (until flowering) was promising in controlling the disorder (4). However, this practice has not gained acceptance among U.A.E. farmers, yet; probably due to its high cost. At present, none of the tested commercial melon (*Cucumis melo* L.) cultivars were found resistant to this disorder (1,2). Only a low level of susceptibility to the disorder was found by the Department of Agriculture and Animal Production in Al-Ain (5) in melon cvs. 'Caribe', 'Maggar Kings', 'Muskotaly', 'Midstar', and 'Rock Sweet'. The use of resistant cultivars would be the simplest and most efficient method of controlling this disorder. Hence, the objective of this study was to search for sources of resistance to the YSD in a large collection of domestic and wild *C. melo* accessions.

Nine hundred seventy-five wild and domestic *C. melo* accessions were evaluated for resistance to the YSD under field conditions in Al-Ain, U.A.E. in the spring and autumn planting of 1988 and 1989. The 1988 spring and autumn trials included 488 U.S.D.A. *C. melo* plant introductions (PIs) and 7 commercial melon cultivars that were recommended by the Department of Agriculture and Animal Production for re-evaluation. Four of these cultivars were previously reported to be only slightly susceptible to the disorder (5). A different lot of 480 PIs was evaluated in the 1989 spring trial. Twenty-five of the most promising PIs in these trials (12 of the 1988 spring and autumn trials and 13 of the 1989 spring trial) were selected for re-evaluation in the 1989/1990 autumn trial in comparison with the susceptible cultivar 'Ananas'. Seeds of PIs evaluated were kindly provided by the U.S.D.A. through Dr. Raymond Clark (Plant Introduction Station, Ames, Iowa), while seeds of commercial cultivars were provided by local seed dealers.

Field seed sowing was during the third week of March for the spring trials and the first week of September for the fall trials in both years of the study. After thinning, individual plants were spaced 50 cm apart in rows 1.5 m-wide. Each accession was assigned to one 8.0 m row in the first, second and third trials and to 3-6 similar rows in a completely random design in the last (1989/1990 autumn) trial. The numbers of plants evaluated of each accession were as follows: 3-16 in the 1988 spring trial, 3-16 (x = 7.7, Sd = 2.98, mode = 4) in the 1988/1989 autumn trial, 3-14 (x = 9.8, Sd = 2.43, mode = 10) in the 1989 spring trial, and 2-6/replicates in the 1989/1990 autumn trial.

Natural infection by the YSD was enhanced by placing heavily whitefly-infested melon plants showing severe symptoms of the disorder nearby the evaluated plants one week after germination. No pesticides were applied to maintain a high population of viruliferous whiteflies which were continuously observed on the plants in all trials. In the 1988 spring trial, each accession was

classified as either resistant (R) or susceptible (S). The former group did not show any visible symptoms of the YSD on any of the plants when they were examined 8 weeks after seed sowing. In other trials, each plant was given a diseased score 8 and/or 10 weeks after seed sowing according to the following scale: 1, no symptoms; 2, slight; 3, moderate; and 4, severe symptoms. Individual plant ratings for each accession were added and divided by the number of evaluated plants to obtain the corresponding mean disease scores (MDS). Then, accessions were classified as either R, slightly susceptible (SS), moderately susceptible (MS), or highly susceptible (HS) when their MDSs were 1.0-1.5, 1.6-2.5, 2.6-3.5, and 3.6-4.0, respectively. In the 1989 spring trial, accessions classified as R or SS 8 weeks after seed sowing were re-evaluated 2 weeks later. The second readings were assigned to the respective accessions which were signaled out in the delayed symptoms development category.

Based on the final (1989/1990 autumn) trial (Table 1), none of the accessions evaluated were found highly resistant or immune. Fourteen PIs were significantly different from cv. 'Ananas' in mean disease score (MDS) 8 weeks after seed sowing, 9 of which were different at the 1% level; but the number diminished to 5 PIs when disease rating was repeated 2 weeks later, with only PI 403994 remaining highly significantly different from 'Ananas'. The others were PIs 255478, 2922007, 381766, and 390452. PI 403994, the best performing accession in these trials, was given MDSs of 1.68 and 2.95 (or was classified as slightly and moderately susceptible) at 8 and 10 weeks after seed sowing, respectively; while 'Ananas' was rated 3.77 and 4.00 at the 2 dates, respectively.

In line with previous reports, cultivars 'Jupiter' and 'Muskotaly' showed only slight symptoms of the disorder, while 'Caribe', 'Midstar', and 'Rock Sweet' were rated 8 weeks after seed sowing as slightly susceptible.

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Table 1. Response of selected *C. melo* accessions re-evaluated in the 1989/1990 autumn planting to the yellow stunting disorder.

Accessions (PI)	Response in previous trials: Spring 1988[z]	Autumn 1988[z]	Spring 1989[z]	No. of replicates	Mean disease score after weeks indicated from seed sowing: 8 weeks[y]	10 weeks[y]
164797	R	HS	-	3	3.66 n.s.	3.83 n.s.
179680	R	R	-	4	3.48 n.s.	3.88 n.s.
179922	S	MS	-	4	2.66 **	4.00 n.s.
211116	S	SS	-	6	2.50 **	3.85 n.s.
214154	S	MS	-	5	3.49 n.s.	3.90 n.s.
222984	R	MS	-	6	3.27 n.s.	4.00 n.s.
229555	R	-	-	3	2.18 **	3.77 n.s.
255478	R	-	-	3	1.33 **	3.40 *
255953	R	-	-	6	2.57 **	3.87 n.s.
288233	-	-	SS-d	4	2.45 **	3.88 n.s.
292007	-	-	SS-d	4	2.74 *	3.35 *
323498	-	-	SS-d	5	3.28 n.s.	4.00 n.s.
353451	-	-	HS-d	6	2.56 **	3.97 n.s.
378062	R	-	-	5	2.80 *	4.00 n.s.
378063	-	-	SS-d	4	3.06 n.s.	4.00 n.s.
378064	R	MS	-	5	2.75 *	3.90 n.s.
381762	-	-	MS-d	6	3.36 n.s.	3.93 n.s.
381772	R	HS	-	6	3.38 n.s.	3.97 n.s.
381766	-	-	SS-d	6	2.30 **	3.50 *
381800	-	-	SS-d	6	3.05 n.s.	3.92 n.s.
381802	-	-	SS-d	6	3.13 n.s.	3.92 n.s.
385966	-	-	SS-d	5	3.27 n.s.	4.00 n.s.
390452	-	-	SS-d	6	2.51 **	3.52 *
401624	-	-	MS-d	5	2.97 *	3.85 n.s.
403994	-	-	R-d	6	1.68 **	2.95 **
cv. 'Ananas'	-	-	-	6	3.77 (control)	4.00 (control)

[z]R, resistant; S, susceptible; SS, slightly susceptible; MS, moderately susceptible; HS, highly susceptible; the letter 'd' refers to delayed symptoms' development at 10 weeks after seed sowing.

[y]L.S.D. between accessions with 3, 4, 5, and 6 replications and the control (cv. 'Ananas') were, respectively, as follows:

P = 0.05 at 8 weeks : 0.91, 0.83, 0.78, and 0.74;
P = 0.01 at 8 weeks : 1.20, 1.10, 1.03, and 0.98;
P = 0.05 at 10 weeks : 0.49, 0.44, 0.42, and 0.40;
P = 0.01 at 10 weeks : 0.64, 0.59, 0.55, and 0.53.

Accessions were either not significant (n.s.), significant (*), or highly significant (**) from the control.

Search for Sources of Resistance to a Whitefly Transmitted Yellowing Disease in Melon

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The principal Spanish area for greenhouse vegetable cultivation is the south-east coast. For some time, a yellowing disease has been a serious problem for greenhouse melon cultivation on this area (3,4,7). The causal agent of melon yellowing disease could be a virus whose particles are long, flexuous and have a length of 900 nm (6). The transmission and host range of melon yellowing disease and the symptoms of infected plants suggest that this disease is very similar to beet-pseudo-yellows virus and cucumber yellows virus (1,2,3,8).

About 200 accessions of melon were evaluated over a period 1989 under conditions of natural infection (4). The lines of Asiatic origin "Nagata Kin Makuwa" and PI 161375 showed a certain level of inheritable tolerance (5). A *Cucumis melo* var. *agrestis* accession and a Spanish landrace which belong to Tendral type showed a high level of resistance (4).

Another 169 melon accessions (Table 1) have been tested in the last two years, 103 of them under conditions of natural infection. Twenty plants of each one of these 103 accessions were tested. The remaining accessions were evaluated using a technique of artificial inoculation which utilizes the vector of yellowing disease causal agent, the greenhouse whitefly (*Trialeurodes vaporariorum* W.) (7): "Non viruliferus" whiteflies were placed for 48 h on melon plant leaves with symptoms of yellowing. After this period, groups of 40 whiteflies were removed and transferred to feed for 72 h on the plants of accessions to be tested. These plants had two true leaves when were inoculated. Later they were removed and transplanted to a greenhouse where they finished their cultivation period under natural conditions. Ten plants of each one of these accessions were tested. The aforementioned *C. melo* var. *agrestis* and the Spanish landrace of Tendral type were included among the accessions artificially inoculated.

All the accessions evaluated under conditions of natural infection showed susceptibility to melon yellowing disease. Among those artificially inoculated only the *C. melo* var. *agrestis* accession displayed a high level of resistance as these plants remained symptomless during the entire trial. (Only one plant showed light yellowing symptoms when the tests were finished.)

A considerable effort has been made to find resistance to melon yellowing disease. The present work confirms the existence of one source of resistance in *C. melo*. This fact opens up hopeful prospects to the melon breeding for resistance to this yellowing disease.

Table 1. Origin of accessions tested for resistance to melon yellowing disease.

Origin	Accessions tested
Conditions of natural infection	
Zimbabwe	4
Spain	53
South of Balkans, USA, Portugal	1
Unknown	27
Conditions of artificial infection	
South of Balkans	4
Yugoslavia	17
China	5
USA	2
Libya	10
Zambia	3
Pakistan	20
Turkestan, Hungria, USA, Afghanistan, Creta, Spain	1
Unknown	8

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Reproductive Patterns of Three Melon Cultivars in Response to Temperature Accumulation

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Patterns of reproductive development in plants are partially controlled by the temperature experienced during growth. The correlation is sufficient that heat-unit accumulation has been used to model the initiation and maturation of reproductive structures for major agronomic crops (3,4). Perry and Wehner (5) recently developed a heat-unit model to predict fruit maturity for cucumber cultivars. Similar information is of great value to the melon industry where cultivar maturities are used as part of the production strategy. Phenotypic responses unique to individual melon cultivars have already been observed for seed germination and plant growth rates (1,2). Therefore, a study was established to obtain preliminary information of flowering and fruit maturity patterns in melon (*Cucumis melo* L. *reticulatus*) as affected by cultivar and growing temperature.

Methods. Two field plantings of 3 commercial melon cultivars were established in February and March, respectively. Plots were direct-seeded into 155 cm raised beds with approximately 20 cm between plants within a row. Plots were cultured according to commercial recommendations. Fully open female flowers were tagged each morning and the fruit followed to harvest. Fruit were harvested daily at full slip. Temperatures were monitored at 2-4 cm below the soil surface and at 1 m above ground using thermistors and a Campbell CR-21 data logger. The accumulated degree-days were computed by summing the differences between air or soil temperature and a base temperature of 10C.

Results. The results of this initial study are summarized in Table 1. There was no difference in the mean flowering date for all cultivars in the early planting. Only two days separated the earliest and latest maturing cultivar. Despite warmer growing temperatures in the later planting, fruit maturity was 3 to 4 days later in all cultivars. However, the accumulated degree-days increased by more than 200 for each cultivar. Fruit maturity was insensitive to very large differences in temperature accumulation regardless of the cultivar. The days from seeding to flowering also remained constant at approximately 50 to 56 days and appeared unrelated to increasing degree-days. The air and soil temperature were highly correlated with an R^2 of 0.85. Using soil temperatures to compute accumulated degree-days did not improve the relationship with fruit maturity.

The lack of relationship between cultivar fruit maturity and temperature was unexpected. However, fruit size is generally smaller for early planted melon cultivars. The primary effect of temperature may be on fruit size rather than maturity. Our initial observations also indicate that the distribution of female flowering and fruit set may be responsive to increasing temperatures. The small sampling of cultivars and single-season data set may contribute to the absence of any clear growth relationship to temperature. Additional studies are planned for succeeding years to construct and validate a heat-unit model for predicting melon maturity by cultivar.

Table 1. The time (mean Julian day) and sum of growing degree-days (SGDD) required for maturity of three melon cultivars established on two different dates in the Texas Lower Rio Grande Valley.

Planting Date	Cultivar	Flowering	Harvest	SGDD (^{0}C)	Maturity (days)
February 2	Laguna	85	123	573	38
(Julian 33)	Easy Rider	85	124	585	39
	Durango	85	125	585	40
March 8	Laguna	117	158	775	41
(Julian 67)	Easy Rider	123	166	831	43
	Durango	123	166	831	43

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Pollen Competition in Melon and Effects on Seed and Fruit Vigor

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Recent research in *Cucurbita* has indicated that pollen competition can improve seed quality (1). In the course of a breeding program to develop bush melons using the *si-1* (short internode) gene (5), I encountered difficulties in seed germination of some lines, similar to that reported for the compact gene in cucumber (2). I decided to see whether seed germination could be improved by pollen parent choice. Since one of the poorly germinating lines was also white-fleshed, the experiment was designed so that I could also observe the proportion of seed set by each pollen parent, as well as the resulting seed vigor of the respective offspring.

The female parent line chosen was 87C57, an F_4 progeny from a single plant selection in the previous generation and originally derived from a cross of an advanced bush crenshaw breeding line to a commercial crenshaw cultivar. The germination of 87C57 was 22%, and the line was homozygous for *si-1* and *wf* (white flesh). Three pollen parents were used: (1) 87C57 for self pollen; (2) 'Charentais Improved', $si\text{-}1^+/si\text{-}1^+$ wf^+/wf^+ and 95% germination (from Stokes Seeds, Buffalo, NY); and (3) 87C54, also an F_4 line in our same breeding program and from the same cross as 87C57, except that a sib selection of the advanced bush crenshaw breeding line had been used. 87C54 also had reduced germination (43%). It was used because it was $si\text{-}1/si\text{-}1$ wf^+/wf^+ and its larger fruit size was expected to expressed in the F_1. 'Charentais Improved' was chosen because its European origin was likely to provide greater genetic differences from the female parent than American $si\text{-}1^+$ wf^+ cultivars.

Following the suggestion of den Nijs (4), pollination partitioning was acomplished by treating each of the three stigmatic lobes per flower of the female parent differently. All pollinations were done in the field. Female flowers were emasculated in the bud stage on the day previous to pollinations. Pollinations were accomplished by peeling off the perianth of a male flower and using the remaining portion as a brush to apply pollen, transferring as much as possible. Female flowers were protected before and after pollination with gelatin capsules, and benzyladenine was applied after pollination (3).

Three randomly selected plants each of 'Charentais Improved' and 87C54 were chosen at the start of the experiment and used throughout. Each pollination with either of these 2 parents involved the use of 3 freshly opened flowers, 1 from each of the 3 selected plants. The pollen from all 3 flowers was transferred to 1 stigmatic lobe. For the self pollinations, 3 freshly opened male flowers from the specific female parental plant were used per stigmatic lobe. In the case of mixed pollinations, therefore, each female flower received the pollen from 9 flowers.

At the start of the experiment, 87C57 comprised about 30 plants, and the reduced vigor of this family meant that plants could produce only 1-2 fruits each. Therefore, plants were randomly chosen to be used for each of the 5 pollination schemes indicated in Table 1, and 2 fruits were set on each plant, both of the same pollination scheme. Four plants were used for each scheme, but F_1 and F_2 generations were only grown from those plants having sufficient viable seed to conduct the experiment, since some of the plants set almost no seed or the seeds were completely inviable.

The F_1 seeds were planted in a greenhouse maintained at a minimal night temperature of 21C and later transplanted to the field. One typical open-pollinated fruit per plant was weighed, and all seeds were saved for weighing and germination tests. F_2 germination tests were conducted in a growth chamber set to a constant 32.2C and 16 hr daylength. Nearly all seeds germinated in 1 week and none after 2 weeks.

Segregation data for the progeny from mixed pollinations are given in Table 2. 57-20-1 and 57-20-2 are different fruits on the same plant; a contingency test indicated that they were segregating in the same ratio (p=0.3), so the results were pooled and tested against those for plant 26. The contingency test here gave p, so pollen transmission rate differed significantly in these 2 female parents, with pollen of genotype *si-1* wf^+ being more frequently effective on plant 20, whereas *si-1*$^+$ wf^+ pollen was more effective on plant 26. Although these data are limited, because only mature plant characters were used, the results indicate unequal gametic transmission for the genes concerned and that maternal parent genotype, even of full sibs within a line, altered transmission frequency. The similarity of segregation ratios for fruits 1 and 2 of plant 20 suggests that these are real differences and not merely the result of small sample size.

As shown in Table 1, pollen genotype influenced offspring vigor, as measured by seed germination and fruit weight. Data for F_1 fruit weight are averages for 5 randomly selelcted plants of that phenotype, 1 fruit per plant. The F_2 germination data are averages for 1 sample of 100 seeds per fruit for each of the 5 plants.

As expected, the larger fruit size of the *si-1* wf^+ parent was reflected in the larger fruits produced by F_1 plants of that phenotype in the mixed pollinations, as well as by the control in which that genotype was the only pollen parent, in comparison to the female parent selfed, either in the mixed pollinations of the 2 selfed controls. Total seed weights for the parental and F_1 generations were also obtained, but differences were not as pronounced as for fruit weight. For example, mean seed weight for the F_1 from mixed pollinations was 14.32 gm for *si-1* wf^+ pollen and 11.68 gm for selfed pollen.

When I examined seed germination, however, some of the results were unexpected. For the control in which *si-1*$^+$ wf^+ was the pollen parent, the mean F_2 germination was 85.5%, whereas for the mixed pollination offspring from the same parent, the mean was only 53.0% (Table 1). The higher F_2 values for plant 27 do not seem to be due to plant 27 itself, because its own seed germinated so poorly (F_1 mean = 20.0%). Consequently, the effect here appears to be due to the 'Charentais Improved' pollen parent, although why this effect was not similarly expressed in the offspring of the same phenotype in the mixed pollinations is not known.

Insufficient seed production did not permit testing of these results with other plants besides 27, nor do I know whether this effect would carry over to the F_3 *si-1* segregants from the crosses of 87C57-27 x 'Charentais Improved'. These results indicate that mixed pollinations by the present method using the genes tested here do not enhance offspring vigor in *si-1* melons, as measured by improved seed germination.

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Table 1. Seed germinations percentage and fruit weight values (gms) for F_1 and F_2 generations for mixed pollinations and controls.

♀ Parent (*si-1 wf*)			Pollen genotype: *si-1*⁺ *wf*⁺		*si-1 wf*⁺		*si-1 wf*	
Plant no.	Fruit weight[z]	F_1 Germination	F_1 Fruit weight	F_2 Germination	F_1 Fruit weight	F_2 Germination	F_1 Fruit weight	F_2 Germination
Mixed pollinations (♂♂: *si-1*⁺ *wf*⁺-*1* lobe, *si-1 wf*⁺-*1* lobe, *si-1 wf* = ⊛-*1* lobe)								
57-20-1	1878	38	1914	50	3456	57	1533	54
57-20-2	2028	20	1706	50	4264	61	1923	58
57-26-1	2531	55	1651	59	2676	65	1987	56
Mean	2146	37.7	1757	53.0	3465	61.0	1814	56.0
♂ *si-1*⁺ *wf*⁺ (1 lobe)								
57-27-1	2268	16	1460	85				
57-27-2	1692	24	1352	86				
Mean	1980	20.0	1411	85.5				
♂ *si-1 wf*⁺ (1 lobe)								
57-21-2	1570	5			3112	50		
57-23-2	1765	6			3583	48		
Mean	1668	5.5			3348	49.0		
♂ ⊛ (1 lobe)								
57-7-1	2041	30					1932	37
57-19-2	1275	19					1451	73
Mean	1658	24.5					1692	55.0
♂ ⊛ (3 lobes)								
57-24-1	1510	11					1642	44
57-24-2	1882	16					1715	65
Mean	1696	13.5					1678	54.5

[z]Mean fruit weights for male parents: *si-1*⁺ *wf*⁺, 2268 gm; *si-1 wf*⁺, 4128 gm.

Table 2. Phenotypes of F_1 progeny from mixed pollination and their frequencies.

	F_1 phenotype (= P_2 pollen genotype)					
	si-1⁺	*wf*⁺	*si-1*	*wf*⁺	*si-1*	*wf*
Parent	No.	%	No.	%	No.	%
57-20-1	19	22	50	57	19	22
57-20-2	28	25	69	62	15	13
57-26-1	52	48	37	34	19	18
Expected		33		33		33

Techniques to Overcome Barrier of Interspecific Hybridization in *Cucumis*

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Our efforts to achieve the interspecific hybrid in *Cucumis* species through conventional breeding procedure were unsuccessful because of the existence of a pre-fertilization barrier. The barrier was characterized by non-germination of pollen even up to 72 h after pollination. To overcome this problem, techniques like bud pollination, use of mentor pollen, brush pollination, growth regulator application, and treatment of stigma with organic solvent, suggested in other crop plants and in *Cucumis* were employed to find out their potential to overcome the barriers of interspecific hybridization.

