

Cucurbit Genetics Cooperative

Report No. 10 June, 1987

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Resolution and notes of organization meeting, October 28, 1976, Denver Hilton, Denver, Colorado, U.S.A.

The following resolution was adopted by research workers interested in organizing a Cucurbit Genetics Cooperative:

The Cucurbit Genetics Cooperative is organized to develop and advance the genetics of economically important cucurbits.

Membership to this Cooperative is voluntary and open to workers who have an interest in Cucurbit Genetics (an invitation to participate is extended to all Horticulturists, Entomologists, Plant Pathologists, Geneticists, and others with an interest in Cucurbits).

Reports of the Cooperative will be issued on an annual basis. The reports will include articles submitted by members for the use of the members of the Cucurbit Genetics Cooperative. 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 the consent of the authors.

Further, dues for the Cucurbit Genetics Cooperative (CGC) will be \$2.50\* per year and will be used to defray cost of preparation and mailing of the annual report. Members from outside the U.S.A. are encouraged to pay dues in at least two-year increments because of bank charges incurred for clearing checks. Only postal money orders or checks drawn on U.S. banks are acceptable. The annual report will include four sections: Research Notes, Stocks and Germ Plasm desired or for Exchange, Membership Directory, and Financial Statement. Other sections will be added in future reports as desired, i.e. gene lists, linkage groups, etc.

In accordance with the above resolution, we requested that an invitation to join the CGC be published in the following:

|               |                                |
|---------------|--------------------------------|
| Agronomy News | Journal of Economic Entomology |
| Euphytica     | Journal of Heredity            |
| HortScience   | Phytopath News                 |

We are most pleased to acknowledge the assistance of the editors of these publications.

\*Dues Structure and Biennial Membership, effective 1986:

| <u>Subscriber</u> | <u>Dues</u><br><u>Biennial Membership</u> | <u>Back Issue</u><br><u>Fee</u> |
|-------------------|-------------------------------------------|---------------------------------|
| Individual        | \$12.00                                   | \$ 6.00                         |
| Libraries         | 24.00                                     | 12.00                           |

## REPORT OF TENTH ANNUAL MEETING

The tenth annual meeting of the Cucurbit Genetics Cooperative was held in conjunction with the XXII International Horticultural Congress and the 83rd Annual Meeting of the American Society for Horticultural Science on 11 August 1986 at University of California, Davis. The meeting was called to order by J. D. McCreight, Chairman.

CGC No. 9 was published at the University of Wisconsin and mailed on 18 July. This was the largest CGC report to date, and consisted of 35 reports, requests for gene stocks by the Gene Curators and an updated muskmelon gene list.

Membership stood at 210 including 60 members partially or completely in arrears. Thirty members from eight different countries including the United States were present.

Cash reserve on 9 August 1986 was \$2,595.84 US. The cost of printing and mailing CGC No. 9 was approximately \$900.00 US.

Analysis of the total cost of publishing CGC No. 8 revealed that the domestic membership dues were not sufficient to cover the cost of publishing and mailing Reports and other CGC expenses, whereas foreign membership dues were just covering these costs. The Coordinating Committee proposed a new membership structure and biennial dues (see page vi) which was motioned, seconded, and unanimously approved by the membership.

R. W. Robinson's term on the Coordinating Committee will expire in 1987.

Gene Stock Curators are T. C. Wehner (cucumber), E. Cox (muskmelon), B. B. Rhodes (watermelon), and R. W. Robinson (Cucurbita sp.). Someone is needed to serve as Curator for Other Genera stocks. Members were requested to send stocks of mutants to the respective Curator.

## MEETINGS

The Eleventh Annual Meeting of the CGC will be held in conjunction with the annual meetings of the American Society for Horticultural Science and the Inter-American Society for Tropical Horticulture, Orlando, Florida, 6 - 12 November 1987. Three cucurbit groups will meet in Orlando following the above annual meetings:

Vine Crops Advisory Committee, 12 November. (Contact J. D. McCreight)  
Squash Breeders, 13 November. (Contact G. L. Elmstrom)  
The National Muskmelon Research Group (NMRG), 13, 14 November. (Contact G. L. Elmstrom)

Pickling Cucumber Improvement Committee will meet, 28-30 October 1987, Colorado Springs, Colorado. (Contact R. A. Buescher, Food Science Dept., Rt. 11, Univ. of Arkansas, Fayetteville, AR 72701, or W. R. Moore, PPI, Box 31, Lake Charles, IL 60174.)

"Cucurbitaceae 88", Eucarpia Meeting, 1988 (see next page).

## COMMENTS FROM THE COORDINATING COMMITTEE

The Call for Papers for the 1988 Report will go out in August, 1987. Papers should be submitted to the respective Coordinating Committee member by 31 December 1987. The Report will be published by July 1988.

We are eager to hear from the membership regarding the future direction of CGC.