Studies on receptivity of bud of *C. figarei* indicated that the stigma of *C. figarei* is receptive 24 h before the anthesis. Hence *C. figarei* x *C. melo* (M4), *C. zeyheri* x *C. melo* (M4), *C. meeusii* x *C. melo* (M4), *C. meeusii* x *C. melo* (M4) and their reciprocal crosses were attempted at bud stage (24 h prior to anthesis). However, this technique was ineffective in overcoming the barriers. Brush pollination (scratching of stigma before pollination) was also ineffective. On the other hand, techniques like mentor pollination as suggested by Oost and den Nijs, 1979 in *Cucumis*, application of growth regulator, and treatment of stigma with organic solvent proved to be useful to obtain fruit set in interspecific crosses. Mentor pollen was prepared by exposing the *C. figarei* pollen for 5-6 h in sunlight and then storing them for 24 h at room temperature. The dried anthers were then put on a filter paper and the mass of pollen was broken up with spatula, after which these were transferred into a glass vial. Pollinations of pre-bagged flowers were done with (a) compatible pollen (self), (b) mentor pollen only, and (c) mentor pollen + compatible pollen (*C. melo* [PM]). The viable pollen of *C. melo* was mixed with mentor pollen in approximate proportions of 2 parts mentor to 1 part viable. When *C. figarei* was pollinated with compatible pollen of *C. figarei*, 30% fruit set was obtained, when *C. figarei* was pollinated with mentor pollen only, no fruit set was obtained thereby indicating that the mentor pollen was completely non-viable. When *C. figarei* was pollinated with mentor pollen + compatible pollen (PM) i.e., *C. figarei* x (*C. figarei** + *C. melo* [PM]), 8% fruit set was obtained (Table 1).

Application of benzyl adenine (1%) to the base of ovaries after pollination also proved to be successful in securing the cross *C. figarei* x *C. melo* (PM). BA (1%) in lanolin-water paste (7:3 w/v) was prepared and applied to the base of ovaries with a disposable tuberculin syringe (minus needle) based on the method described by Loy (1982). Four per cent fruit set was obtained in this cross, the fruit was small in size and the pedicel was thick at the point of its attachment. The fruit was harvested 40 days after pollination and it contained 85 seeds. In the reciprocal cross, BA application resulted in fruit set, but the fruits were abnormal in shape and they collapsed after 10 days of pollination. At this stage, the fruits were devoid of seed showing the parthenocarpic development of these fruits. In the cross *C. meeusii* x *C. melo* (PM), three fruits were set when BA was applied at the base of the ovary prior to pollination. However, only one fruit reached maturity. It was completely normal with respect to fruit shape and size. It was harvested after 40 days

and it contained 10 seeds. Six seeds were abnormal in shape, twisted and were empty and the remaining four seeds were normally developed and well filled. The other two fruits collapsed after 12 days of pollination and no seeds were formed in them indicating that they were developed parthenocarpically. In the reciprocal cross, no fruit set was obtained, probably due to the presence of unilateral incompatibility.

In the cross *C. figarei* x *C. melo* (M4) 2% fruit set was obtained when pollination was done after washing the stigma with organic solvent (n-hexane) as per the method described by Willing and Pryor (1976) in Populus.

It is inferred that it is possible to obtain interspecific hybrid(s) by employing techniques like mentor pollination, growth regulator (BA) and use of organic solvent (n-hexane) in the crosses mentioned above. However, confirmation that these actually were interspecific hybrids is needed.

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Table 1. Per cent fruit set obtained in interspecific cross in *Cucumis* through various techniques.

Experiments	Pollen source[z]	No. of flowers pollinated	No. of fruits obtained	% Fruit set	No. of fruits attaining maturity	Average no. of seeds per fruit
A. MENTOR POLLEN						
Crosses:						
C. figarei	S.P.	10	3	30	3	400
C. figarei	M.P.	25	0	0	0	0
C. figarei x (*C. figarei** + *C. melo* (PM)	M.P.+ C.P.	25	2	8	2	220
B. GROWTH REGULATOR (BA 1%)[y]						
Crosses:						
C. figarei x *C. melo* (PM)	-	25	1	4	1	85
C. melo (PM) x *C. figarei*	-	50	3	6	0[x]	0
C. meeusii x *C. melo* (PM)	-	25	3	12	1[w]	10
C. ORGANIC SOLVENT (n-hexane)[y]						
Crosses:						
C. figarei x *C. melo* (PM)	-	50	4	8	4	250
C. figarei x *C. melo* (M4)	-	100	2	2	2	200

[z]S.P.: Self pollen, M.P.: Mentor pollen, M.P. + C.P.: Mentor pollen + compatible pollen (*C. melo* [PM]), *C. figarei**: Pollen made non-viable by storing for 24 h (M.P.).

[y]PM: Pusa Madhuras, M4: Monoecious 4.

[x]A: 6 per cent fruits were obtained, however, all of them developed parthenocarpically for up to 10 days after pollination and then abscised.

[w]B: Remaining 2 fruits developed parthenocarpically for up to 12 days and then collapsed.

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Interspecific Hybridization in *Cucumis* spp.

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Research work conducted in this Institute revealed that high level resistance to cucumber green mottle mosaic virus (CGMMV) is not available in cultivated forms of melon (*Cucumis melo* L.). Nevertheless, five wild *Cucumis* species viz, *C. figarei, C. zeyheri, C. meeusii, C. myriocarpus* and *C. africanus* were identified as immune to CGMMV (4,5). Besides, in the first four species, a complete resistance to Fusarium wilt caused by *Fusarium oxysporum* sp. *melonis* was confirmed from the field as well as artificial inoculation screenings (Thomas and More, 1990). Hence, attempts were made to cross these wild *Cucumis* species with cultivated forms of *C. melo*.

Sixteen interspecific crosses were made by crossing four wild species with two cultivated forms of melon (Pusa Madhuras and Monoecious-4) and their eight reciprocal crosses were attempted under field conditions by conventional hybridization procedure. Fruit set was not obtained in any of the crosses (Table 1) nor was it obtained in their reciprocal crosses. This indicated the presence of strong barrier to interspecific hybridization of *Cucumis*.

In order to determine the exact nature of barrier, all the 16 crosses were subjected to fluorescence microscopic study, based on method described by Kho and Baer (1968) and modified by Tomer and Gottreich (1975). Cross-pollinated stigmas were collected 24, 48 and 72 h after pollination and immediately fixed in 3:1 AA (absolute alcohol 3, acetic acid 1). After 24 h in fixative the stigmas were washed in water and sectioned free hand longitudinally. Each section was softened in 8N NaOH for 1 h, washed with water and stained with 0.005 per cent aniline blue dissolved in 0.05M Na_2HPO_4 (Sodium-bi-phosphate, pH 8.2 containing 20% glycerol). The stained tissues were then placed on a slide in glycerine and gently squashed by applying pressure on the cover slip. Observations were made on Nikon microphot equipped with a 200W high pressure mercury lamp illuminated at 380-490 nm by use of transmission filter BA 530 in the oculars. Selfed stigmas in each case were also observed as control. In crosses, *C. figarei* x *C. melo* (M4), *C. meeusii* x *C. melo* (M4), and *C. zeyheri* x *C. melo* (M4) and their reciprocals the pollen grains were bound on the stigma without any change even 72 h after pollination. Not a single pollen grain germinated in any of these crosses. As expected, in the selfings of *C. figarei, C. meeusii* and *C. zeyheri* several pollen grains had germinated and pollen tubes reached to the ovule.

Inability of pollen of *C. melo* to germinate on the stigmas of *C. figarei, C. meeusii* and *C. zeyheri* and the pollen of these species on *C. melo* stigmas, even up to 72 h after pollination, indicated that a pre-fertilization barrier is involved in the failure of interspecific hybridization in *Cucumis*. Interestingly, the present results provided evidence for the existence of a strong barrier at very first stage of interspecific hybridization (i.e., non-pollen germination). Similar observations were made for *C. metuliferus, C. ficifolius, C. prophetarum, C. zeyheri* and *C. myriocarpus* interspecific crosses with *C. melo* (1). In most of the earlier reports on this aspect, the pollen germinated and the failure occurred only after the post-pollen

germination stage. Only in *C. melo* x *C. metuliferus* cross was there evidence of a post-fertilization abortion (3). Based on these results, some advanced biotechnological tools such as somatic hybridization might be useful in solving the problem of interspecific hybridization in *Cucumis*.

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Table 1. Fruit set in interspecific crosses.

	Cross	No. of flowers pollinated	No. of fruits obtained
1.	*C. figarei* x *C.melo* (PM)	50	0
2.	*C. melo* (PM) x *C. figarei*	100	0
3.	*C. zeyheri* x *C. melo* (PM)	50	0
4.	*C. melo* (PM) x *C. zeyheri*	100	0
5.	*C. myriocarpus* x *C. melo* (PM)	50	0
6.	*C. melo* (PM) x *C. myriocarpus*	100	0
7.	*C. meeusii* x *C. melo* (PM)	50	0
8.	*C. melo* (PM) x *C. meeusii*	100	0
9.	*C. figarei* x *C. melo* (M4)	50	0
10.	*C. melo* (M4) x *C. figarei*	100	0
11.	*C. zeyheri* x *C. melo* (M4)	50	0
12.	*C. melo* (M4) x *C. zeyheri*	100	0
13.	*C. myriocarpus* x *C. melo* (M4)	50	0
14.	*C. melo* (M4) x *C. myriocarpus*	100	0
15.	*C. meeusii* x *C. melo* (M4)	50	0
16.	*C. melo* (M4) x *C. meeusii*	100	0
	Total	1200	0

Organogenic and Embryogenic Potential of Several Commercial Lines of *Cucumis melo* L.

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Introduction. Availability of efficient methods for plant regeneration of melon is required to take advantage of biotechnological alternatives for crop improvement. A reliable protocol for regeneration from primary explants can be useful in somaclonal variation as a way for producing new genetic variability. Furthermore, successful plant regeneration could permit the introduction of desirable traits by the "leaf disc transformation" procedure.

Nowadays, regeneration in tissue culture of *Cucumis melo* from several sources of explants has been successfully achieved (1,3,4,5,6,7,8,9). However, the frequency of regeneration appears to be genotype dependent (10). Therefore, some study of the morphogenic response of the agronomically important lines or varieties is needed if we aim towards the improvement of this important crop by new technologies.

The purpose of the present study is the selection of suitable genotypes for somaclonal variation programs and genetic transformation studies by screening commercial lines of melon.

Materials and Methods. The genotypes of *Cucumis melo* L. tested were lines of 'Pinyonet Piel de Sapo', 'Amarillo Canario' and 'Amarillo Golda' (kindly provided by G. Anastasio, Petoseed Ibérica S.A.). Seeds were surface-sterilized and explants were excised from cotyledons and hypocotyls of 9 day-old seedlings and from leaves of axenic plants as described previously (7,8). The only difference was that cotyledonary explants were prepared by cutting in three segments instead of two. For each explant source, six explants were placed into 250-ml jars containing 40 ml of medium and, in all cases, at least 12 replicate jars were included. Incubation conditions were identical to those previously used in other studies (7,8).

The organogenic response of cotyledonary and leaf explants was determined when cultured on IK 15/60 medium, consisting of MB3 basal medium (8) + 1.5 mg/l indole-3-acetic acid (IAA) + 6.0 mg/l kinetin (K). Recently, the efficiency of this protocol has been significantly enhanced (4). In spite of this, the present study was focused to evaluate the morphogenetic capacity of lines, cultivars or varieties, and with this idea we considered IK 15/60 medium more suitable in order to reveal the genotype effect. Organogenic response was rated after 30 days of culturing. Explants were scored for growth (fresh weight), shoot-bud and shoot formation (both frequency and index) (see Tables).

Embryogenic response was tested using hypocotyl explants cultured on NP 25/25 medium (MB3 + 2.5 mg/l naphthalenacetic acid NAA + 2.5 mg/l 2- isopentyl adenine 2-iP) in order to obtain unorganized calli. After 25 days incubation in the dark, calli were subcultured onto MB3 medium without growth regulators. Once calli showed embryoid-like structures, they were transferred to low intensity light conditions and after an additional 3 days were incubated under the standard intensity light (8). Embryogenic response was rated 25 days after culturing in MB3. Calli were scored for growth and embryoid-like structures formation.

Results and Discussion. The organogenic response of the primary calli from cotyledonary and leaf explants is outlined in Tables 1 and 2.

When cotyledonary explants were used, the highest overall organogenic response was obtained with 'Pinyonet Piel de Sapo'. It should be noted that the response of this line was similar to or slightly higher than that obtained with the standard line of 'Cantaloup Charentais' (C.Ch.) used in our previous studies. Although the frequency of shoot-bud formation of C.Ch. in the same medium was 76.8%, this result was obtained when cotyledons were excised in two segments. Later studies have confirmed that organogenic response decreases significantly when smaller explants are used (68% in C.Ch., see 4).

Table 1. Growth and organogenic response in cotyledonary explants on IK 15/60 medium.

	P. PIEL SAPO	A. CANARIO	A. GOLDA
Growth[z]	2.29 ± 0.07	1.34 ± 0.06	1.39 ± 0.07
Shoot-buds (%)[y]	77.08 ± 3.50	52.78 ± 5.88	41.67 ± 5.81
Shoots (%)[y]	37.50 ± 4.03	44.44 ± 5.86	30.56 ± 5.43
Organogenic Index[x]	1.39 ± 0.06	0.89 ± 0.09	0.74 ± 0.08
Shoots Index[w]	0.66 ± 0.09	0.94 ± 0.16	0.65 ± 0.15

Table 2. Growth and organogenic response in leaf explants on IK 15/60 medium.

	P. PIEL SAPO	A. CANARIO	A. GOLDA
Growth[z]	2.35 ± 0.08	1.86 ± 0.06	1.68 ± 0.05
Shoot-buds (%)[y]	16.66 ± 4.39	63.89 ± 5.66	8.33 ± 3.26
Shoots (%)[y]	4.16 ± 2.35	41.67 ± 5.81	4.17 ± 2.36
Organogenic Index[x]	0.51 ± 0.05	0.95 ± 0.08	0.18 ± 0.04
Shoots Index[w]	0.05 ± 0.03	0.87 ± 0.16	0.04 ± 0.02

[z]Fresh weight (g) expressed as mean ± 1 SE.
[y]Frequencies of calli with shoot-buds or developed shoots $\sqrt{p(1-p)/n}$(%), p= number of calli with response/total number of calli (n).
[x]Organogenic Index = weighted average obtained from arbitrary values (0 to 3) corresponding to the organogenic response level shown in each callus ± 1 SE.
[w]Shoots Index = average number of shoots per callus ± 1 SE.

The results obtained when leaves were used as an explant source indicates that cv. Amarillo canario showed the highest shoot-bud and shoot formation at a frequency of 63.89% and 41.67%, respectively. Interestingly, the highest regenerating capacity from leaf explants was obtained with the above mentioned genotype among all lines screened for response in tissue culture. Colijn-Hooymans et al. (2) found in cucumber that the number of endopolyploid cells pre-existing is larger in cotyledonary than in leaf tissues. If this is the case in melon, the explant source will be very important depending on the purpose of the research. Leaves especially could be a useful tissue source for applying "leaf disc transformation."

Somatic embryogenesis response was very high in all cases. The frequency of calli which showed embryoid-like structures, after 10-15 days of culturing, were 78.47%, 83.33% and 75.00% in 'Pinyonet Piel de Sapo', 'Amarillo canario' and 'Amarillo Golda', respectively. After 30 days, these values increased up to 98.88%, 98.61% and 88.70%. However, embryoids usually showed abnormal development. Consequently, results indicate that these lines require further optimization for efficient regeneration by way of somatic embryogenesis.

In conclusion, it has been confirmed that the regenerating protocol established can be applied in all genotypes tested in order to obtain calli- derived plants in a somaclonal selection program. In addition, it has been proved that cultivars tested in the present study will be suitable for experiments on the production of transgenic plants using the technology of *Agrobacterium*-mediated gene transfer by "leaf disc transformation. "

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Preliminary Studies on the Growth and Organogenic Response of Cotyledon- derived Calli of *Cucumis myriocarpus* Naud

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Introduction. *Cucumis myriocarpus* is a cucurbitaceous wild species in which resistances to *Tetranychus urticae*, CGMMV, powdery mildew (PM1), and yellowing disease have been reported (1, 2, 3, 4, 5). It is, therefore, an ideal partner for use in experiments of somatic hybridization with cultivated species of *Cucumis* with the aim of introgressing those resistance carrying genes into their genetic context. In order to approach these hybridizations efficiently the availability of good selection methods for the hybrid cells after fusion of protoplasts would be desirable. *Agrobacterium*-mediated transformation offers a rapid and efficient method for the introduction of antibiotic-resistance genes carried in engineered Ti plasmids into the plants regenerated from callus grown after co-cultivation of primary explants with the bacteria. These transgenic plants are particularly useful biochemical markers for protoplast fusion experiments, so it is very important to develop efficient and reproducible methods for plant regeneration at high rates from primary callus of this wild species.

A considerable handicap in undertaking studies on morphogenesis in explant- derived callus with several interesting accessions of this wild species is related to their very low rate of in vitro seed germination under the photoperiod conditions normally used by us with other cucurbitaceous species (6,7). Studies carried out in our laboratory in order to improve the germination rate have indicated that short periods of seed incubation under darkness and higher temperature dramatically increase the percentage of germination.

In this paper, the results of this study and the preliminary results of a series of studies aimed at establishing the best cultural conditions leading to the attainment of plant regeneration from explant-derived callus of this wild species, are described.

Material & Methods. Seeds of *Cucumis myriocarpus* L-2 (accession CuC 31/1983, kindly supplied by Dr. Chr. Lehmann, Zentralinstitut fur Genetik und Kulturpflanzen-forschung, Gatersleben, DDR), were decoated, surface sterilized by immersion in 30% commercial bleach (50 g/l active chlorine) for 30 min, followed by three rinses with sterile distilled water and germinated on MG medium (6), either under 16 h photoperiod (1300 lux, Gro-lux, Sylvania) at 27 ± 2^{o}C day/24 ± 2^{o}C night or under continuous darkness at 32^{o}C.

Explant sources for the experiments on morphogenesis were cotyledons taken off from seedlings at the stage of first true leaf apparition (5-7-days- old). This stage was accomplished under four different conditions: seed germination and growth of seedling always under photoperiod (D-1 seedling treatment condition), seed germination in darkness until plumule apparition and transfer to photoperiod for seedling growth (D-2 condition), seed germination and growth until hypocotyl development around 1 cm length in the dark and the remaining growth under photoperiod (D-3 condition), and seed germination and growth in continuous darkness (D-4 condition).

Cotyledon explants were obtained as previously described for melon (7) and placed onto a solid basal medium consisting of M&S salts (9), 2% sucrose, 100 mg/l myo-inositol, 1 mg/l thiamine-HCl, RT vitamins (7) and 0.8% agar (Technical No.3, Oxoid), with 0.1 mg/ indole-3-acetic acid (IAA) and four levels of 6-benzylaminopurine (6BA): 0.1, 0.5, 1.0 and 5.0 mg/l (henceforth denominated as NB 01/01, NB 01/05, NB 01/10 and NB 01/50, respectively). The incubation was carried out for 21 days under the same photoperiod conditions described for germination. Variables scored were the growth of callus (expressed as growth Index = weighted average of all the calli per treatment, calculated by assigning an arbitrary value, ranging from 0 to 4, to each callus growth estimated qualitatively: 0=no growth, 1=little growth, 2=middle growth, 3=great growth, and 4=enormous growth), and the organogenic response of the calli (expressed as percentage of calli giving shoot-buds).

Results:

1. Seed germination. As can be seen in Figure 1, seeds incubated under photoperiod conditions present a germination rate much lower than those incubated under darkness and the time for germination is even longer. Thus, under photoperiod, germination does not occur until the 4th day of incubation and the maximum rate (41%) is reached after 6 days in culture, whereas under darkness at 32°C a germination of 90% at the 3rd day and almost 100% after 4 days in culture can be obtained. Therefore, a simple change in the incubation conditions makes the quick attainment of high rates of germination feasible and also the assessment of the necessary amount of explant sources for further in vitro experiments.

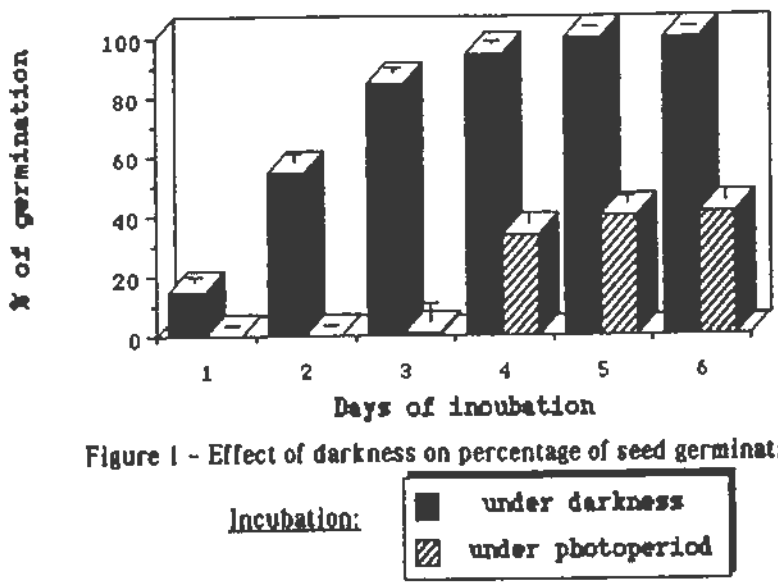

Figure 1 - Effect of darkness on percentage of seed germination.

2.- Morphogenesis in vitro. Once the problem of germination was solved and to study their morphogenetic response in vitro, the cotyledon halves of seedlings grown under the four different conditions (D1-D4) were used as explants and put in the four culture media (NB 01/01, NB 01/05, NB 01/10 and NB 01/50). The results of this study are shown in Figure 2.

Concentration of 6BA in the culture media greatly affects the growth of calli (see figure 2A): callus fresh weight increases as the level of 6BA increases. This effect seems to be independent of any treatment given to the seedlings, so seed germination under darkness can be used without significant detrimental effect on the callus growth given that the appropriate culture medium is selected.

The culture media also affects the organogenic response of the calli (Figure 2B), but in a different manner: with the lower level of 6BA (0.1 mg/l) there is no response at all, independently of seedling treatment, but the response at higher levels of 6BA does depend of the incubation conditions of the seedlings. Thus, when seedlings were germinated and grown under photoperiod, the 6BA concentration did not affect the regeneration rate (50-60% of the calli in all the cases), while when seeds were germinated under darkness, 6BA levels did affect the results, being 1.0 mg/l (medium NB 01/10) the best of those studied, giving rise to frequencies of 40-70% of calli forming

shoot-buds, similar to those obtained under photoperiod. This means that by choosing this culture medium, one can use seeds germinated in darkness (at whatever time they were incubated) without decreasing the organogenic response of their explant-derived callus and ensuring the highest germination rate.

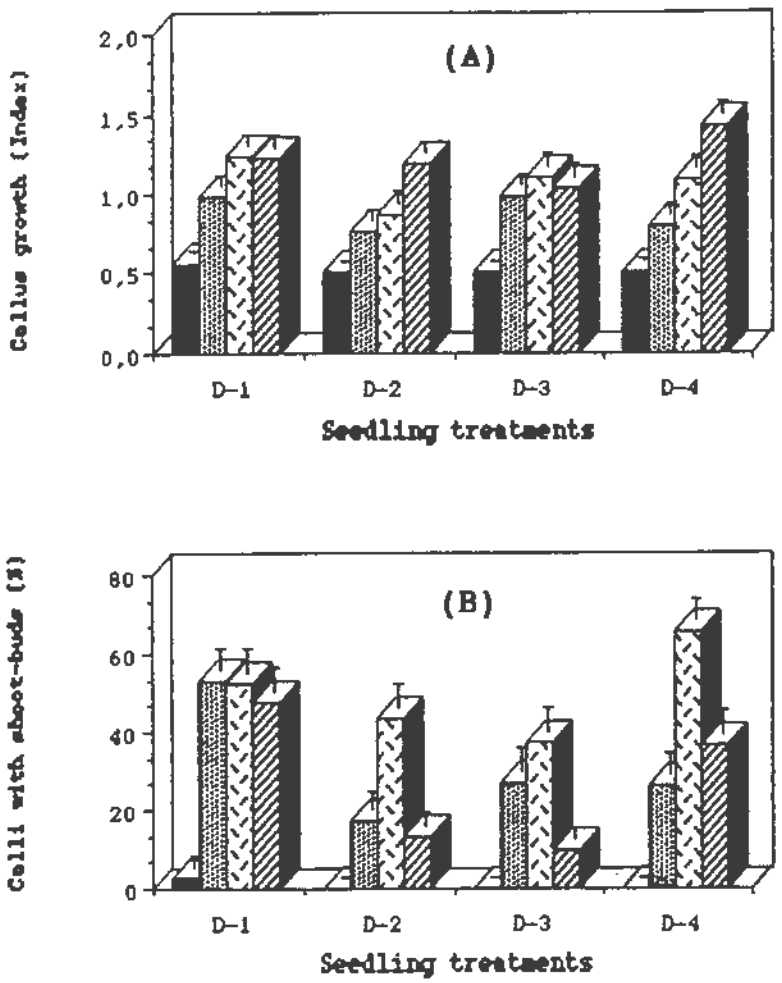

Figure 2: Effect on callus growth (A) and organogenic response as percentage of calli with shoot-buds (B).

In conclusion, both a method for overcoming the in vitro germination problems and the cultural conditions for the attainment of plant regeneration from cotyledon-derived calli of this wild species at high rates, have been achieved. Experiments aimed at obtaining biochemical markers through *Agrobacterium*-mediated transformation by applying these results, are in progress.

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Plant Regeneration of Protoplasts from Different Cultivars of Melon (*Cucumis melo*)

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Introduction

Successful plant regeneration from explants of melon have been reported by a number of authors (for review see 2). Publications on plant regeneration from protoplasts still remain scarce and the genotypes used are limited (1,3,4,5). In the publications by Debeaujon and Branchard (3) and by Moreno et al. (5), plants of 'Cantaloupe charentais'-type were used as starting material, while Li et al. (4) used a Chinese cultivar 'Xinjang'. Here we present a method by which normal plants were obtained by organogenesis from protoplasts of different genotypes.

Materials and Methods

Nine different genotypes were tested in total, of which 4 commercial varieties and 5 parental lines. The cotyledons of 7-days-old-in vitro plants were used for isolation. The cotyledons were placed for a few days (2-3 d) in dark at room temperature, on a M/S medium supplemented with 1.0 mg/l NAA and 1.0 mg/l BAP, prior to isolation. Isolation was carried out overnight (16 h) at 28°C in an enzyme solution (0.8%[w/v] Cellulase RS Onozuka, 0.4%[w/v] Macerozyme R10 Onozuka, 0.1M glycine, and 0.4M mannitol in ¼ strength MS salt solution). Isolated protoplasts were suspended at a density of 5 x 10^4 protoplasts/ml in a modified B5 medium consisting of B5 salts, KM8p vitamines, 0.25 M mannitol, 0.17 M glucose and 0.034 M sucrose [550 mosm], 0.2 mg/l 2,4-D, 1.0 mg/l NAA and 0.5 mg/l BAP. After 3 weeks, fresh culture medium without mannitol was added. At this time, Seaplaque agarose was added to give a final concentration of 0.2%[w/v]. After another 3-4 weeks, minicalli appeared and could be transferred to a shoot-initiation medium. For five of the cultivars, several different hormone combinations were tested. Four different cytokinins were tested at a range of concentrations: KIN (1.0-10.0 mg/l), BAP (0.2-5.0 mg/l), ZEA (0.1-5.0 mg/l) and 2-IP (0.1-0.5 mg/l). The different cytokinins were used alone or in combination with IAA (0.01-2.5 mg/l). For four other cultivars, an addition of 0.5 mg/l ZEA was used as standard. The shoot-initiation phase took about 3 weeks, after which the calli with the induced shoot primordia were transferred to a medium for the development of shoots. Also in this case, different combinations of the following hormones were tested for five of the cultivars: IAA, NAA, BAP, ZEA, and GA3. For the other four tested cultivars, the combination of 0.1 mg/l IAA and 0.2 mg/l BAP was used. At this stage, developing shoot primordia could be dissected out for subculturing on the same medium. Also different gelling agents were used in different concentrations: 1.0%[w/v] of agar (Merck), agarose 13.20 (Duchefa) and Microagar (Duchefa), while Gelrite (Duchefa) was tested in concentrations of 0.2, 0.5, and 1.0%[w/v]. Finally, a medium supplemented with 0.03 mg/l NAA was used for rooting.

Results and Discussion

The highest plating efficiency was obtained when cotyledons were used as explant material. The preculture was also seen to increase the division frequency substantially. After one week in culture medium, the division

frequency varied between 30 to 90% according to genotype. Our best responding cultivar is of 'Charentais' type, also used in the majority of publications of plant regeneration of melon. With this cultivar, 90% of the calli gave rise to shoot primordia on a shoot-inducing medium with 0.5 mg/l ZEA (Fig. 1). This shoot-inducing medium gave the best response for most genotypes. For some genotypes however, an addition of auxin, 0.1 mg/l IAA, gave a better result. For development of shoots, a combination of 0.1 mg/l IAA and 0.2 mg/l BAP gave the best results for the five tested genotypes. With two of the other four genotypes good results were obtained with this medium. The other two gave only shoot primordia which only occasionally developed into shoots. In total, 30 to 90% of the calli gave rise to shoot primordia with this sequence depending on genotype. From each of those calli, 1 to 50 shoots could be obtained. To prevent vitrification of the plants, 1%[w/v] agarose 13.20 was used in the shoot-induction medium, while in the shoot-development medium, 0.5-1.0%[w/v] Gelrite gave the best results. The concentration of Gelrite added depended on the condition of the plants. The plants could then be rooted and transferred to soil (Fig. 2). In total, 6 months were needed for the regeneration of plants from protoplasts.

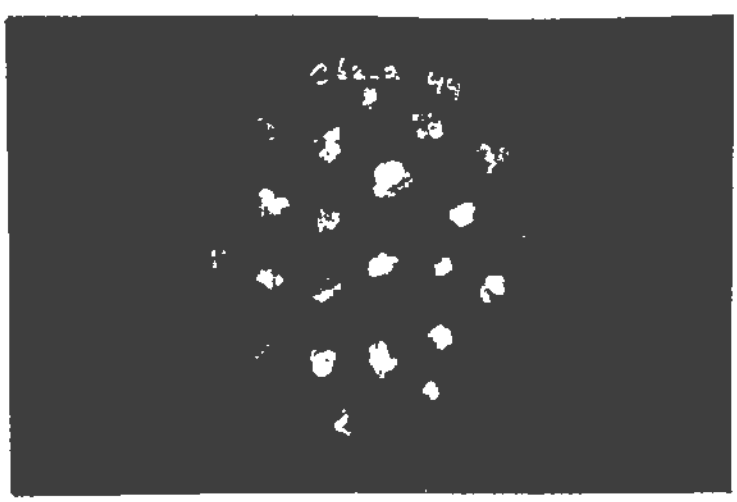

Fig. 1. Shoot primordia obtained with our best cultivar, a 'Cantaloupe charentais' type.

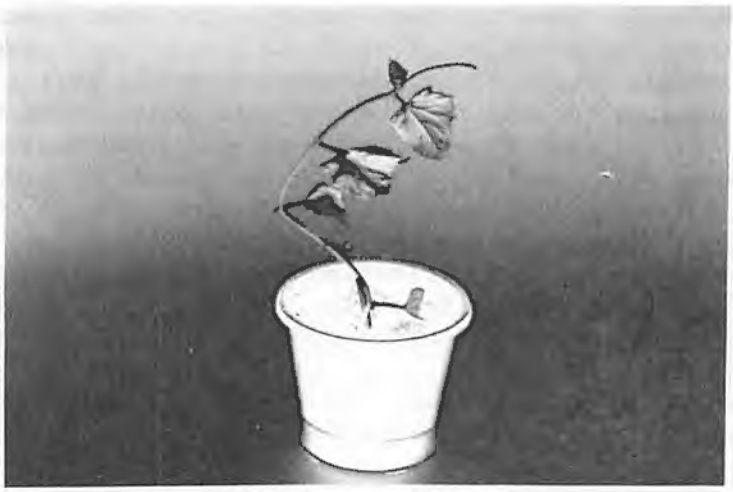

Fig. 2. Potted plant regenerated from protoplasts.

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Selective Methods for the Recovery of Somatic Hybrids of *Cucumis melo* x *C. metuliferus* and *C. sativus* x *C. metuliferus*

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Introduction. Methods for the isolation and culture of *Cucumis metuliferus* protoplasts have been previously reported by our group (18) and by other authors (8,17,20). These studies have given of valid information about the cultural response of protoplasts from this cucurbitaceous wild species and they are relevant works since, in accessions of this species, resistances to root- knot nematode (2), SqMV (16), WMV-1 (16), ZYMV (17), powdery mildew (PM1) (1) and aphids (1), have been described. In spite of the sexual cross between a feral *Cucumis melo* (PI 140471) and a line of *Cucumis metuliferus* (PI 292190) published in 1969 (14), other authors (3,6,7,15) reported the impossibility of reproducing this particular cross, even using the same parental lines. As a consequence, efforts for introgressing those resistance carrying genes into the genetic context of cultivated cucurbitaceous species by conventional sexual crosses have been severely impeded by the existence of incompatibility barriers (1,7). Somatic hybridization by protoplast fusion should allow the attainment of genetic bridges through which the transfer of interesting traits, from the wild species to the cultivated ones like melon or cucumber, should be feasible.

In this paper, the results of isolation and culture of *C. metuliferus* protoplasts and those from a study carried out about their morphogenetic response in sequential culture media that have been designed by our group to regenerate plants from protoplasts of melon (through organogenesis) or of cucumber (trough somatic embryogenesis), are described. These results have provided adequate information about the cultural as well as the morphogenetic capability of protoplast-derived cells from the wild species and they will allow us to design more suitable and efficient selection systems for use in experiments on somatic hybridization.

Materials and Methods. Seeds of *Cucumis metuliferus* (kindly supplied by Dr. Jacobs, Stellenbosch University, South Africa) were surface sterilized and germinated on MG medium as previously described (9,12). Cotyledons from 5 day-old seedlings were used for protoplast isolation after pre-culturing them in C medium (12) during three days. Strips of 1-2 mm width from these explants were placed in 100 ml Erlenmeyer flasks containing 8 ml of a filter sterilized enzyme solution consisting in LG medium (M&S macroelements at 1/4 strength, 0.4 M mannitol, 0.1 M glycine, 1 mM CaCl2·2H2O, 0,51 mM MES) and 1.5% cellulase Onozuka R-10 (Yakult Pharmaceutical Ind. Ltd.) at pH 5.7. The incubation was performed under darkness at 28°C for 12 h in a reciprocal shaker at 100 strokes/min (amplitude 20 mm). After incubation, the protoplasts were filtered through a 85 µm nylon mesh, purified by flotation over F6 medium (0.6 M sucrose, 0.05 M glycine, 0.01 M CaCl2·2H2O at pH 5.7) and rinsed twice in LG medium. After protoplast counting, they were cultured in 35 mm diameter glass Petri dishes containing 2.5 ml of the culture liquid medium at a final concentration of 1×10^5 protoplasts/ml.

Two different media were used for protoplasts culture: DNB 10/05/05 and DIB 10/05/05 (henceforth denominated DNB and DIB). Both of them contained B5 mineral solution (4), 0.058 M sucrose, 0.055 M glucose, 0.55 mM myo-inositol and 0.51 mM MES. Additionally, DNB medium contained 0.03 mM thiamine-HCl, 8.12 5M nicotinic acid, 4,86 5M pyridoxine-HCl, 0.44 M mannitol, 4.52 5M 2,4-D, 2.68 5M NAA and 2,22 5M 6-BA. DIB medium contained Shahin's vitamins (19), 100 mg/l casein hydrolysate, 50 ml/l coconut milk, 0.27 M adenine sulfate, 0.02 M xylitol, 0.36 M mannitol, 4.52 5M 2,4-D, 2.85 5M IAA and 2.22 5M 6-BA. The pH was adjusted to 5.7 in both media before autoclaving. Incubation took place under darkness at 28°C. Four consecutive reductions in the osmolarity (0.50-0.42-0.32-0.25-0.21M) of the culture media were effected by the addition of mannitol-free fresh medium every 7 days.

After 30 days in culture, the microcalli grown in each of two media were subcultured into different media following two different ways: those grown on DNB followed the embryogenic pathway designed for cucumber protoplasts (in preparation) and the ones grown on DIB followed the organogenic pathway established for melon protoplasts (10). Thus, microcalli grown in DNB medium were subcultured in two media consisting in MB basal

medium (M&S salts + 0.09M sucrose + 0.55 mM myo-inositol + 1.71 mM glutamine + 0.27M adenine sulfate + Sh-vitamins) supplemented with 4.52 5M 2,4-D + 4.44 5M 6-BA (DB 10/10 medium) or 13.43 5M NAA + 4.92 5M 2-iP (NP 25/10 medium), and incubation was made under darkness at 28^{o}C, while those proceeding from DIB medium were subcultured onto IB 25/10 medium (5) and further transferred to solid IK 01/60 Cu1 medium and incubated under photoperiod as described for melon protoplasts (10).

A double-layer ("soft-hard") technique was utilized for subculturing the microcalli. It consists of removing the worn-out protoplast culture medium and replacing it with 3 ml of the new liquid medium to be tested. The microcalli are resuspended in this liquid medium and mixed with 3 ml of the same, but agarified (1%), liquefied medium. The resulting mixture (final agar concentration 0.5%) is agitated and rapidly distributed ("soft layer") over the "hard layer" consisting of 20 ml of the same medium solidified with 0.8% agar and previously distributed in 90 mm diameter plastic Petri dishes. Final cell count concentration is 1-2 x 104 minicalli/ml in each replicate. Fifteen replicates were used per treatment.

Results.

1.-Protoplasts isolation and culture. Protoplast yield was 1-2 x 10^{6} protoplasts/g of tissue. After 2 days, cell wall regeneration took place in 80% of the protoplasts in both DNB and DIB culture media. First divisions began on the third day and cell aggregates could be observed on the 7th day in culture in DNB medium, while in DIB medium first divisions started a little later (6 days). Nevertheless in both media the growth is similar and considerable after 30 days in culture: the liquid medium has disappeared and replaced by a continuous mass of microcalli.

2.-Cultural and embryogenic response of *C. metuliferus* p-calli. *C. metuliferus* protoplast-derived microcalli grew slowly in both media DB 01/10 and NP 25/10 and gave rise to small minicalli that originated only one embryogenic callus in all the DB medium replicates and three embryogenic calli when NP medium was used. These calli were subcultured to hormone-free medium to promote the development of proembryos, but they did not grow anymore and became necrosed in 10 days.

On the contrary, media as well as cultural conditions utilized in this experiment allow an intense mitotic activity and the regeneration of plants through somatic embryogenesis in protoplast-derived calli of *Cucumis sativus* (in preparation). As a consequence, this completely different response in both cucurbitaceous species could be efficiently used to perform selective methods for the recovery of somatic hybrids after protoplast fusion between cucumber and *C. metuliferus*. The selective scheme would imply the use of protoplasts from a recessive mutant of cucumber (such as *glabrous* or *yellow-green*) and their fusion with protoplasts from a normal line of *C. metuliferus*. It would be based, therefore, on the complementation between the embryogenic capability of cucumber and the wild phenotype of the wild species. Only the hybrid cells would expect to be able to regenerate normal hairy or green plants, respectively.

3.-Cultural and organogenic response of p-calli. In this case the response of *C. metuliferus* protoplast-derived calli was not positive either. After 21 days in culture in the first sequential medium (IB 25/10), the microcalli became small minicalli and did not grow (or did so very slowly) even when transferred to the IK 01/60 solid medium, becoming necrotic, although occasionally some calli grew a little more and showed small cream-greenish globular structures that did not develop anymore.

This response is, again, completely different to that normally found when protoplasts of *Cucumis melo* are used following the same sequential media and incubation conditions designed by our group (10). Melon protoplasts cultivated in DIB liquid medium regenerate the cell wall and start cell division very quickly; after subculturing the microcalli into IB 25/10 medium they grow fast up to the point of 5-10 mm sized cream-greenish minicalli. The transfer of these minicalli to IK 01/60 solid medium causes the formation of big calli carrying organized growing zones with shoot-buds from which plants can be regenerated.

Again, these results pointed out the possibility of applying the differences found between *C. melo* and *C. metuliferus* to the selection of hybrid cells after their protoplast fusion. The scheme should be the same as that

mentioned above but taking advantage this time of the organogenic response of melon in the sequence of media described: only the hybrid cells would regenerate normal hairy or green plants through shoot formation.

Experiments aimed at the attainment of somatic hybrids between *C. metuliferus* and cucumber or melon lines are in progress.

Acknowledgments: The authors express their appreciation to CICYT (Comision Interministerial de Ciencia y Tecnologa, Ministry of Education and Science, Spanish Government) for financial support (Project BIO89-0446). M. Dabauza is grateful for her Grant from the Autonomous Government of the Valencian Community (Spain).

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Late Male Fertility in a Glabrous, Male-Sterile (*gms*) Watermelon Line

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The glabrous character can be fixed in lines heterozygous for *gms* (2,3). Although Whitaker and Bemis (4) noted the possibility of using late male fertility for maintaining the *gms* line, no further study of this phenomenon has been reported. This study was begun when late male-fertility was observed in a putative tetrasomic line segregating for *gms* that had been cloned in tissue culture from nodal cuttings. This line had previously behaved as a tetrasomic (1).

A sib-pollinated fruit, which received pollen from a hairy, male-fertile sibling, produced 155 seed. Selfs and sibcrosses of glabrous, male-sterile x hairy, male-fertile plants were made from the original sibcross. Lines exhibiting male fertility were designated GMF (glabrous male fertile) and lines segregating only for glabrous, male sterile plants were designated GMS. Germination data were recorded as an index of seed viability in the first two generations.

The F_1 seedlings from the first fruit segregated 1:1 hairy:glabrous (Table 1). Previous crosses had indicated that the genotypes in the parent line were *gms gms gms gms* and + *gms gms gms* and the F_1 genotypes were expected to be the same. Hairy plants from the F_1 population were selfed and glabrous plants from this population were sibbed to produce the F_2 populations. The hairy plants (+ *gms gms gms*), when selfed, were expected to produce populations segregating 3:1 hairy:glabrous, but a ratio of 8:1 was obtained. Germination rates of 70 and 74% were less than the above-90% germinations obtained before with seed from the line. Similarly, two out of 5 F_2 populations from sib crosses fit a 2:1 ratio instead of the expected 1:1 hairy: glabrous ratio, and germination rates were less than normal in 4 out of 5 of these F_2 populations. Ratios of 8:1 from the selfed hairy F_1 plants and 2:1 for the sibbed F_1 plants are genetic ratios consistent with that of trisomics if only n+1 gametes are functional. The reduced germination percentages are also consistent with aneuploidy. The original tetrasomic may have lost the extra pair of chromosomes in tissue culture.

The small F_3 populations (Table 1) were from selfs of hairy plants, one from a sibbed F_2 and one from a selfed F_2 plant. The F_4 populations were from selfs and sibs of F_3 plants. Late male fertility was observed again in the F_2 and F_5 populations grown at the same time in the greenhouse. GMF.1 was the glabrous, male-fertile line which arose from the F_3 seed obtained from selfing a glabrous F_2 plant from the sibcross (19x18); GMF.2 from the F_3 seed obtained from selfing a glabrous F_2 plant from the sibcross (25x28). GMF.3 and GMF.4 were from the F_4 seed obtained from selfing the same F_3 plant from the sibcross (4x1) twice at a single node. A line segregating for *gms*, designated GMS.1, which did not exhibit late male fertility, was maintained by repeated sibbing from an F_3 line arising from the original cross. Another glabrous, male-sterile line, designated GMS.2, was not a descendant line of the original late male-fertile plant. GMS.2 was maintained by sibbing and used in crosses with the late male-fertile lines.

Descriptions of the original four GMF fruit and the two GMS fruit are summarized in Table 2. All progeny of the GMF selfs were glabrous, but only a portion of GMF.2, 3 and 4 were male fertile Pollen fertility paralled seed set in the three populations observed (Table 3).

Functional male flowers on the GMF lines were three weeks later than male flowers on the hairy plants from the GMS lines (Table 2).

Sterile, non-glabrous plants were not found, as expected if recombination had occurred between two closely linked genes. Rather, a more apt description of late male fertility appears to be a partial restoration of fertility in some glabrous plants. Glabrous male fertile plants, when selfed, produced all glabrous progeny, more of them male sterile than male fertile. Glabrousness behaves strictly as a recessive character, but male sterility does not.

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Table 1. Segregating populations of hairy and glabrous watermelon plants from an original cross between a glabrous, male-fertile plant x a hairy, male-sterile tetrasomic plant and the origins of four glabrous, male-fertile and one glabrous, male-sterile line.

ID	% Germination	Hairy:Glabrous	Ratio	X^2	p
F_1	88	19:23	1:1	0.214	0.50-0.70
Hairy F_1 selfed: (F_2):					
1	70	54:6	8:1	0.0009	>0.95
18	74	32:5	8:1	0.041	0.50-0.75
Glabrous x Hairy F_1 sibbed: (F_2):					
19x18	100	32:19[z]	2:1	0.125	0.50-0.75
4x1	88	39:22	2:1	0.101	0.75-0.90
21x18	46	12:11	1:1	0.044	0.75-0.90
22x27	82	20:21	1:1	0.024	0.75-0.90
25x28	58	16:13[y]	1:1	0.138	0.50-0.75
Hairy F_2 selfed: (F_3):					
4x1	NR*	12:8	2:1	0.446	0.50-0.70
1	NR	3:1	NR	NR	NR
Hairy F_3 selfed: (F_4):					
4x1	NR	21:6[x]	3:1	0.360	0.50-0.75
4x1	NR	15:3	5:1	0	>0.95
4x1	NR	4:0	NR	NR	NR
4x1	NR	16:3	5:1	0.150	0.50-0.75
Glabrous x Hairy F_3 sibbed: (F_4):					
12.1 x 13.1	NR	16:14	1:1[w]	0.134	0.50-0.75
15.3 x 15.1	NR	8:1	1:1	5.444	0.010-0.025
15.4 x 15.1	NR	6:5	1:1	0.090	0.75-0.90

[z,y,x]One glabrous plant was selfed from each of these glabrous populations to produce four glabrous male-fertile families: GMF.1, GMF.2, GMF.3 and GMF.4, respectively. Separate pollinations on the same plant from c produced GMF.3 and GMF.4.

[w]Sib-pollinations each generation were made to maintain the glabrous, male-sterile line designated GMS.1. A glabrous, male-sterile line unrelated to the late male fertile lines listed above was designated GMS.2 and maintained by sib-pollinations.

*NR = Not Recorded

Table 2. Comparison of four glabrous male-fertile (GMF) families from self-pollinations of three glabrous plants with two families (GMS) maintained by sib pollinations of glabrous male-sterile x hairy, male-fertile plants.

		FAMILY[z]					
		GMF.1	GMF.2	GMF.3	GMF.4	GMS.1	GMS.2
Parent Population	H:G	36:19	16:13	21:6	21:6	16:14	29:20
	selfed:	G	G	G	G	H	H
F_1 Population	H:G	0:35	0:12	0:33	0:19	35:11	6:2
% Germination		87.5	50.0	89.0	79.0	92.0	NR*
Plants with Male Flowers		0/35	1/11	6/32	5/20	H:all G:none	H:all G:none
Days after[y] 1st Female		2	0	1	3	H:5 G:11	H:2 G:11
1st Male[w]		none	22	21	27	H:5 G:none	H:2 G:none
Seed/Fruit		23	37	100	68	101	154
S.E.		24	33	55	68	59	107

[z]GMF is glabrous, male-fertile; GMS is glabrous, male-sterile phenotype. The notation *gms* refers to the glabrous male-sterile gene. GMS.1 and GMS.2 are lines that segregate 1:1 for hairy (H) male-fertile plants and glabrous, male-sterile (G) plants. GMF.3 and GMF.4 came from fruit at the same node of the same plant.

[y]The first female occurred on GMF.2. A day later, a female occurred on GMF.3, *etc.*

[w]The first male occurred 21 days after the first female, *etc.*

*NR = Not Recorded

Table 3. Comparison of seed number in four glabrous, male-fertile (GMF) families and seed number from GMF pollen.

Pollen Source		FAMILY			
		GMF.1	GMF.2	GMF.3	GMF.4
		Seed Number +/- S. E.			
SC7 pollen[z]	Mean	12	30	93	76
	S.E.	2	30	52	71
GMF pollen:	Mean	none	none	200	178
SC7 fruit	S.E.			225	90
GMF pollen:	Mean	38	90	127	123
sibcross	S.E.	10	0	101	103
GMF pollen:	Mean	29	32	85	63
GMS.1 fruit	S.E.	18	26	46	46

[z]Pollen from a diploid line SC7 unrelated to line with glabrous character. Normal seed number of SC7 in the greenhouse is not different from that obtained with GMF pollen.

Segregation Data Suggest Male Sterility Genes *gms* and *ms* In Watermelon Are Not in the Same Linkage Group

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The *gms* and *ms* traits in watermelon have both been previously reported as the expression of single recessive nuclear genes in apparent non-allelic relationship (1,2,3). We now report data that suggest *gms* and *ms* exhibit independent assortment.

The cross [(*Ms Ms gms gms*) x (*Ms ms Gms Gms*)] produced all pubescent, fertile F1 progeny (1). Twelve of these F1 plants were self-pollinated and 10-15 F2 progeny from each were evaluated for presence of *gms* and *ms* conditioned sterility. One of the F1 plants (termed P42) was therefore determined to have possessed a (*Ms ms Gms gms*) genotype. The remaining F2 progeny of P42 were then planted and staminate flowers evaluated both before and after fruit set. Germination was 98.5% (139/141).

P42 produced 139 F2 plants which segregated for pubescence and glabrousness in a 3:1 ratio (105 pubescent : 34 glabrous) P=0.90. However, four phenotypic classes could be recognized within this F2 population.

1. **Pubescent fertile.** All pubescent fertile plants had normal anthers. It should be noted that fertile anthers produced on (*Gms gms*) and (*Ms ms*) are normal and indistinguishable from one another.

2. **Pubescent sterile.** The only staminate sterility manifested in the pubescent plants was that usually found in (*ms ms*) plants. In such genotypes only very small, undeveloped anthers are present. The *gms* conditioned sterility found only in glabrous plants (hence the "g" of the *gms*) has much larger, more developed non-functional anthers and is easily distinguishable from the *ms* condition if comparative evaluation is made throughout the growing season.

3. **Glabrous sterile with "*gms*" type anthers,** and

4. **Glabrous sterile with "*ms*" type anthers.**

   The analytical crux of this report is based upon the observation that the sterility produced by *gms* and *ms* are distinguishable even when the *ms* conditioned sterility is found within glabrous plants. The *gms* conditioned sterility produces anthers which, although they do not dehisce, are much larger than the ms conditioned type. The sterile anthers of this *gms* conditioned sterility can sometimes approximate the size of fertile anthers, especially late in the season. The *ms* conditioned sterility produces only small, shrunken anthers throughout the season. In large unopened flowers, *ms* conditioned male-sterility produces a very "hollow" flower bud when simply pressed between the fingers. This can be compared to the *gms* conditioned male-sterility, with its sometimes much larger yet nonfunctional anthers, which can manifest very "full" unopened flower buds during its life cycle when sensed by a similar finger-tip evaluation. Our preliminary cytological analysis of the *gms* and *ms* conditioned sterilities seem to confirm the distinctions made from these simple visual observations and touch.

By distinguishing 4 phenotype classes within the F2 generation of P42, a Chi-Square analysis based on independent assortment of *gms* and *ms* was possible. These data are listed in Table 1.

Table 1. Segregation ratios of the F2 of P42 into 4 phenotypic classes and Chi-Square analysis assuming independent assortment of *gms* and *ms*.

Phenotypic class	Putative genotype	Number of plants	Expected if 9:3:3:1 segregation
Pubescent fertile	(*Ms _ Gms _*)	85	78.18
Pubescent sterile with "*ms*" type anthers	(*ms ms Gms gms*)	20	26.06
Glabrous sterile with "*gms*" type anthers	(*Ms _ gms gms*)	25	26.06
Glabrous sterile with "*ms*" type anthers	(*ms ms gms gms*)	9	8.68
		Chi-Square = 2.05; 3 d.f. P=0.50-0.70	

Assumptions necessary to interpret our observations in this manner are (1) the glabrousness of homozygous *gms* overrides any expression of pubescence, and (b) the male sterility of homozygous *ms* overrides the expression of homozygous *gms*. Further evaluation of the glabrous material in this F2 population has been thwarted by extreme female sterility.

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R309, A Selection of Citrullus colocynthis with Multigenic Resistance to Colletotrichum lagenarium race 2

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Anthracnose, caused by Colletotrichum lagenarium (Pass.) Ell. and Halst., is a widespread disease of curcurbits (5). It is especially destructive on watermelon (Citrullus lanatus (Thunb.) Matsum and Nakai) in humid growing regions of the world. The discovery of anthracnose resistant watermelon germplasm and the nature of inheritance of anthracnose resistance has been reported (5,7,8,9). In 1980, Sowell et al. (7) screened 450 plant introductions of C. colocynthis for resistance to race 2 C. lagenarium. Suvanprakorn et al. (8) concluded that three of these, PI 271778, PI 326515 and PI 189225, had a single dominant gene for resistance.

Seven races of C. lagenarium have been described (2,3,4). Resistance to races 1, 3 and 7 have been incorporated into commercial watermelon cultivars. The predominant race of C. lagenarium in the Southeastern United States is now race 2. A shift in race prevalence from race 1 has made the use of expensive fungicides the only effective control measure (4).

The use of single gene resistance to eliminate the threat of anthracnose invites the same sequence of events, namely a rapid change in race prevalence, as occurred previously (1). This paper introduces the selection R309, a new source of resistance to race 2 C. lagenarium with multigenic resistance.

R309, the resistant line characterized in this study, is a selection of C. colocynthis. Citrullus colocynthis crosses easily with C. lanatus and only occasionally results in sterility or genetic abnormality. Two additional genotypes, the resistant PI 189225 and the susceptible cultivar New Hampshire Midget were included as parents. PI 189225 was included to provide direct comparison with previous work (8,9). The study was conducted in the field in 1983. Seed from, each parent, reciprocal F_1 , F_2 and BC generations, were planted in a randomized complete block with four replications.

An isolate of C. lagenarium was obtained from naturally infected watermelons at the Edisto Research and Education Center in Blackville, SC. Using hot differentials (4), the isolate was determined to be race 2. Methods used for culture of the fungus and spore production were adapted from Littrell and Epps (6). The plots were inoculated at sundown by spraying six-week old plants with a suspension of 20,000 conidia^{-1}. After five weeks, the mature vines were rated for resistance using a two-part rating system. The rating system combined the percent defoliation with the percent of remaining leaves showing lesions. This resulted in a zero to 200 scale (0=immune, 200=dead). Plants with a score of 70 or less were considered resistant; plants with scores greater than 70 were considered susceptible.

Chi-square analysis was used to determine if resistance was simply inherited. Orthogonal contrasts were also used to clarify the relationships among parents and F_1 populations.

In the inheritance study R309 demonstrated the same level of resistance for which it had been selected at the Edisto Research and Education Center. A mean rating score of 48.7 was recorded, which was slightly higher (more susceptible), than the score of 25.5 for PI 189225 and much lower than the 115.2 recorded for New Hampshire Midget. This level of resistance is more than adequate for commercial production of watermelons without the use of fungicides.

Chi-square analysis of progeny from PI 189225 x New Hampshire Midget confirmed the conclusion of Suvanprakorn et al. (8) that resistance from this source is due to a single dominant gene.

Chi-square analysis revealed a poor fit for a single dominant gene hypothesis in the progeny of R309 (Table 1). Neither did the data fit a two dominant gene model. From the observed ratios, it was concluded that the resistance of R309 is multigenic in nature. This type of resistance has not been available for breeding resistance to race 2 C. lagenarium and may prove valuable.

The actual inheritance of the resistance expressed by R309 has not been determined but orthogonal contrasts were used to provide more information (Table 2). Reciprocal F_1 (R309 x NHM) populations were not significantly different indicating that resistance is due to nuclear rather than cytoplasmic genes. The parent R309 and the F_1 (R309 x NHM) were not significantly different, but the F_1 was significantly more resistant than the midparent, indicating that resistance in R309 is due to complete dominance.

Due to the small difference in resistance between PI 189225 and R309, Chi-square analysis could not define the genetic relationship of the resistance fouind in each line. Comparison of progeny means from R309 x PI 189225 indicated that the level of resistance displayed by PI 189225 is higher and is also due to dominance (Table 3). The gene(s) that impart a higher level of resistance to PI 189225 is presumably the same dominant factors described previously (8).

In conclusion, R309 is a selection of C. colocynthis with intermediate resistance to race 2 C. lagenarium. The genes conditioning resistance in R309 are different from any previously reported. Multiple dominant genes are responsible for the resistance expressed by R309 and the use of an appropriate breeding scheme should make it possible to successfully exploit this new source. It is likely that this multigenic source of resistance will be more stable than single gene sources.

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Table 1. Reaction of R309, 'New Hampshire Midget' (NHM) watermelon and progeny to inoculation with C. lagenarium race 2 in the field. Chi-square test for single gene resistance.

Poplulation	Total Plants	Resistant[a]	Susceptible	Expected ratio	x^2	p
NHM	49	3	46	0:49		
R309	9	9	0	9:0		
F_1	45	43	2	45:0		
F_2	69	24	45	3:1	14.880	.01
BC x NHM	34	6	18	1:1	0.059	20 -.50
BC x R309	10	6	4	10:0		

[a]All plants were rated on a 0 to 200 scale (0 = no symptoms; 200 = dead). Plants with a score from 0 to 70 were considered resistaant; those above 70 susceptible.

Table 2. Disease rating of R309, New Hampshire Midget (NHM), F_1 and midparent in the 1983 field study.

Populations	Rating[a]
NHM	115.2
R309	48.7
F_1	45.9
Midparent	82.0
Contrast	**Significance[b]**
NHM vs R309	**
R309 vs F_1	ns
F_1 vs midparent	**

[a]Plants were rated on a) to 200 scale (0 = no symptoms; 200 = dead). Plot means are given.

[b]Nonsignificant (ns) or significant at the 5% (*) or 1% (*) level. Comprisons were made using orthogonal contrasts.

Table 3. Disease rating of R309, PI 189225, F_1 and midparent in the 1983 field study.

Populations	Rating[a]
R309	53.3
PI 189225	33.0
F_1	34.0
Midparent	43.2
Contrast	**Significance[b]**
R309 vs PI 189225	**
PI 189225 vs F_1	ns
F_1	**

[a]Plants were rated on a 0 to 200 scale (0 = no symptoms, 200 = dead).

[b]Nonsignificant (ns) or significant at the 5% (*) or 1% (**) level. Comparisons were made using orthogonal contrasts.

Screening of Domestic and Wild Citrullus Germplasm for Resistance to the Yellow-Stunting Disorder in the United Arab Emirates

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Watermelon, Citrullus lanatus (Thunb.) Matsum & Nakai, is one of the most important vegetables in the U.A.E. However, it's production has been seriously curtailed in recent years due to a yellow-stunting disorder (YSD) caused by a new virus of the closterovirus-like group similar to the lettuce infectious yellows virus (LIYV) (2). It was observed in most samples examined by electron microscopy as long filamentous particles (3). It infects all cucurbits commercially grown in the U.A.E. and is thought to be transmitted by the tobacco whitefly, Bemisia tabaci Genn.

All attempts made locally to control the disorder through the control of whiteflies has failed. The use of spunponded polysters and polypropylene plant covers for about 35 days (until flowering) was promising in controlling the disorder in muskmelon (4). However, this practice has not been tried with watermelon and has not gained acceptance among U.A.E. melon farmers, probably due to its high cost. At present, none of the tested commercial watermelon cultivars were found resistant to this disorder (1,2). The use of resistant cultivars would be the simplest and most efficient control method. Therefore, the objective of this study was to search for sources of resistance to the YSD in C. colocynthis and in a large collection of domestic and wild C. lanatus accessions.

Four hundred fifty-eight wild and domestic Citrullus accessions were evaluated for resistance to the YSD under field conditions in Al-Ain, U.A.E. in the spring and autumn plantings of 1989. The spring trial included 441 and 16 USDA plant introductions (PIs) of C. lanatus and C. colocynthis, respectively. The most promising accessions were re-evaluated in the autumn trial in two experiments: (a) 15 C. lanatus PIs in comparison with cv. Crimson Sweet (Table 1) and (b) C. colocynthis PIs in comparison with a local (U.A.E.) strain of the same species, and the watermelon cv. Charleston Gray (Table 2). Seeds of PIs evaluated were kindly provided by the USDA through Dr. G. Lovell (Plant Introduction Station, Experiment, Georgia).

Seeds were sowed on March 10 and September 3 in the spring and autumn trials, respectively. After thinning, individual plants were spaced 50 cm apart in rows 2.0 m-wide. Each accession was assigned to one 8.0 m row in the spring trial, and to 2 to 6 similar rows in a completely random design in each of the autumn experiments. The number of plants evaluated of each accession were as follows: 3-16 C. lanatus ($\bar{x}$ = 9.5, sd = 2.74, mode = 10) and 3-11 C. colocynthis in the spring trial; and 2-6 per replicate of either species in the autumn trial.

Natural infection to produce the YSD was enhanced by placing heavily whitefly-infested melon plants, showing severe symptoms of the disorder, nearby the evaluated plants one week after germination. No pesticides were applied to maintain a high population of viruliferous whiteflies which were continuously

Table 3. Disease rating of R309, PI 189225, F_1 and midparent in the 1983 field study.

Populations	Rating[a]
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Contrast	**Significance[b]**
R309 vs PI 189225	**
PI 189225 vs F_1	ns
F_1	**

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Seeds were sowed on March 10 and September 3 in the spring and autumn trials, respectively. After thinning, individual plants were spaced 50 cm apart in rows 2.0 m-wide. Each accession was assigned to one 8.0 m row in the spring trial, and to 2 to 6 similar rows in a completely random design in each of the autumn experiments. The number of plants evaluated of each accession were as follows: 3-16 *C. lanatus* ($\bar{x}$ = 9.5, sd = 2.74, mode = 10) and 3-11 *C. colocynthis* in the spring trial; and 2-6 per replicate of either species in the autumn trial.

Natural infection to produce the YSD was enhanced by placing heavily whitefly-infested melon plants, showing severe symptoms of the disorder, nearby the evaluated plants one week after germination. No pesticides were applied to maintain a high population of viruliferous whiteflies which were continuously

observed on the plants in both trials. Plants were examined for symptoms of the YSD 10 and 12 weeks after sowing. Each plant was given a disease score according to the following scale: 1=no symptoms; 2=slight; 3=moderate; and 4=severe symptoms. Individual plant ratings for each accession were added, then divided by the number of plants evaluated to obtain the corresponding mean disease score (MDS). Then, accessions were classified as either resistant (R), slightly susceptible (SS), moderately susceptible (MS), or highly susceptible (HS) when their MDSs were 1.0-1.5, 1.6-2.5, 2.6-3.5 and 3.6-4.0, respectively. Only the second reading was assigned to accessions which were classified in the first reading of the spring trial as R or SS. These accessions were signaled out in the delyaed symptoms development category (Table 1).

C. lanatus accessions: Out of 441 C. lanatus PIs evaluated in the 1989 spring trial, only 2 (0.45%) were rated R, 4 (0.91%) and 30 (6.80%) were found SS and MS, respectively; while 405 (91.84%) PIs were found HS. All R and SS PIs, 9 of the MS PIs and 4 HS PIs showed delayed symptom development. In the autumn trial, PIs 186490, 189318, 346082, 482258, 482264 and 482269 had significantly lower mean disease scores (MDSs) than 'Crimson Sweet' at 10 weeks after sowing. However, these significant differences disappeared two weeks later, though some variations in MDSs remained among accessions. PIs exhibiting the lowest MDSs at 12 weeks after sowing were 189316, 189318 and 482258. Delayed symptom development, regardless of the final MDS assigned to an accession, was believed to be a dependable criterion for differentiating genotypes in their response to the YSD.

C. colocynthis accessions: Out of the 16 C. colocynthis PIs evaluated in the spring trial, one (PI 235118) was highly susceptible, 2 were MS, 6 were SS, while 7 (PIs 386015, 386016, 386024, 386026, 494528, 494529 and 494530) were R (Table 2). Eleven out of 13 accessions evaluated in the autumn trial remained significantly lower ($P = 0.01$) than 'Charleston Gray' in MDS 12 weeks after sowing. The highest level of resistance (MDS = 1.0-2.0) was found in PIs 386015, 386016, 386018, 386025, 494528, 494530 and the U.A.E. strain.

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Table 1. Response of selected C. lanatus accessions re-evaluated in the 1989/1990 autumn planting to the yellow stunting disorder.

Accessions (PI)	Response in spring, 1989[a]	No. of replicates	Mean disease score (weeks after sowing)	
			10 weeks[b]	12weeks[c]
186490	MS	6	3.26*	3.52
189316	HS-d	6	3.62 n.s.	3.27
189318	HS-d	6	2.93*	3.12
249008	MS-d	6	3.89 n.s.	4.00
346082	SS-d	3	3.11*	3.57
457916	SS-d	2	3.38 n.s.	3.65
481871	R-d	3	3.44 n.s.	3.90
482247	MS-d	4	4.00 n.s.	4.00
482258	MS-d	6	2.93*	3.32
482264	MS-d	4	2.71*	4.00
482269	MS-d	5	3.27*	3.60
482275	SS-d	3	3.44 n.s.	3.50
500349	R-d	4	3.63 n.s.	3.75
500350	SS-d	3	3.78 n.s.	4.00
500352	MS	4	3.25 n.s.	4.00
'Crimson Sweet' (Control)		5	4.00	4.00

[a] R=resistant; SS=slightly susceptible; MS=moderately susceptible; HS=highly susceptible. The letter 'd' refers to delayed symptom development at 12 weeks after sowing.

[b] LSD at P = 0.05 between accessions with 2, 3, 4, 5 and 6 replications and the control (cv. 'Crimson Sweet') were, respectively 0.95, 0.82, 0.76, 0.72 and 0.69. Accessions were either not significant (n.s.) or significant (*) from the control.

[c] Accessions were not significantly different from the control at P=0.05.

Table 2. Response of C. colocynthis accessions to the yellow-stunting disorder.

Accession	Spring 1989		Autumn 1989/1990		
	Number evaluated	MDS[a]	No. of replicates	Mean disease score weeks after sowing)	
				10 weeks[b]	12 weeks[b]
235118	7	4.0	-	-	-
386015	11	1.5	2	1.30**	1.80**
386016	6	1.0	5	1.32**	1.72**
386018	9	1.9	4	1.44**	1.50**
386019	11	2.4	-	-	-
386024	6	1.2	4	1.51**	2.38**
386025	6	2.3	5	1.28**	1.64**
386026	9	1.2	5	1.51**	2.26**
388770	8	3.0	-	-	-
432337	3	2.0	2	2.43**	2.15**
494527	6	2.5	6	2.81**	3.15 n.s.
494528	9	1.3	6	1.51**	1.72**
494529	9	1.0	5	2.52**	2.44**
494530	10	1.0	5	1.60**	1.88**
494531	7	3.0	-	-	-
494532	10	3.4	2	2.92 n.s.	3.25 n.s.
U.A.E. (local)	-	-	2	1.25**	2.00**
'Charleston Gray' (Control)	-	-	6	3.76	4.00

[a] MDS=mean disease score. Readings were recorded 12 weeks after sowing.

[b] LSD between accessions with 2, 4, 5 and 6 replications and the control (cv. Charleston Gray) were respectively, as follows:
P = 0.05 at 10 weeks: 0.99, 0.78, 0.73 and 0.70.
P = 0.01 at 10 weeks: 1.32, 1.04, 0.98 and 0.93.
P = 0.05 at 12 weeks: 1.21, 0.99, 0.94 and 0.90.
P = 0.01 at 12 weeks: 1.61, 1.32, 1.25 and 1.20
Accessions were either not significant (n. s.) or highly significant (**) from the control.

A Strategy Toward Varietal Resistance to Watermelon Fruit Blotch

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The disease called watermelon fruit blotch (WFB) appeared in the summer of 1989, first in Florida and subsequently in South Carolina and in other states on the eastern seaboard as the summer progressed. It was also reported in Indiana. It was particularly devastating in Florida and South Carolina, sometimes spoiling large fields of fruit. Hopkins (1) described the disease. The disease also appeared in recent years on 'Charleston Sweet' and 'Xin Hong Bao' varieties under humid conditions in China. Observations by Zhang suggest that the disease was transferred to China by seed.

A grower in South Carolina realized a high yield of marketable fruit from the triploid variety 'Tri-X 313' despite the fact that the fruit of the pollinator variety, 'Prince Charles', was completely ruined by watermelon fruit blotch. The tetraploid parent of 'Tri-X 313' reputedly came from the USDA variety, 'Congo'. In 1979, Sowell and Schaad (2) screened 740 plant introductions and several varieties for seedling resistance to a pathogen similar to that described for WFB. The three most resistant hosts possessed genes from 'South Africa' and 'Congo' was the third most resistant host.

One hundred 3-week old seedlings from each of nine sources of 'Congo', 'PI 295843', 'PI 299378' and 'Tri-X 313' were sprayed with a solution of 10^6 cells ml^{-1} from a WFB culture obtained from Hopkins and left covered with polyethylene for two days. Two unrelated lines, one from the CU breeding program and one from Gansu Agricultural University, were used as checks.

Visual ratings of seedlings two weeks later (Table 1) suggested that 'PI 295843' was virtually immune and 'PI 299378' was susceptible to the pathogen. Lots of 'Congo' varied somewhat in resistance. Original breeders' seed from Charleston appeared to be the most resistant. 'Tri-X 313' also showed resistance. Numerous plants on the perimeters of the flats did not acquire the disease and were not included in the ratings.

Some selections were made from lots of the variety 'Congo' within flats where inoculations were obviously successful. These selections were grown to maturity in the greenhouse. Only one of these selections eventually developed a characteristic blotch on a fruit. Plants from seed recovered from resistant selections did not show WFB symptoms under similar greenhouse conditions.

"Escapes" were primarily plants from peat cups that became desiccated between waterings due to the wicking effect of an exposed portion of the peat cup. Older plants behaved in a similar fashion. Under our greenhouse conditions, only after foliage of the entire plant was kept wet every day did WFB lesions appear on fruit. These observations are consistent with all the reports of WFB symptoms being associated with high humidity or rainfall.

In summary, initial observations suggest that resistance does exist in the variety 'Congo' and in a triploid hybrid derived from 'Congo'. If these observations are correct, then triploid hybrids, and perhaps diploid hybrids derived from 'Congo' can be synthesized quickly to take advantage of this resistance. A long-term strategy would be to attempt to transfer the higher level of resistance from 'PI 295843' to commercially acceptable lines and varieties.

Table 1. Severity of Foliage Destruction of Watermelon Seedlings Inoculated with the WFB Pathogen

Identification	Severity of Disease
PI 295843	1 (least affected)
USDA WR Congo	1
USDA Congo	1
Coffey Congo	2
Hollar Congo	2
Shumway Congo	2
Harris Moran Congo	2
Tri-X 313	2
Coffey WR Congo	3
90.7 CU	3
Early GAU	3
Musser Congo	3
PI 299378	4 (most affected)

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Allozyme Diversity at the *Pgi-2* Locus in Landraces of *Citrullus lanatus* from Zimbabwe

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Toll and Gwarazimba reported in 1983 to have collected 134 landraces of watermelon (*Citrullus lanatus)* in Zimbabwe (5). In 1986, we received from the USDA Germplasm Resources, 56 accessions of that collection kindly provided by V. Gwarazimba of the Harare Research Station. The primary purpose of our request was to find sources of resistance to strains of zucchini yellow mosaic virus (ZYMV).

A few isozyme loci have been demonstrated to be ideal molecular markers for viral resistance genes in some vegetables (6,7). Using the information generated by Navot and Zamir (2), regarding the linkage relationship of 19 isozyme genes in *C. lanatus* and *C. colocynthis*, we directed our efforts toward finding a locus which might be useful as a marker for ZYMV resistance. In a preliminary study involving single plant selections resistant to the Florida strain of ZYMV and those of several commercial cultivars, we noted that although all lines possessed two loci for phosphoglucoisomerase (*Pgi-1* and *Pgi-2*), two different banding patterns were evident for the *Pgi-2* region. One band was common to all the commercial cultivars, while a second band occurred in plants resistant to the virus. Since multiple allelism for the *Pgi-2* locus has been reported for other crops (3), we extended our tests to all accessions from Zimbabwe, as well as a number of watermelon cultivars of domestic and foreign origin. Enzyme extraction, starch gel electrophoresis, and enzyme staining were performed according to known protocols (4,8). Individual samples of cotyledon or leaf tissue of six plants of each line were homogenized in 0.01M potassium phosphate extraction buffer (pH 7.0) and crude extracts were subjected to electrophoresis in a pH 6.5 histidine-citrate buffer, 11% starch gel at 250 VDC, for 3 to 4 hr at 4C.

The results can be summarized as follows:

a) All PI's, from Zimbabwe, as well as commercial watermelon cultivars, contained *Pgi-1* and *Pgi-2* loci;

b) The *Pgi-1* locus appeared to be monomorphic, a condition which has been reported for other plant species (1). Polymorphism was noted in the *Pgi-2* region, where three bands were noted: *a*, *b*, and *c*. In each individual plant they occurred either as a single-band (homodimer) for homozygous condition, or as a triple-band (heterodimer) for heterozygous condition;

c) The fast homodimer band (*aa*) was confined to PI's: 482273, 482308, and 482361;

d) The intermediate homodimer band (*bb*) was noted in PI's: 482252, 483353, 482256, 482261, 482273, 482286, 482289, 482293, 482398, 482399, 482316, 482318, 482319, 482321, 482322, 482333, 482342, 482356, and in the cultivar Knight from Taiwan.

e) The slow homodimer band (*cc*) was present in PI's: 482250, 482253, 482256, 482267, 482269, 482289, 482318, 482334, 482341, 482342, 482343, 482345, 482346, 482347, 482348, 482349, 482362, 482363, 482364, 482365, 482366, 482368, 482369, 482370, 482371, 482373, 482375, 482376, 482377, 482378, and 482381. It occurred also in the domestic cultivars Charleston Gray, Crimson Sweet, Dixie Queen, Florida Giant, Kleckley Sweet, Jubilee, New Hampshire Midget, Sugar Baby and in the Taiwanese Early Klondike, Empire No. 2, Farmer's Giant, Fengshan No. 1, Flower Dragon, Grand Baby, Jumbo, New Dragon, Petite Yellow, and Yellow Baby.

f) The original seeds (collected in Zimbabwe) were from open pollinated fruits, hence, a certain degree of heterogeneity was anticipated. Consequently, some plants contained triple-banded patterns resulting in a combination of *ab*, *bc*, or *ac*. The combination *ab* was present in a few plants of PI's: 482273, 482286, 482289, 482308, and 482333; the combination *bc* was evident in some plants of PI's: 482253, 482316, and 482356; and the combination *ac* was encountered in one plant of PI 482348. The generation of active heterodimers between different monomers is typical of allelic products.

All the plants possessing *aa* or *bb* bands shared a number of common characteristics including large size, late maturity, large fruits with a yellowish flesh and low sugar content. Although fruit shape and skin color varied considerably, all had long storage capabilities. In addition, most of the plants appeared to be cold tolerant. Our designation *a*, *b*, and *c* implies that there is a multiple allelic system at the *Pgi-2* locus of *C. lanatus*. Genetic studies are presently under way to confirm this hypothesis and determine whether homodimers *aa* or *bb* can be used as markers for ZYMV resistance and cold tolerance.

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Plant Regeneration from Callus Cultures of *Citrullus colocynthis*

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Citrullus colocynthis (L.) Schrad. belongs to the Cucurbitaceae family and produces edible seeds rich in oil and protein (4). The plant is a long-lived perennial, indigenous to Africa and the Middle East and is probably an ancestral type of watermelon (5). Preliminary studies in our laboratory indicated that *C. colocynthis* may possess resistance to a number of watermelon diseases including Fusarium wilt. Tissue culture is a powerful technique in the selection of resistance to pathogens and in the creation of genetic variability from cell cultures (1). There have been few reports on tissue culture of *C. colocynthis* (3). In this report, we describe regeneration of plantlets from callus cultures of *C. colocynthis*.

Callus Induction: Seeds of *C. colocynthis* were obtained from University of Port-Harcourt, Nigeria, West Africa. The seeds were surface- sterilized by immersion in 2.5% (v/v) sodium hypochlorite for 15 min, rinsed with sterile distilled water, germinated aseptically and grown in potting soil in the greenhouse. Cotyledons and leaf petioles of 14-day- old seedlings were used as explants. Explants were surface-disinfected in 2.5% sodium hypochlorite for 10 min, rinsed three times with sterile distilled water, sliced into 3 mm sections and transferred onto a callus induction medium. The callus induction medium consisted of a basal medium of Murashige and Skoog (2), MS salts plus sucrose (30 g l^{-1}), myo-inositol (100 mg l^{-1}), thiamine HCl (0.8 mg l^{-1}), nicotinic acid (2 mg l^{-1}), pyridoxine HCl (0.8 mg l^{-1}) and Difco Bacto agar (0.8 g l^{-1}) supplemented with 4 mg l^{-1} 2,4-D and 0.1 mg l^{-1} kinetin. The pH was adjusted to 5.7 prior to autoclaving for 20 minutes at 121°C, 124 kPa for 15 minutes. Thirty explants from each source were used in the callus induction and the experiment was repeated three times. All cultures were grown under 16 hr photoperiod at 25°C.

Plant Regeneration: Callus cultures were transferred to a maintenance medium consisting of basal medium supplemented with 1.5 mg l^{-1} 2,4-D. Shoots were induced on MS basal medium containing 1.0 mg l^{-1} kinetin and 0.05 mg l^{-1} 1-napthaleneacetic acid (NAA). At the end of 4 weeks, shoots were excised and placed on the rooting medium which consisted of basal medium supplemented with 0.5 mg l^{-1} NAA and 0.05 mg l^{-1} 6- benzyladenine (BAP). Rooted shoots were transferred to sterile peat and sand soil mixture. When shoot and roots began active growth, regenerated plants were evaluated for somaclonal variation.

Results and Discussion: In all cases, cotyledon-derived callus grew faster and had greater morphogenetic potential than petiole-derived callus. Although the present study focused on the effect of explant source, a previous pilot study on the growth regulator combinations and concentrations required for optimum callus growth or shoot and root regeneration of *C. colocynthis* indicated similar requirements for both explant sources (data not shown). Studies are under way to use highly regenerative *C. colocynthis* callus in *in vitro* selection for disease resistance.

Table 1. Effect of explant source on callus induction and plant regeneration of *Citrullus colocynthis*.

Explant source	% Explant with callus	Number of shoots obtained	% shoot with roots	Number of plantlets
cotyledon				
Exp. #1:	38	25	96	24
#2:	34	32	72	23
#3:	41	26	77	20
leaf petiole				
Exp. #1:	16	7	24	1
#2:	10	3	0	0
#3:	23	7	29	2

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Obtention of haploïd plants induced by irradiated pollen in watermelon (*Citrullus lanatus*).

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The first haploïd plant in Cucurbitaceae was observed spontaneously in cucumber in 1958 (1). The first induced haploïd was obtained in muskmelon by pollination of *Cucumis melo* with *C. ficifolius* pollen (3). However, the use of haploïdy in breeding programs was actually possible only after discovering the induction of gynogenesis *in situ* with gamma rays irradiated pollen of *C. melo* followed by rescue of haploïd embryos by *in vitro* culture (4). And then this technic was also applied to cucumber (2).

Until now, in the literature, two studies of haploïdy in watermelon have been reported. In 1958, one haploïd branch was observed on a watermelon plant irradiated by X-ray (5). In 1983, it was reported that some haploïd tissue as well as haploïd plantlets were obtained by anther culture, but they did not develop into complete plants (6).

Here we report the obtention of haploïd watermelon plants by gynogenesis induced by gamma rays irradiated pollen (300 Gy) with the method proposed by Sauton (4) on muskmelon.

The plant material consists of a F1 hybrid 'Panonia' and three open pollinated cv 'Crimson sweet', 'Sugar baby' and 'Halep Karasi'. In order to find an effect of the season and the genotypes on haploïd embryo production, seeds were sown for 7 times with 15 days intervals from 5th January to 6th April, 1990. According to the results, haploïd embryo formation was influenced by genotypes and sowing and pollination periods.

Embryos obtained were at different development stages: 72 % globular, 28% heart shaped (8% soft, 19% necrotic and 1% normal heart shaped). A total number of 761 embryos were obtained from 13,844 seeds and only 17 embryos regenerated into plants. One from 'Crimson sweet', 5 from 'Halep Karasi', 5 from 'Sugar baby' and 6 from 'Panonia'. The percentage of plant regeneration (2.2%) is very low probably because most of the embryos were in the globular stage and most of the heart shaped were soft or necrotic. Only 5 normal heart shaped embryos were observed. They were obtained from the last pollination period (May 31 to June 13). This indicated that, in order to increase the success of the method, the pollination period must be during the period where the plant growth is better.

The DNA content has been estimated by the fluorescence of propidium iodure (analysis made by B. Longhi and J. Roustan, I.M.I.M., Montpellier, France). The regenerated plants have between 33 and 46 % of their cells which were haploïd compared with 0 % in the diploïd control (Table 1 and Figure 1). There is a tendency to natural doubling with around 10 % of the nuclei at the 4C level and even some 8C.

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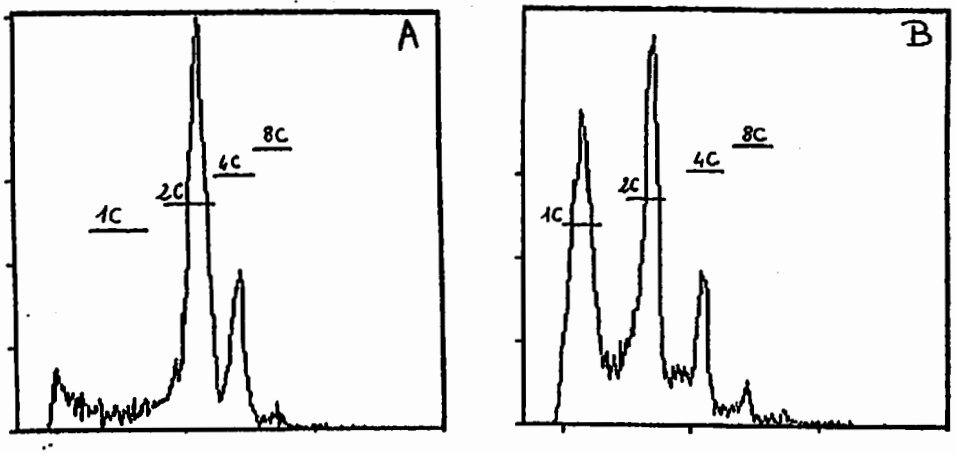
Table 1. Frequency of leaf cells with different DNA content in regenerated watermelon plants.

Plant	Conditions*	1C	2C	4C	8C
Crimson sweet	A	33.5	39.5	11.5	2.5
Crimson sweet	B	35.5	35.5	11.5	2.5
Halep Karasi	A	42.5	27.5	7.5	2
Sugar baby	B	46.5	30	9.5	1.5
Diploïd control	C	0	59.5	19	2

* A = Cuttings transplanted in pots in the greenhouse ; B = *in vitro* cuttings ; C = Plants from seeds in the greenhouse.

Figure 1. Distribution of the nuclei (Y-axis = number) of a diploid control watermelon (A) and a regenerated plant of 'Crimson sweet' (B) according to the DNA fluorescence (X-axis).



Fused Vein Trait in *Cucurbita pepo* L.

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Few morphological markers are known for *Cucurbita pepo* that can be used to detect outcrossing during commercial seed production (1). A new mutant leaf form, fused vein, shows potential as a roguing marker. The fused vein trait was first observed in 1987 in breeding material for high yielding hull-less seeded pumpkins, descending from a cross between 'Trickijack' and 'Minijack'.

The trait is characterized by a partial fusion of the lateral leaf veins to the main central vein. The fusion begins at the distal point of the petiole and extends 10 to 15 centimeters into the leaf blade. Branching of the veins is delayed and there is a reduction of the intraveinal leaf blade. Consequently, the dorsal leaf surface appears puckered or wrinkled. The trait is expressed beginning at the fourth to sixth leaf stage and then throughout vegetative growth. The extent of fusion along the central vein is less on early leaves and becomes pronounced by the tenth leaf stage. There is no apparent fusion of other plant parts associated with this trait. Comparison of fused vein and normal sister lines shows no appreciable differences in fruit yield per plant or seed yield in open pollinated fruit. There is, however, greater variation in seed yield per fruit from fused vein self pollinations than from normal self pollinations.

The fused vein trait is stably inherited once fixed. The trait is recessive; reciprocal F_1s between fused vein and normal plants appear normal. In reciprocal F_2 and backcross generations the percent recovery of the fused vein phenotype varies with each pollination event. F_2 fused vein recovery ranges from 0 to 19%, well below the expected 25% for a single recessive gene (Table 1). In backcross generations, recovery ranges from 3 to 63% (Table 2). Total fused vein recovery for all backcross generations is 39% which is between the expected 50% for one recessive gene and 25% for two recessive genes. One interpretation of the data is that the trait is governed by a single gene (or genetic event) with associated subvitality in both male and female gametes.

Gametic subvitality, observations of rare partially fused leaves in F_1 plants, and occasional fused vein sectoring within otherwise normal F_2 and backcross segregants, suggest that the fused vein trait may be caused by a chromosomal aberration.

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Table 1. F_2 fused vein inheritance data.

crosses	fused	normal	% fused	X^2 one gene	P
Fused x Normal F_2					
1	0	200	0.0	66.66	<.001
2	0	373	0.0	124.33	<.001
3	0	225	0.0	75.0	<.001
4	0	48	0.0	16.0	<.001
5	2	46	4.2	11.11	<.001
6	3	45	6.3	9.0	.001 -.01
7	9	74	10.8	8.47	.001 -.01
8	7	41	14.6	2.78	.05 -.10
9	18	76	19.1	1.72	.10 -.20
Normal x Fused F_2					
1	0	48	0.0	16.0	<.001
2	0	48	0.0	16.0	<.001
3	0	48	0.0	16.0	<.001
total	39	1128	3.3		

Table 2. Backcross fused vein inheritance data.

crosses	fused	normal	% fused	X^2 one gene	P
Fused (Fused x Normal)					
1	4	26	13.3	16.13	<.001
2	4	26	13.3	16.13	<.001
3	7	23	23.3	8.53	.001 -.01
4	21	37	36.2	4.41	.01 -.05
5	34	39	46.6	0.34	.50 -.70
6	14	16	46.7	0.13	.70 -.80
7	14	16	46.7	0.13	.70 -.80
8	22	17	56.4	0.64	.30 -.50
9	62	38	62.0	5.76	.01 -.05
(Fused x Normal) Fused					
1	1	29	3.3	26.13	<.001
2	2	26	7.1	20.57	<.001
3	9	29	23.7	10.52	.001 -.01
4	33	76	30.3	16.96	<.001
5	10	17	37.0	1.81	.10 -.20
6	21	21	50.0	0.00	1.0
7	19	11	63.3	2.13	.10 -.20
Fused (Normal x Fused)					
1	9	19	32.1	3.57	.05-.10
2	10	20	33.3	3.33	.05-.10
3	11	19	36.6	2.13	.10-.20
4	15	15	50.0	0.00	1.0
(Normal x Fused) Fused					
1	13	17	43.3	0.53	.30-.50
2	13	17	43.3	0.53	.30-.50
total	348	544	39.0		

Expression of the B Genes in Cucurbita.

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B_1 vs B_2. Table 1 shows the effect of the two B genes on different plant organs. B_1B_1 stands for $B_1B_1B_2{}^+B_2{}^+$ in tests 1 to 6, and B_2B_2 stands for $B_1{}^+B_1{}^+B_2B_2$ in tests 7 to 11. It is not certain yet whether NA159 (test 12), a derivative of NJ-B x IL-B, is $B_1{}^+B_1{}^+B_2B_2$ or $B_1B_1B_2B_2$. Inbreds similar in their phenotype to NA159 were obtained from crosses of $B_1{}^+B_1{}^+B_2B_2$ (e.g., PI-165558) x $B_1{}^+B_1{}^+B_2{}^+B_2{}^+$ (e.g., 'Buttercup') and these are presumably $B_1{}^+B_1{}^+B_2B_2$.

JGA (tests 1 & 2) carries a modifier gene, Ses - $B_1{}^+$, that enables B_1 to "turn on" in leaf blades in response to relatively low temperatures (below 15 ° C). In contrast, JGA, II carries Ses - B_1 that selectively suppresses the action of B_1 in leaf blades.

Circumstantial evidence suggests that a similar mechanism, including "selective activators" and "selective suppressors" is responsible for the regulation of B_2 in stems. But the regulators of B_2 in stems have not been identified. Furthermore, the control mechanism in this case may be more complicated, because the expression of B_2 in stems can be intense and stable even at very high temperatures, as shown by the performance of IL-B (tests 10 & 11).
Unlike NA159 (test 12), inbred PI-165558 (tests 7 & 8) is vigorous and very productive. This is largely due to the fact that the expressivity of B_2 in PI-165558 is low (or incomplete) and fluctuating in organs other than the ovary and pedicel.

Phenotypic variation among $B_2B_2{}^+$ heterozygotes. The data in Table 2 show that heterozygotes can exhibit either a relatively narrow or a relatively wide range of phenotypic variations. Thus, the expression of B_2 in fruits and stems is incompletely dominant in heterozygotes of some crosses (tests 3 & 4). In heterozygotes of other backgrounds, the expression of B_2 ranges from incomplete dominance in both fruits and stems to incomplete dominance in fruits but complete recessiveness in stems (tests 5 to 9). The progeny of 807-34 (test 5) gives an excellent fit to a 1:2:1 ratio when all heterozygotes are grouped together (22 B_2B_2: 56 $B_2B_2{}^+$: 28 $B_2{}^+B_2{}^+$; X^2 = 0.4; P = 0.80-0.90). Moreover, selection of heterozygotes for a very low level of expressivity is effective (test 9). Note also that all the 53 (22+6+7+8+10) B_2B_2 segregates in progenies of tests 5 to 9 exhibited a lower level of expressivity of B_2 than IL-B (test 1), the original B_2B_2 parent. The lower expression of B_2 in these 53 individuals was manifested by their green petioles.

Expressivity of B_2 is influenced by the environment, but the genetic background is a major factor, implying that modifiers are at work.

The basis for the excess of green-fruited plants in progenies of tests 7 & 8 is not known. However, the pooled data from progenies of tests 5 to 9 give a fairly good fit to 3:1 ratio (202:53; deviation X^2 = 2.42; P = 0.10-0.20; heterogeneity X^2 = 0.69; P = 0.95-0.98).

The above data support R. A. Fisher's hypothesis on the role of modifier genes in the evolution of dominance as do the data of several previous workers. For review of evolution of dominance see Chapter 8 in P. M. Sheppard. 1960. Natural Selection and Heredity. Harper Torchbooks (a 1959 revision of the original 1958 edition).

Table 1. Effect of B_1 and B_2 on different plant organs in Cucurbita. Relationship of effect to genetic background and environment. Effect = chlorophyll deficiency; + = organ was effected; − = organ was not effected.

Test	Species	Inbred	Genotype	Environment[z]	Stems	Petioles	Leaf blades	Pedicel	Ovary	Calyx of staminate flowers
1	C. pepo	JGA[y]	B_1B_1	Spring, NJ	-	-	+[v]	+	+	-
2	"	"	"	Fall, FL	-	-	-	+	+	-
3	"	JGA, II	"	Spring, NJ	-	-	-	+	+	-
4	"	"	"	S & F, FL	-	-	-	+	+	-
5	C. moschata	NJ-B	"	Spring, NJ	-	-	-	?	+	-
6	"	"	"	S & F, FL	-	-	-	?	+	-
7	C. maxima	PI-165558[x]	B_2B_2	Spring, FL	+	+	+[uv]	+	+	+
8	"	"	"	Fall, FL	-	+	+[u]	+	+	+
9	"	NA701	"	S & F, FL	-	-	-	+	+	+
10	C. moschata	IL-B	"	Winter, NJ	+	+	+[v]	+	+	?
11	"	"	"	S & F, FL	+	+	-	+	+	?
12	"	NA159[w]	"	S & F, FL	+	+	+	+	+	+

[z] Spring, NJ = Late May field transplanting in New Brunswick; Winter, NJ = greenhouse culture in New Brunswick.
Spring, FL = Early March field transplanting in Naples; Fall, FL = late September field transplanting in Naples.

[y] 'Jersey Golden Acorn' (JGA) is the source of B_1 in inbreds JGA, II and NJ-B.

[x] PI-165558 is the source of B_2 in inbreds NA701, IL-B and NA159.

[w] The whole-plant phenotype of inbred NA159 is manifested as severe yellowing. The extent of yellowing may be partial or complete, and may be associated with lethality or semi-lethality depending on local environmental variations.

[v] Leaf blades exhibit varying degrees of diffused yellowing.

[uv] Leaf blades often appear as "galaxies" of golden spots or exhibit varying degrees of diffused yellowing.

[u] Leaf blades often appear as "galaxies" of golden spots.

CGC 14:114 (1991)

Table 2. Phenotypic and breeding behavior of some $B_2B_2^+$ heterozygotes in Cucurbita moschata. Naples, Florida.

Test[z]	Parents: Pollination of breeding material	Parents: Phenotypes[x]	Parents: Genotypes	Parents: Growing season	Offspring: Growing season	Offspring: PDC-UO PDC-S PDC-P GB	Offspring: PDC-UO PDC-S GP GB	Offspring: GOT PDC-SL GP GB	Offspring: GOT GS GP GB	Offspring: GOT-VL GS GP GB	Offspring: GO GS GP GB	Offspring: Number of classified plants
1	IL-B, self	PDC-UO,PDC-S,PDC-P,GB	B_2B_2	Fall, '89	S & F, '90	15	0	0	0	0	0	15
2	NA124, self	GO,GS,GP,GB	$B_2^+B_2^+$	"	"	0	0	0	0	0	25	25
3	F_1, IL-B x NA124	-	$B_2B_2^+$	"	"	0	0	25[v]	0	0	0	25
4	BC_1, F_1 x NA124	-	-	"	Spring, '90	0	0	294[v]	0	0	264	558[u]
5	807-34, self	GOT,GS,GP,GB	$B_2B_2^+$	"	"	0	22	41	13	2	28	106[t]
6	807-34-8, self	GOT,PDC-SL,GP,GB	"	Spring, '90	Fall, '90	0	6	0	16	0	8	30
7	807-34-14, self	GOT-L,GS,GP,GB	"	"	"	0	7	0	14	0	20[s]	41
8	807-34-34, self	GOT,GS,GP,GB	"	"	"	0	8	0	13	0	17[s]	38
9	807-34-110, self	GOT-VL,GS,GP,GB	"	"	"	0	10	0	0	18	12	40

z Tests 1 to 4 serve as checks.

y IL-3 is an inbred that carries B_2 of PE-165558 (Table 1); NA124 is a dark green inbred derived from the cross IL-B X NJ-B (Table 1); 807-34 is an F_2 segregate of IL-B X NJ-B.

x See Table 1 in the preceding report for the key to phenotypic symbols. Additional symbols: B = leaf blades; P = petioles; VL = very low level of expressivity, chlorophyll deficiency being limited to a small area (5-10mm in diameter) affecting the proximal end of the fruit and the pedicel.

v Include a few plants that are close to the PDC-UO, PDC-S, GP,GB phenotype.

u In addition to 558, 68 plants died before they could be classified with certainty.

t In addition to 106, 4 plants died before they could be classified with certainty.

s Classification was uncertain.

The Two B Genes of Cucurbita are Unlinked

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The available data (in reference 4 and in the present report) support the conclusion that the two B genes of Cucurbita are unlinked. One of the B genes originated in C. pepo and the other in C. maxima.

In order to ascertain the nature of the relationship between the two B genes by breeding tests, these genes were transferred to C. moschata. As a result, two different inbreds were established in C. moschata: NJ-B carries the B of C. pepo and IL-B carries the B of C. maxima (1). The evidence for their unlinked relationship is based on inheritance studies of the cross NJ-B x IL-B as well as of testcrosses.

Each of the B genes conditions precocious depletion of chlorophyll. This effect is presumably due to blockage of normal transformation of proplastids into chloroplasts, indirectly inhibiting chlorophyll synthesis.

The two genes were designated years ago by symbol B because they are responsible for the bicolor variations that potentially effect fruit pigmentation. Since they are now believed to be unlinked, the B of C. pepo is designated as B_1 and the B of C. maxima, as B_2.

B_1 and B_2 are similar in two respects. First, their primary target is the fruit. Specifically, the fruit "skin" (the outer layer of cells) is the site at which they are active in all known genetic backgrounds. Second, when these genes are inserted in certain backgrounds they effect some or all other aerial organs.

B_1 and B_2 also differ from one another. First, the expression of B_1 is strictly limited to pre-anthesis stages, whereas the expression of B_2 can be manifested over a wide range of time during fruit development, from very early pre-anthesis stages to post-anthesis stages. Second, apart from fruit, B_1 can effect a relatively few other organs (e.g., cotyledons and true leaf blades), whereas B_2 can effect all other aerial organs, depending upon the genetic background. Particularly relevant to the discussion here is the fact that, unlike B_1, B_2 can bring about precocious depletion of chlorophyll in stems.

The objective of the present report is to summarize and interpret the cumulative data bearing upon the relationship between the B genes. It should be pointed out, however, that the long-term aim is to identify the major genetic elements that play a role in sustaining the capacity of plants to synthesize chlorophyll throughout development. This broader aim includes testing the HPT (the nuclear homeostat of plastid transformation) hypothesis (2).

In a preliminary study of inheritance (1), the F_1 plants of NJ-B x

IL-B exhibited precocious depletion of chlorophyll, essentially as in IL-B, and the F_2 varied widely, ranging from lethal seedlings (albino or yellow seedlings died at the cotyledon stage) to completely green plants. The intermediate plants manifested chlorophyll deficiency selectively in different organs, at different times and in varying extent during development. Especially striking were the variegated patterns of green and yellow that effected fruits and leaves.

This preliminary study was conducted under greenhouse conditions that were not very favorable to plant growth; classification of phenotypes was often difficult or uncertain; and no firm conclusions were drawn from it. Nevertheless, the 4.1% of completely green plants in the F_2 (9 out of 217) did not disagree with the hypothesis of duplicate genes (for 15:1 ratio, chi-square = 1.6372 and P = 0.20-0.30), alluding that these plants might be conditioned by a homozygote for two standard ("wild-type") unlinked genes.

Subsequent studies of inheritance were conducted under field conditions in four growing seasons. During this period no lethal seedlings were evident in the F_2. But semi-lethal (partially yellow) plants consistently occurred in the F_2, albeit in varying proportions and in varying degrees of yellowing. The majority of these plants survived in each season, but they were difficult to reproduce. Although clear-cut lethals appeared in one environment and "semi-lethals" appeared in other environments, the overall picture of the F_2 variation was the same in its complexity. For sake of simplicity the present study of inheritance was limited to the effect of chlorophyll deficiency on fruits and stems exclusively.

Results and interpretation. The system of classification of individual plant phenotypes is described in Table 1. The data in Table 2 are based on the use of this system. The hypothetical relationship between genotypes and phenotypes is shown in Figure 1.

The data in Table 2 are of special significance. These data were gathered under field conditions during the fourth growing season, after some experience in classification was gained in the previous three seasons. In obtaining these data each plant was observed at least three times before its phenotype was determined. Furthermore, segregates of questionable phenotypes received special examination. For example, no plant was classified as completely green (GO, GS) unless it produced in sequence over 15 green fruits, and exhibited green stems throughout the season. This examination was essential because some potentially bicolor-fruited individuals produce green fruits (not more than 10) early in development, and because other individuals that potentially can exhibit chlorophyll deficiency in their stems have completely green stems early in life.

The hypothesis on the relationship between genotypes and phenotypes in Figure 1 tells us that the data in Table 2 should give a good fit to 12:3:1 ratio as well as to 15:1 ratio. This is true if the phenotypic classes are grouped according to theory (Figure 1). A good fit to 12:3:1 ratio is obtained if classes are arranged into three groups of 1 to 4, 5 to 6, and 7, i.e., 275:61:23 (X^2 = 0.73,

df = 2, P = 0.50-0.70). An excellent fit to 15:1 ratio is obtained if classes are arranged into two groups of 1 to 6 and 7, i.e., 336:23 (X^2 = 0.0150, df = 1, P = 0.90-0.95).

The grouping of the phenotypic classes (1 to 7) according to theory is supported by several observations. First, there is little doubt that the overwhelming majority if not all the persistently green plants of class 7 (GO, GS) breed true.

Second, field notes showed that the phenotypes of classes 5 and 6 reflect respectively the expression of homozygotes and heterozygotes of gene B of C. pepo (as represented here by NJ-B). These observations are supported by the excellent fit to the 12:1:2:1 ratio (275:21:40:23, X^2 = 0.7586, df = 3, P = 0.80-0.90).

Third, the lumping of classes 1 to 4 is justified by two considerations. (i) The F_1 phenotype shows that the expression of the B of C. maxima (as represented here by IL-B) is epistatic to the B of C. pepo with respect to chlorophyll deficiency in stems. (ii) The evidence in the following report shows that the phenotypes of classes 2, 3 and 4 are conditioned by the heterozygotes of the B of C. maxima; that the expression of the heterozygotes of class 2 varies greatly and that some of their phenotypes may be mistaken occasionally with the phenotype of class 1.

Finally, the data summation in Tables 3 and 4 further strengthens the conclusion that the two B of C. pepo and C. maxima are unlinked.

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Table 1. The system employed in phenotypic classification of ovaries, fruits and stems.

Class	Phenotypic symbols[z]	Description
1.	PDC-UO, PDC-S	Ovaries are subject to precocious depletion of chlorophyll over their entire surface. All ovaries and fruits are uniformly pigmented. Stems are also subject to precocious depletion of chlorophyll and appear yellow or golden.
2.	GOT, PDC-SL	Ovaries are green. But sometime following anthesis the immature fruits are subject to chlorophyll depletion. Usually only their upper region is affected, resulting in bicolor (green and yellow) pigmentation. Stems are subject to precocious depletion of chlorophyll, but expressivity is low.
3.	GOT, GS	Ovaries and fruits are as in class 2. Stems are consistently green.
4.	GO, PDC-SL	Ovaries are green. Stems are as in class 2.
5.	PDC-UO, GS	Ovaries are subject to precocious depletion of chlorophyll over their entire surface. All ovaries and fruits are uniformly pigmented. Stems are consistently green.
6.	PDC-BiO, GS	Ovaries are subject to precocious depletion of chlorophyll over their upper region, resulting in bicolor (green and yellow) pigmentation prior to anthesis. But some ovaries on the same plant are unaffected, being completely green. Stems are consistently green.
7.	GO, GS	Ovaries and stems are consistently green.

[z]Key to symbols: Bi = bicolor; G = green; L = low expressivity; O = ovaries prior to anthesis; PDC = precocious depletion of chlorophyll; S = stems; T = turning, i.e., the fruit turns from green to yellow sometime following anthesis; U = uniformly pigmented.

Table 2. Inheritance of precocious depletion of chlorophyll, based on a cross between two "precocious" inbreds of Cucurbita moschata. Seed was sown in a greenhouse on February 15 and seedlings were transplanted into a field on March 2, 1990. Naples, Florida.

	Phenotypic Classes[z]							
	1	2	3	4	5	6	7	Number of
Breeding Material	PDC-UO PDC-S	GOT PDC-SL	GOT GS	GO PDC-SL	PDC-UO GS	PDC-BiO GS	GO GS	classified plants
P_1, NJ-B[y]	0	0	0	0	5	0	0	5
P_2, IL-B[y]	5	0	0	0	0	0	0	5
F_1, $P_1 \times P_2$	5	0	0	0	0	0	0	5
F_2	249	22	1	3	21	40	23	359

[z] See Table 1 in the present report for the system of classification.

[y] See Table 1 in reference 3 for the origin and description of this inbred.

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F_2 genotypes (frequency)	F_2 phenotypic ratio (12:3:1)
1 B_1B_1 B_2B_2	
2 $B_1B_1^+$ B_2B_2	
2 B_1B_1 $B_2B_2^+$	12 B_1-B_2 plus $B_1^+B_1^+$-B_2
4 $B_1B_1^+$ $B_2B_2^+$	Classes 1+2+3+4
1 $B_1^+B_1^+$ B_2B_2	
2 $B_1^+B_1^+$ $B_2B_2^+$	
1 B_1B_1 B_2^+ B_2^+	3 B_1-$B_2^+B_2^+$, Classes 5+6
2 $B_1B_1^+$ $B_2^+B_2^+$	
1 $B_1^+B_1^+$ $B_2^+B_2^+$	1 $B_1^+B_1^+$ $B_2^+B_2^+$, Class 7

Figure 1. Hypothetical relationship between genotypes and phenotypes in the F_2 of NJ-B x IL-B. See classes of phenotypes in Table 1 and Table 2 (above).

Table 3. Tests of the hypothesis that precocious depletion of chlorophylls in two inbreds of Cucurbita moschata is conditioned by two unlinked genes, B_1 and B_2. Summary of field data obtained in Naples, Florida, from fall of 1988 to spring of 1990.

Breeding materials and tests	Classification[z] B_1-B_2 plus $B_1^+B_1^+$-B_2	B_1-$B_2^+B_2^+$	$B_1^+B_1^+$ $B_2^+B_2^+$	Number of classified plants
P_1, NJ-B[y]	0	27	0	27[w]
P_2, IL-B[x]	27	0	0	27[w]
F_1, $P_1 \times P_2$	53	0	0	53[w]
F_2 pooled	832	184	82	1098[w]
	X^2	df	P	
Deviation (12:3:1)	5.02	2	0.05-0.10	
Heterogeneity	1.82	6	0.90-0.95	
F_1 x standards[v] pooled	210	85	112	407[u]
	X^2	df	P	
Deviation (2:1:1)	4.00	2	0.10-0.20	
Heterogeneity	4.79	4	0.30-0.50	
F_1 x P_1	47	43	0	90[t]

[z] See Figure 1 for the relationship between genotypes and phenotypes.

[y] The genotype of NJ-B is $B_1B_1\_B_2^+B_2^+$.

[x] The genotype of IL-B is $B_1^+B_1^+\_B_2B_2$.

[w] Total plants of 4 tests made in 4 seasons from fall '88 to spring '90. The data from fall '88 to fall '89 are in Table 3 of reference 4. The data from spring '90 are in Table 2 of the present report.

[v] The genotype of "standards" is $B_1^+B_1^+\_B_2^+B_2^+$.

[u] Based on the sum of 3 tests made in 1989. See Table 3 in reference 4.

[t] Based on a single test made in the fall of 1988. See Table 3 in reference 4.

Table 4. F_2 data obtained from the cross of NJ-B and IL-B. These two inbreds exhibit precocious depletion of chlorophyll in similar as well as in different ways.

Growing season	Number of plants that exhibited precocious depletion of chlorophyll	Number of completely green plants	Total	X^2 (15:1)
Winter of 1985[z]	208	9	217	1.6372
Fall of 1988[y]	196	18	214	1.7059
Spring of 1989[y]	204	15	219	0.1343
Fall of 1989[y]	280	26	306	2.6362
Spring of 1990[y]	336	23	359	0.0150
				6.1286
F_2 pooled	1224	91	1315	1.0079
				5.1207

	X^2	df	P
Deviation	1.01	1	0.30-0.50
Heterogeneity	5.12	4	0.20-0.30

[z] Grown in New Brunswick, NJ, under greenhouse conditions (see reference 1).
[y] Grown in Naples, Florida, under field conditions.

Resistance to Papaya Ringspot virus-W, Zucchini Yellow Mosaic Virus, and Watermelon Mosaic Virus-2 in C. maxima

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Papaya ringspot virus type W (PRV-W), zucchini yellow mosaic virus (ZYMV) and to a lesser degree watermelon mosaic virus 2 (WMV-2) cause losses in cucurbits in Australia. There are a number of isolates of ZYMV and PRV-W in Australia (Greber et al. 1988, Herrington et al. 1989). Cucurbita ecuadorensis is resistant to PRV-W, ZYMV, WMV-2 and CMV (Provvidenti et al. 1978, 1984).

We have transferred through backcrossing, the resistances to PRV-W, ZYMV and WMV-2 from C. ecuadorensis (Cutler and Whitaker) into C. maxima (Duch) to produce cv. Redlands Trailblazer. The cultivar has fruit of flattened globe shape and a grey slightly-ribbed skin. The skin has cracked severely in some environments. The high resistance to PRV-W, ZYMV and WMV-2 following mechanical inoculation of the cotyledons cv. of Redlands Trailblazer (Table 1) should be useful in breeding programmes. However the degree of resistance appears less than that in C. ecuadorensis. Some systemic movement of virus is apparent. The reaction to PRV-W and ZYMV usually involves necrotic lesions on the cotyledons. These lesions are most obvious following inoculation with our 'K-isolate' of ZYMV. In other segregating populations of C. maxima derived from C. ecuadorensis differences in the size of lesion appear related to the degree of resistance.

Table 1. Response of C. maxima cultivars to inoculation with virus

Cultivar	Disease severity[z]			
	PRV-W	ZYMV-K	ZYMV-G4	WMV-2
Redlands Trailblazer	0.3	2.0	2.3	0.2
Queensland Blue	58.7	83.7	62.8	80.7
Jarrahdale	53.5	72.5	59.3	48.5
LSD (P=0.05)	8.0	5.9	19.5	13.9
Av. S.E.M.	2.46	1.81	5.97	4.27

[z] As percentage of leaf area chlorotic; estimated on the youngest expanded leaf at the 5 leaf stage, 17-21 days after inoculation of cotyledons with infective sap (1:20) in 0.1M phosphate buffer pH 7.0.

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Reaction of *Cucurbita pepo* L. cv. Cinderella to Strains of Cucumber Mosaic Virus

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Cucumber mosaic virus (CMV) is the only virus of any economic importance infecting zucchini and vegetable marrow crops in the United Kingdom (4). Although some resistance was reported in *Cucurbita pepo* L. 'Delicata' (2), this resistance does not appear to have been incorporated into any present day cultivars. Screening at the seedling stage of 64 accessions of C. pepo from Europe, UK, US and South America to two UK strains of CMV revealed resistance in several open pollinated cultivars (6). The highest frequency of resistant plants was found in the pumpkin 'Cinderella', a bush-type Halloween pumpkin bred from the cross UConn x Connecticut Field (Dr. A.M. Rhodes, per. comm.). The resistance was expressed as prevention of systemic infection. Inoculated cotyledons developed chlorotic local lesions identical to those found on susceptible plants but the true leaves remained symptomless. The resistance was shown to be under complex genetic control (3) and was influenced by environment, being more effective at higher temperatures and light intensities (5). The resistance was also equally effective against six strains of CMV including one strain from the US (4). We report here reaction of 'Cinderella' plants to two strains of CMV from NY known to differ in their virulence (1), and describe the effect of varying pre- and post-inoculation temperatures on symptom expression.

Methods. The test plants used were 'Cinderella', 'Cinderella S_3', a line selected from Cinderella, 'Cobham Bush Green' (CBG), a vegetable marrow previously shown to have moderate resistance, and the highly susceptible yellow zucchini 'Goldrush' (6). The seeds were pre-germinated on moist cellulose wadding and transplanted into 12 cm pots filled with Fisons Levingtons compost and placed in troughs containing modified Hewitt's nutrient solution in controlled environment cabinets. The temperature in the cabinets prior to and after inoculation with virus varied between experiments. Single plant randomization was used throughout.

The M strain of CMV was isolated from marrow in Essex, England and has previously been shown to cause severe symptoms in susceptible *C. pepo* lines (6). The FNY and SNY strains originated from muskmelon and differ greatly in aphid transmissibility and replication in muskmelon and summer squash (1). All three strains were maintained in Goldrush. Crude virus inoculum was used in experiments 1 and 2, while partially purified inoculum prepared by low and high speed centrifugation was used in experiments 3 and 4. Seedlings were mechanically inoculated at the cotyledon stage. Plants were scored for systemic infection of the true leaves 12 days after inoculation using a 0 (symptomless) to 5 (severe mosaic) scale (6). Any plant without systemic symptoms or no chlorotic local lesions (CLL) on cotyledons were discarded from the data as being escapes. Selected plants were assessed for their virus content using ELISA or by indexing to *Chenopodium quinoa*.

Results. In experiments 1 and 2 the three strains were tested for their severity on the cultivars selected. In experiment 1 there was no significant difference in virulence between the CMV strains when tested on Cinderella, CBG or Goldrush; however, there were significant differences ($p = 0.001$) between cultivars. The resistance of Cinderella, was equally effective against all strains, and the ranking of cultivars for mean symptom scores was Cinderella (0.7) < CBG (3.4) < Goldrush (4.3). In experiment 2 comparisons were made between Cinderella, Cinderella S_3 and Goldrush. . Cinderella S_3 was slightly more resistant than Cinderella but analysis of variance showed the difference was not significant. The resistance of both lines was effective against all three strains. The strains caused similar levels of symptoms on Cinderella and Cinderella S_3, but CMV-FNY and -SNY caused significantly ($p < 0.001$) less symptoms on Goldrush than did CMV-M.

In experiments 3 and 4, the effect of pre-inoculation temperature and inoculum concentration and the effect of pre- and post- inoculation temperature were studied using Cinderella and Goldrush grown in three cabinets set at 15, 20 and 25°C until inoculation. After inoculation all three cabinets were set at 20°C. Fifty plants each of Cinderella and Goldrush were grown in each cabinet, 45 of these plants were inoculated and five plants were uninoculated. The plants were inoculated with a semi-purified virus preparation of undiluted, 10^{-1} and 10^{-2} dilutions. Each dilution was inoculated to 15 plants of each cultivar in each cabinet. Diluting the inoculum or altering the pre-inoculation temperature had very little effect upon the response of Goldrush to infection by CMV (Table 1). The only significant effect was a reduction in the mean symptom score of plants grown at 25°C prior to inoculation with the 10^{-2} dilution. There was a significant percentage weight loss at any temperature for all treatments involving Goldrush. In contrast all Cinderella plants inoculated with the 10^{-2} dilution suffered no significant weight loss. Plants in these three treatments also had the lowest mean symptom scores with many plants scoring 0. There was, however, a significant ($p < 0.001$) interaction between pre-inoculation temperature and inoculum. Plants inoculated with the 0 and 10^{-1} dilutions had the highest mean symptom scores when grown at 25°C prior to inoculation, in contrast this temperature gave the lowest mean symptom score for plants inoculated with the 10^{-2} dilution.

In experiment 4 plants were again grown at the three temperatures prior to inoculation. Each cabinet contained 48 plants of each cultivar. Thirty-six plants were inoculated with the semi-purified inoculum and 12 plants were uninoculated. After inoculation plants were moved between cabinets to give all possible combinations of pre- and post-inoculation temperature combinations. All Goldrush plants displayed severe symptoms at all pre- and post-inoculation temperature regimes (Table 2). In contrast the growing conditions for the Cinderella plants significantly affected symptom severity. Plants raised at 25°C prior to inoculation had much less severe symptoms than those raised at 15 or 20°C regardless of the post-inoculation conditions. Likewise, a post-inoculation temperature of 25°C resulted in reduced symptom severity compared with 15 or 20°C. Pre- and post-inoculation temperatures of 15 and 20°C had little effect on symptom severity. Mean % weight loss of Cinderella plants was generally correlated with symptom severity (Table 2) except that plants grown continuously at 25°C showed significantly less % weight loss than plants grown under the other temperature regimes despite having a mean symptom score of 4.5.

Table 1. Effect of pre-inoculation temperature and inoculum concentration on mean symptom score (left) and percent weight loss (right) of Goldrush and Cinderella.

	Cultivar					
	Goldrush			Cinderella		
Dilution	0	10^{-1}	10^{-2}	0	10^{-1}	10^{-2}
Pre-inoculation temperature						
15°C	5.0 71.6[a]	4.9 63.8[a]	4.8 48.2[b]	1.7 46.5[c]	0.8 23.6[c]	0.7 0.0
20°C	5.0 51.3[a]	4.9 53.1[b]	4.6 36.0[b]	1.7 20.5[c]	1.5 19.4[c]	0.5 1.9
25°C	5.0 43.5[b]	4.9 55.488	3.7 45.388	2.2 23.78	2.6 22.9[b]	0.1 8.7

Significance of weight loss: [a]p 0.001, [b]p 0.01, [c]p 0.05

Table 2. Effect of pre-inoculation temperature on mean symptom score (left) and percent weight loss (right) of Goldrush and Cinderella.

	Cultivar					
	Goldrush			Cinderella		
Post-inoculation	15	20	25	15	20	25
Pre-inoculation temperature						
15°C	5.0 58.4[a]	4.7 52.5[a]	4.5 47.5[a]	3.6 30.6[b]	3.4 35.9[b]	2.0 28.5[b]
20°C	5.0 36.8[b]	5.0 69.2[a]	4.6 51.9[a]	3.3 39.2[b]	4.2 45.4[a]	2.6 31.0[b]
25°C	5.0 43.6[b]	5.0 54.1[b]	4.5 25.4[b]	1.6 11.7	1.8 18.9[b]	1.8 0.0

Significance of weight loss: [a]p 0.01, [b]p 0.05

In general, the results using ELISA and local lesion assays to estimate virus titer were in good agreement with the subjective symptom scores. Statistical correlations between ELISA results and symptom scores could not be made because it was necessary to dilute samples from plants with scores of 2 and 3 by 10^{-2} and with scores of 4 and 5 by 10^{-3}. However, by taking a spectrophotometer reading of twice that given by the healthy control as a threshold for positive virus detection, CMV was only just detectable in plants with a symptom score of 1 and increasingly higher readings were obtained with plants with scores of 2 up to 5 (A^{405} 0.24-1.16). Virus was also easily detectable in uninoculated cotyledons showing CLL on Cinderella plants with scores of 0, but not in the uninoculated true leaves of these plants. Virus could not always be detected in inoculated cotyledons which did not show CLL.

Discussion. The resistance of Cinderella was effective against the three strains of CMV and agrees with an earlier finding (4). Also, our results agree with an earlier report by Pink and Walkey (5), showing that an increase in post-inoculation temperature from 15 to 25°C produces an increase in CMV resistance in Cinderella. They postulated that this effect might be attributable to a threshold virus concentration being necessary for systemic symptoms to appear. Whether or not this threshold concentration is reached will depend upon the balance between the host's growth rate and the rate of virus replication. The 'resistance' of Cinderella presumably inhibits virus replication to such an extent that systemic symptoms do not appear. Thus in the present study dilution of the inoculum enhanced this effect in Cinderella but had no effect upon CMV symptoms in Goldrush (Table 1). In contrast raising the pre-inoculation temperature from 15 to 25°C had a differential effect depending upon the concentration of the inoculum. At 0 and 10^{-1} dilutions raising the temperature presumably favored virus replications over plant growth and mean symptom severity increased, whereas at 10^{-2} dilution the reverse occurred.

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Gene List for Watermelon

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A list of the genes of watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) was initially published in 1976 (30); lists of supplementary genes were published in 1979 (3) and 1982 (4). In 1985, CGC initiated the policy of printing a complete list of genes for the major cucurbit species on an alternating basis; each list would be updated every four years. A complete listing of watermelon genes was published in 1987 (5), and this list provides an update of the known genes in watermelon.

It is hoped that scientists will consult the following list as well as the rules of gene nomenclature for the Cucurbitaceae (see page 140) before choosing a gene name and symbol.

Gene symbol			
Preferred	**Synonym**	**Character**	**Reference**
a	-	*andromonoecious*. Recessive to monoecious.	24, 25, 32
Aco-1	-	*Aconitase-1*.	19
Aco-2	-	*Aconitase-2*.	19
Adh-1^1	-	*Alcohol dehydrogenase-1*^{1}. One of three codominant alleles, each regulating one band.	20, 21, 42
Adh-1^2	-	*Alcohol dehydrogenase-1*^{2}. One of three codominant alleles, each regulating one band. Found in *C. lanatus* var. *citroides* and *C. colocynthis*.	20, 21, 42
Adh-1^3	-	*Alcohol dehydrogenase-1*^{3}. One of three codominant alleles, each regulating one band. Found in *C. lanatus* and *C. lanatus* var. *citroides*. (Additional alleles reported in *Praecitrullus fistulosus* and *Acanthosicyos naudinianus*.)	20, 21, 42
Af	-	*Aulacophora faveicollis* resistance. Resistance to the red pumpkin beetle. Dominant to susceptibility.	36
Aps-1	*Acph-A*	*Acid phosphatase-1*.	19, 20, 21, 42
Aps-2	-	*Acid phosphatase-2*. Found in *C. lanatus* and *C. colocynthis*. (A second allele reported in *Acanthosicyos naudinianus*.)	19, 20, 21

Gene symbol			
Preferred	**Synonym**	**Character**	**Reference**
Ar-1	*B, Gc*	*Anthracnose resistance to race 1* of *Glomerella cingulata* var. *orbiculare*.	7, 12, 41
Ar-2	-	*Anthracnose resistance to race 2* of *Colletotrichum lagenarium* derived from PI 299379 and PI 189225.	14, 33, 34, 41
C	-	*Canary* yellow flesh. Dominant to pink.	24
d	-	*dotted* seed coat. Black dotted seeds when dominant for *r*, *t*, and *w*.	10, 24, 26
db	-	Resistance to gummy stem blight caused by *Didymella bryoniae* from PI 189225. Recessive to susceptibility.	23
dg	-	*delayed green*. Cotyledons and young leaves are initially pale green but later develop chlorophyll. Hypostatic to *I-dg*.	29
Dia-1	-	*Diaphorase-1*.	19
dw-1	-	*dwarf-1*. Short internodes, due to fewer, shorter cells than normal. Allelic to $dw\text{-}1^{s}$.	13, 17
$dw\text{-}1^{s}$	-	*short* vine. Allelic to *dw-1*. Vine length intermediate between normal and dwarf. Hypocotyl somewhat longer than normal vine and considerably longer than dwarf. $dw\text{-}1^{s}$ recessive to normal.	6
dw-2	-	*dwarf-2*. Short internodes, due to fewer cells.	13, 18
e	*t*	*explosive* rind. Thin, tender rind, bursting when cut.	24, 27
$Est\text{-}1^{1}$	-	*Esterase-1*^{1}. One of two codominant alleles, each regulating one band.	19, 20, 21
$Est\text{-}1^{2}$	-	*Esterase-1*^{2}. One of two codominant alleles, each regulating one band. Found in *C. lanatus* var. *citroides* and *C. colocynthis*. (Additional alleles reported in *C. colocynthis*, *C. ecirrhosus*, *Praecitrullus fistulosus* and *Acanthosicyos naudinianus*.)	19, 20, 21
Est-2	-	*Esterase-2*. (Additional alleles reported in *C. colocynthis*, *Praecitrullus fistulosus* and *Acanthosicyos naudinianus*.)	19, 20, 21
f	-	*furrowed* fruit surface. Recessive to smooth.	24

Gene symbol			
Preferred	**Synonym**	**Character**	**Reference**
Fdp-1	-	*Fructose 1,6 diphosphatase-1.*	19, 20
Fo-1	-	Dominant gene for resistance to *race 1* of *Fusarium oxysporum* f. sp. *niveum.*	9, 22
For-1	-	*Ferrodoxin oxidoreductase-1.*	19
Fwr	-	*Fruit fly resistance* in *watermelon.* Dominant to susceptibility to *Dacus cucurbitae.*	11
g	*D*	light *green* skin. Light green fruit recessive to dark green.	24, 27, 40
g^s	d^s	*striped green* skin. Recessive to dark green but dominant to light green skin.	24, 40
Gdh-1	-	*Glutamate dehydrogenase-1.* Isozyme located in cytosol.	20
Gdh-2	-	*Glutamate dehydrogenase-2.* Isozyme located in plastids.	19, 20
gms	*ms*g	*glabrous male sterile.* Foliage lacking trichomes; male sterile - caused by chromosome desynapsis.	28, 38, 39
go	*c*	*golden.* Yellow color of older leaves and mature fruit.	1
$Got\text{-}1^1$	-	*Glutamate oxaloacetate transaminase-1*^{1}. One of two codominant alleles, each regulating one band.	19, 20, 21, 42
$Got\text{-}1^2$	-	*Glutamate oxaloacetate transaminase-1*^{2}. One of two codominant alleles, each regulating one band. Found in C. lanatus var. citroides (Additional alleles reported in *C. colocynthis*, *Praecitrullus fistulosus*, and *Acanthosicyos naudinianus.*)	19, 20, 21, 42
Got-2	-	*Glutamate oxaloacetate transaminase-2.* (Additional alleles reported in *C. colocynthis*, *C. ecirrhosus*, *Praecitrullus fistulosus*, and *Acanthosicyos naudinianus.*)	19, 20, 21, 42
Got-3	-	*Glutamate oxaloacetate transaminase-3.*	42
Got-4	-	*Glutamate oxaloacetate transaminase-4.*	19, 42
I-dg	-	*Inhibitor* of *delayed green.* Epistatic to *dg*: *dg dg I-dg I-dg* and *dg dg I-dg i-dg* plants are pale green; and *dg dg i-dg i-dg* plants are normal.	29
Idh-1	-	*Isocitrate dehydrogenase-1.*	42

Gene symbol			
Preferred	**Synonym**	**Character**	**Reference**
l	-	*long* seed. Long recessive to medium length of seed; interacts with *s*.	26
Lap-1	-	*Leucine aminopeptidase-1*.	19, 20
m	-	*mottled* skin. Greenish white mottling of fruit skin.	24, 40
Mdh-1	-	*Malic dehydrogenase-1*. (A second allele reported in *Praecitrullus fistulosus*.)	21, 42
Mdh-2	-	*Malic dehydrogenase-2*. (Additional alleles reported in *C. colocynthis* and *Praecitrullus fistulosus*.)	21
Me-1	-	*Malic enzyme-1*. (Additional alleles reported in *C. colocynthis* and *Praecitrullus fistulosus*.)	19, 20, 21, 42
Me-2	-	*Malic enzyme-2*.	42
nl	-	*nonlobed* leaves. Leaves lack lobing; dominance incomplete.	16
O	-	Elongate fruit. Incompletely dominant to spherical.	25,40
p	-	*pencilled* lines on skin. Inconspicuous; recessive to netted fruit.	24, 40
Pgd-1	*6 Pgdh-1*	*6-Phosphogluconate dehydrogenase-1*. (Additional alleles reported in *Praecitrullus fistulosus* and *Acanthosicyos naudinianus*.)	19, 20, 21, 42
Pgd-2	*6 Pgdh-2*	*6-Phosphogluconate dehydrogenase-2*.	21, 42
Pgi-1	-	*Phosphoglucoisomerase-1*. (Additional alleles reported in *C. colocynthis* and *Acanthosicyos naudinianus*.)	19, 20, 21
Pgi-2^{1}	-	*Phosphoglucoisomerase-2*^{1}. One of three codominant alleles, each regulating one cytosolic band.	19, 20, 21, 42
Pgi-2^{2}	-	*Phosphoglucoisomerase-2*^{2}. One of three codominant alleles, each regulating one cytosolic band. Found in *C. lanatus* and *C. colocynthis*.	19, 20, 21, 42
Pgi-2^{3}	-	*Phosphoglucoisomerase-2*^{3}. One of three codominant alleles, each regulating one cytosolic band. Found in *C. lanatus* var. *citroides*. (Additional alleles reported in *Praecitrullus fistulosus* and *Acanthosicyos naudinianus*.)	19, 20, 21, 42

Gene symbol			
Preferred	**Synonym**	**Character**	**Reference**
Pgm-1	-	*Phosphoglucomutase-1.* (Additional alleles reported in *C. colocynthis*, *Praecitrullus fistulosus* and *Acanthosicyos naudinianus.*)	19, 20, 21, 42
Pgm-2^{1}	-	*Phosphoglucomutase-2*^{1}. One of two codominant alleles, each regulating one cytosolic band.	21, 42
Pgm-2^{2}	-	*Phosphoglucomutase-2*^{2}. One of two codominant alleles, each regulating one cytosolic band. (Additional alleles reported in *Praecitrullus fistulosus* and *Acanthosicyos naudinianus.*)	21, 42
pm	-	*powdery mildew* susceptibility. Susceptibility to *Sphaerotheca fuliginea.*	31
Prx-1^{1}	-	*Peroxidase-1*^{1}. One of two codominant alleles, each regulating one band.	19, 20, 21
Prx-1^{2}	-	*Peroxidase-1*^{2}. One of two codominant alleles, each regulating one band. (Additional alleles reported in *C. colocynthis*, *C. ecirrhosus*, *Praecitrullus fistulosus* and *Acanthosicyos naudinianus.*)	19, 20, 21
Prx-2	-	*Peroxidase-2.*	19
Prx-3	-	*Peroxidase-3.*	19
r	-	*red* seed coat. Interacts with w and t.	26
s	-	*short* seeds. Epistatic to *l.*	26
Skdh-1	-	*Shikimic acid dehydrogenase-1.*	42
Skdh-2	-	Shikimic acid dehydrogenase-2. (Additional alleles reported in *C. colocynthis*, *C. ecirrhosus*, *Praecitrullus fistulosus* and *Acanthosicyos naudinianus.*)	19, 20, 21
Sod-1	-	*Superoxide dismutase-1.* (Additional alleles reported in *C. colocynthis* and *Acanthosicyos naudinianus.*)	19, 20, 21, 42
Sod-2	-	*Superoxide dismutase-2.* (A second allele reported in *Acanthosicyos naudinianus.*)	21
Sod-3	-	*Superoxide dismutase-3.* (A second allele reported in *Praecitrullus fistulosus.*)	21

Gene symbol			
Preferred	**Synonym**	**Character**	**Reference**
Sp	-	*Spotted* cotyledons, leaves and fruit.	29
Spr-1	-	*Seed protein-1.*	20
Spr-2	-	*Seed protein-2*	20
Spr-3	-	*Seed protein-3*	20
Spr-4	*Sp-4*	*Seed protein-4*	19, 20
Spr-5	*Sp-5*	*Seed protein-5*	19, 20
su	*Bi*, su^{Bi}	*suppressor* of bitterness. Non-bitter fruit. Bitterness in *C. colocynthis* is due to *Su Su* genotype.	2, 19
t	b^{t}	*tan* seed coat. Interacts with *r* and *w*.	26
Tpi-1	-	*Triosephosphatase isomerase-1.* (Additional alleles reported in *C. colocynthis*, *Praecitrullus fistulosus* and *Acanthosicyos naudinianus*.)	19, 20, 21
Tpi-2	-	*Triosephosphatase isomerase-2.* (Additional alleles reported in *Acanthosicyos naudinianus* and *Praecitrullus fistulosus*.)	21
Ure-1	-	*Urease-1.*	21
w	-	*white* seed coat. Interacts with *r* and *t*.	26
Wf	*W*	*White flesh. Wf* is epistatic to a second gene (*y*) which conditions yellow and red flesh. *Wf_ y y* and *Wf_ Y_* are white fleshed; *wf wf Y_* is red fleshed; *wf wf y y* is red fleshed.	33
y	*r,rd,red*	*yellow* flesh ('Golden Honey' type). Recessive to red flesh and hypostatic to orange flesh (*yo*). Red flesh (*Y_*) is epistatic to orange flesh.	8, 24, 27
yo	-	*orange* flesh from 'Tendersweet Orange Flesh'. Orange flesh (*yo yo*) epistatic to yellow flesh (*y y*) from 'Golden Honey' and hypostatic to red flesh (*Y_*).	8
Yl	-	*Yellow leaf* (from 'Yellow Skin'). Incompletely dominant to green leaf.	37

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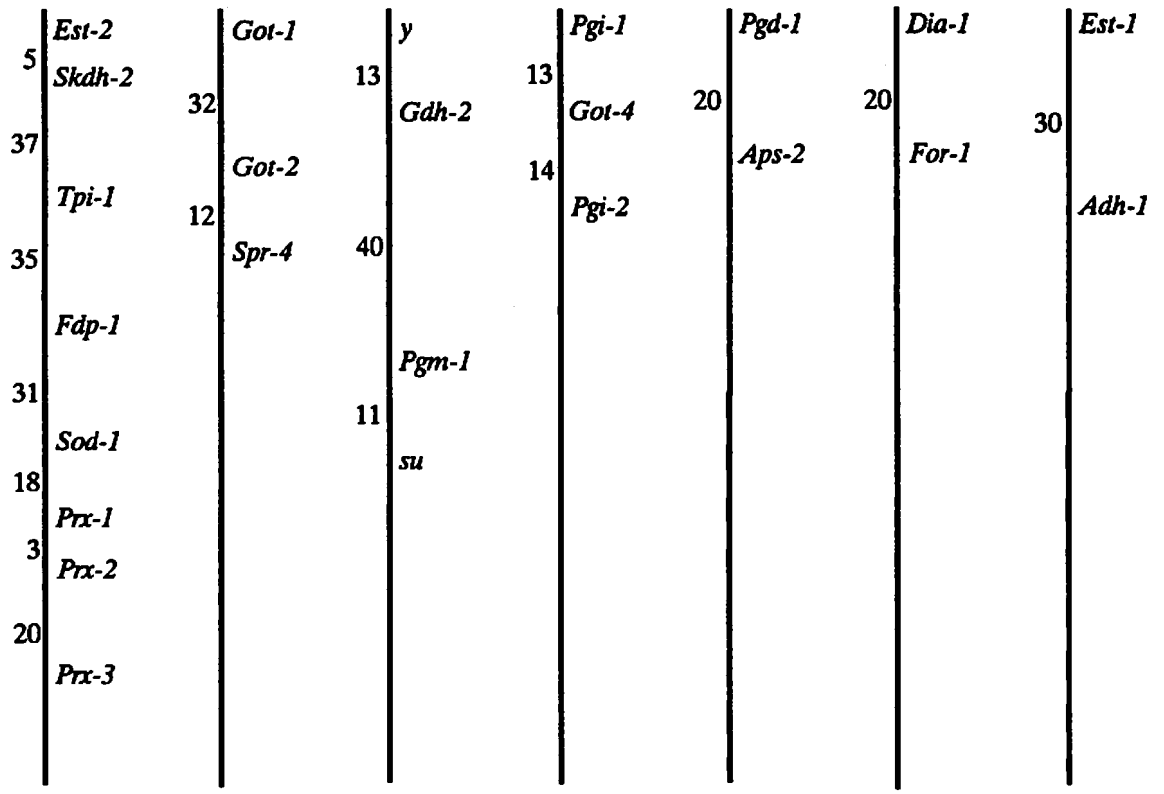
Watermelon:	W. R. Henderson
Cucurbita spp.:	R. W. Robinson
Other Genera:	R. W. Robinson

Linkage Groups in Watermelon

Seven linkage groups with 24 loci have been identified so far in watermelon. A schematic linkage map is shown below:



Me-1, *Spr-5*, *Lap-1* and *Aco-2* were independent of the above linkage groups.

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Stocks and Germplasm Desired or For Exchange

Request from the Gene Curators

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

Curators are responsible for collecting, maintaining and distributing stocks of the known marker genes upon request.

MEMBERS ARE REQUESTED TO FORWARD SAMPLES OF CURRENTLY HELD MARKER GENE STOCKS TO ONE OF THE RESPECTIVE CURATORS

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Gene Nomenclature for the Cucurbitaceae

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

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

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

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

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Hector Quemada The Upjohn Company, 9612-50-1, Kalamazoo, MI, 49001. Ph.: (616) 384-2642. FAX:(616) 384-2725.

Dennis Ray Department of Plant Sciences, University of Arizona, Tucson, AZ, 85721. Ph.:(602) 621-7612. New crops; cytogenetics.

Billy B. Rhodes Clemson Univ./Horticulture, Poole Agricultural Center, Clemson, SC, 29634-0375. Ph.:(803) 656-0410. Watermelon genetics, breeding, micropropagation, disease resistance, male sterility, triploids.

Georgette Risser Centre de Recherches Agronomiques de Avignon, Stat d'Amelior des Plantes Mar, Domaine St-Maurice, 84140 Montfavet, France.

R.W. Robinson Dept. Horticultural Sciences, New York State AES, Hedrick Hall, Geneva, NY, 14456-0462. Ph.:(315) 787-2237. FAX:(315) 787-2397. Breeding and genetics of cucurbits.

Jose Pablo Rodriguez 25 De Mayo 75, 2930-San Pedro, Buenos Aires, Republica Argentina.

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

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

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

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

Douglas M. Scheirer Libby, McNeill & Libby, Inc., P.O. Box 198, Morton, IL, 61550. Ph.:(309) 263-2651. Processing pumpkin; breeding and cultural practices.

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

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

Terry T. Sekioka Kauia Branch Station, University of Hawaii, Kapaa, HI, 96746. Ph.:(808) 822-4984. Cucumber and bitter melon breeding; disease resistance.

V.S. Seshadri 15-A/12 WEA, Karol Bagh, New Delhi 110 005, India.

Oved Shifriss 21 Walter Avenue, Highland Park, NJ, 08904.

Toshio Shiga Plant Biotech. Ctr., Sakata Seed Corp., 358 Uchikoshi, Sodegaura, Chiba, 299-02, Japan. Ph.:0438-75-2369. FAX:0438-75-7276.

Yurie Shintaku 2-10-2, Shimizu, Suginami-ku, Tokyo, 167, Japan.

Philipp W. Simon 5125 Lake Mendota Drive, Madison, WI, 53705. Ph.:(608) 264-5406. FAX:(608) 262-4743. Breeding and genetics.

Robert M. Skirvin Univ. Illinois, Dept. Horticulture, 1707 S. Orchard St., Urbana, IL, 61801. Ph.:(217) 333-1530. Micropropagation; somaclonal variation.

Jim Snyder PetoSeed Co., Inc., RR 2, Box 80A, Bridgeton, NJ, 08302-8723. Ph.:(609) 451-6231.

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

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

Joseph Stern Royal Sluis Inc., 910 Duncan Road, San Juan Bautista, CA, 95045.

Mario Steta Petoseed - Juan de Dios Batiz 109 PTD, Colonia Guadalupe, Culiacan, Sinaloa, 80220 Mexico.

M. Allen Stevens Petoseed Company, Inc., Route 4, Box 1255, Woodland, CA, 95695. Ph.:(916) 666-0931.

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Turan Tatlioglu Institut of Applied Genetics, Univ. Hannover, Herrenhauser Str. 2, 3000 Hannover, Fed. Rep. Germany.

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

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

Claude E. Thomas USDA-ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC, 29407.

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

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

David Unander P.O. Box 168, Downington, PA, 19335.

Ph.:(215) 873-9131. FAX:(215) 728-3574. Virus resistance, anti-viral natural products, vegetable breeding.

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

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

Loes van Leeuwen Sluis y Groot Semillas, Apartado 57, El Ejido (Almeria), Spain.

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

Ruud Verhoef Bruinsma Selectiebedrijven B.V., P.O. Box 24, 2670 AA Naaldwijk, The Netherlands.

Deena Decker Walters Fairchild Tropical Garden Science Center, 11935 Old Cutler Road, Miami, FL, 33156. Ph.:(305) 665-2814.

Terrence Walters Fairchild Tropical Garden Science Center, 11935 Old Cutler Road, Miami, FL, 33156. Ph.:(305) 665-2814.

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

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

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

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

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

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

Tom V. Williams Northrup King & Co., 10290 Greenway Road, Naples, FL, 33962. Ph.:(813) 775-4090. FAX:(831) 774-6852. Watermelon breeding.

Mingzhu Wu Hort. Inst., Xinjiang Acad. Agric. Sciences, Nanchang Road NO. 38, Urumqi, Xinjiang, People's Rep. China.

Colen Wyatt PetoSeed Company, Inc., Rt. 4, Box 1255, Woodland, CA, 95695.

Hisako Yamanaka Yamato-Noen Co., Ltd., 110, Byodobo-cho, Tenri-City, NARA, Japan 632.

Shyi-Dong Yeh Dept. Plant Pathology, National Chung Hsing Univ., Taichung, Taiwan, Republic of China.

Paul Yorty Musser Seed Company, Box 1406, Twin Falls, ID, 83301. Ph.:(208) 733-0077. Cucurbit breeding.

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

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

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

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

Geographical Distribution of CGC Members in the United States

Alabama
Fenny Dane
J.D. Norton

Arizona
Dennis Ray

Arkansas
Ted Morelock

California
Warren S. Barham
G.W. Bohn
Al Burkett
N.C. Chen
Paul Chung
Edward Cox
J.W. DeVerna
James C. Hollar
Krystyna M. Ladd
J.D. McCreight
Chris Miller
Brian J. Moraghan
Alison Morgan
Ken Owens
Lawrence Pierce
Vicki Pierce
Robert H. Schroeder
Joseph Stern
M. Allen Stevens
Paul Thomas
Anna Trulson
Jon Watterson
T.W. Whitaker
Colen Wyatt
Frank Zink

Colorado
Larry A. Hollar

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

Georgia
David Groff

Hawaii
Fure-Chyi Chen
Terry T. Sekioka

Idaho
Stephen L. Love
Gary Whiteaker
Paul Yorty

Illinois
John Juvik
Douglas M. Scheirer
Robert M. Skirvin

Indiana
Orie J. Eigsti
Dae-Geun Oh

Iowa
Glenn Drowns

Kansas
C.D. Clayberg

Kentucky
M. Brett Callaway

Maine
Laura C. Merrick

Maryland
Joseph H. Kirkbride Jr.
Timothy J Ng

Michigan
Rebecca Grumet
Hector Quemada

Missouri
Jeanne G. Layton

Nebraska
Dermot P. Coyne

New Hampshire
R. Bruce Carle
J. Brent Loy

New Jersey
Mark Hutton
Oved Shifriss
Jim Snyder

New Mexico
Marisa Maiero

New York
Molly Kyle
Richard McArdle
James R. McFerson
H.M. Munger
Rosario Provvidenti
R.W. Robinson
Thomas Zitter

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

Ohio
Greg Tolla

Oklahoma
E. Glen Price

Oregon
Louis Di Nitto
August C. Gabert

Pennsylvania
David Unander

Puerto Rico
Linda Wessel-Beaver

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

Texas
James R. Dunlap
Joseph O. Kuti
Wayne A. Mackay
Marvin E. Miller
Ted Rosario

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

Geographical Distribution of CGC Members in Countries Other than the United States

Argentina
Jose Pablo Rodriguez

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

Austria
Oswald Baumgartner
Herwig Teppner

Belgium
J. P. Goblet

Brazil
Wilson Roberto Maluf
Hiroshi Nagai
Seikoh Tasaki

Bulgaria
Maria Alexandrova

Canada
Zamir K. Punja

China, Peoples Republic of
Ma Kechi
Depei Lin
Wang Ming
Mingzhu Wu

China, Republic of
Lih Hung
Shyi-Dong Yeh

Colombia
Juan Jaramillo-Vasquez

Denmark
Hans Henrik Kampmann

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

England
Iraj Poostchi

France
Sofia Ben Tahar
Daniel Chambonnet
Bernard Charpiot
Robert Dumas de Vaulx
Graines Gautier
Christine Giraud
Yves Gonon
Frederic Ignart
Michel Lecouviour
Florence Picard
Michel Pitrat
Georgette Risser
M. Sockell

Germany, Federal Republic of
Martin G. Schnock
Turan Tatlioglu

Greece
Nicholas E. Fanourakis
Demetrios J. Vakalounakis

Hungary
Peter Milotay

India
K.V. Peter
V.S. Seshadri

Israel
Yigal Cohen
Haim Davidi
Ran Herman
Zvi Karchi
Shulamit Nechama
Shlomo Niego
Harry Paris
Yaacov Ventura

Italy
Paola Crino'
Andrea Lari
Alessandro Nencini

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

Jordan
Mahmoud Kaswari

Korea, Republic of
Soo Nyeon Kwack
Haktae Lim
Y.H. Om
Hyo Guen Park
Jin-Soo Song

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

The Netherlands
Wim van der Arend
A.G.B. Beekman
P.A. Boorsma
Erik de Groot
A.C. de Ruiter
K. Hertogh
Carin I. Jarl
Ad. A. Klapwijk
S. van Deursen
Ruud Verhoef
Sierd Zylstra

Peru
Edward E. Carey
Miguel Holle

Poland
Henryk O. Mackiewicz
Katarzyna Niemirowicz-Szczytt

Portugal
Antonio A. Monteiro

Romania
Virgil Poli

Saudi Arabia
Abdul Mohsen I. Al-Sulaiman

Spain
Ma Cruz Ayuso
Pilar Corella
Maria Luisa Gómez-Guillamón
Peter Kraakman
Fernando Nuez
Luis A. Roig
Loes van Leeuwen

Sweden
Louis Carl Lehmann

Thailand
Likhit Maneesinthu

Zimbabwe
Tandai Mutangadura

Covenant and By-Laws of the Cucurbit Genetics Cooperative

ARTICLE I. Organization and Purposes

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

ARTICLE II. Membership and Dues

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

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

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

ARTICLE III. Committees

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

ARTICLE IV. Election and Appointment of Committees

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

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

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

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

ARTICLE V. Publications

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

ARTICLE VI. Meetings

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

ARTICLE VII. Fiscal Year

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

ARTICLE VIII. Amendments

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

ARTICLE IX. General Prohibitions

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

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

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

ARTICLE X. Distribution on Dissolution

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

Approvals:

W. Bemis

J. D. Norton

R. W. Robinson

W. R. Henderson

M. L. Robbins

R. L. Lower

Cucurbit Genetics Cooperative

FINANCIAL STATEMENT

31 December 1990

Balance (31 December 1989)		**$3,743.40**
Receipts:		
Dues & CGC back issue orders		$4,218.00
Cucurbitaceae '89 back issue orders		$170.00
Interest on savings		$248.18
	Total	$4,636.18
Expenditures:		
CGC Report No. 13 (1990)	(Printing)	$1,198.00
	(Mailing)	$403.00
Reprint CGC back issues	Rept. 1 (1978)	$244.13
	Rept. 2 (1979)	$279.51
	Rept. 4 (1981)	$301.88
	Rept. 7 (1984)	$423.62
Call for papers (Rept. No. 14)		$94.75
Miscellaneous (postage, envelopes, supplies)		$225.11
U.S. FDIC bank fees		$12.00
	Total	$3,182.00
Balance (31 December 1990)		**$5,197.58**