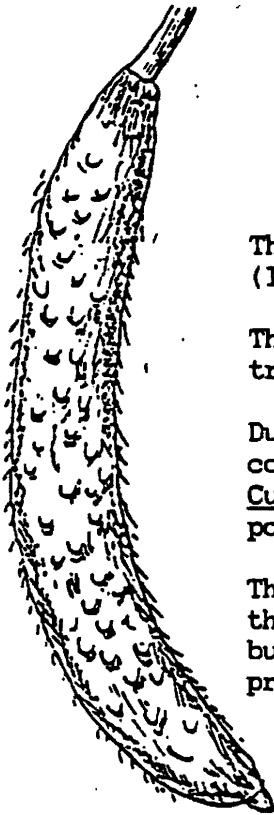
It is a pleasure to acknowledge the assistance of three people who did much for CGC this past year: Julie Flores and Tameron Wilson for typing correspondence and assisting in the day-to-day business of CGC. And Madelyn Alt in the printing and binding of CGC No. 10 at the University of Wisconsin.

### Coordinating Committee:

G. W. Elmstrom (muskmelon)  
W. R. Henderson (watermelon)  
J. A. Juvick (Cucurbita spp.)  
T. C. Wehner (cucumber)  
J. D. McCreight, Chairman

# Cucurbitaceae 88

EUCARPIA Meeting on Cucurbits Breeding  
May 31 - June 2, 1988



This meeting will be held in the Agronomic Research Center (INRA), Avignon-Montfavet, France

The official language will be English. Simultaneous translation is not planned.

During the three days of the meeting, there will be communications, posters, and visits related to Cucumis, Cucurbita, and Citrullus breeding. There will be a post-meeting tour on June 3.

The participation fee will be about 1,000 FF., and includes three lunches, a social evening, coffee and refreshments, busing from hotel to INRA and visits, and a copy of the proceedings.

"Cucurbitaceae 88" Organizing Committee: G. Risser, M. Pitrat, C. Trousse  
INRA - Station de Amelioration des Plantes Maraicheres  
Domaine St. Maurice, B.P. 94  
84140 Montfavet (FRANCE)



Sources of Combined Resistance to Powdery Mildew and Corynespora Leafspot in Cucumber

Munger, Henry M. and David P. Lane

Departments of Plant Breeding and Vegetable Crops, Cornell University, Ithaca, NY 14853.

Lane and Munger reported (1) an association between resistance to powdery mildew (PMR) and susceptibility to target leafspot (TLS) caused by Corynespora cassicola (Berk. and Curt.) Wei. This unfortunate situation has been found in most U.S. cucumber varieties. Wisconsin 2757 from C.E. Peterson was the first U.S. source in which we found resistance to both diseases. Lane crossed it with Poinsett 83, our backcross derived cucumber mosaic resistant version of Poinsett 76. Having been told that in the South Poinsett is extremely susceptible to target leafspot and finding the same true in greenhouse tests of Poinsett 83, we released the latter as germplasm only, pending incorporation of TLS resistance. This has now been accomplished through 5 backcrosses. Four sublines of Poinsett 83 with TLS are available as Poinsett 87, a germplasm rather than a variety release.

In another approach to resistance, linkage was broken in crosses between 2 near-isogenic pairs of lines with and without PMR, Cornell PMR551 X SR551 and Cornell PSMR18 X Wis. SMR18. Later, the same was done in the F<sub>2</sub> of Marketmore 80 (PMS) X Marketmore 76 (PMR). We now have lines breeding true for combined resistance to mildew and Corynespora from all three crosses. In addition we backcrossed TLS resistance from W2757 into PMR551 and Marketmore 76 because of the possibility that a source of TLS resistance with PMR might give higher PMR than one originally linked with mildew susceptibility. This seems not to be the situation; the lines with TLS resistance from mildew susceptible Marketmore 70 and SR551 have as high PMR as those with resistance from W2757 which has a slightly higher level of PMR than either Marketmore 76 or PMR551. Likewise, Poinsett 87 has no greater PMR than Poinsett 83 even though the donor of TLS resistance, W2757, had much higher PMR.

It seems clear that the single dominant gene for Corynespora resistance is the same in all the sources we studied, and that it is near the locus that determines whether a cucumber has a minimal level of PMR. It is not associated with the modifier genes required for higher levels of PMR. Nevertheless, it may be advantageous to use the coupled resistances by selecting for the dominant TLS resistance gene and thereby carrying the basic recessive gene for PMR in most of the progeny.

Literature Cited

1. Lane, David P. and Henry M. Munger. 1985. Linkage between Corynespora leafspot resistance and powdery mildew susceptibility in cucumber (Cucumis sativus). HortScience 20(3):593. (Abstr.).

## Comparison of Fruit-Set Concentration of Pickling Cucumbers under Greenhouse and Field Conditions

Haim Nerson, Harry S. Paris, Zvi Karchi, Anneke Govers, Menahem Edelstein and Yosef Burger

Department of Vegetable Crops, Agricultural Research Organization, Neve Ya'ar Experiment Station, P.O. Haifa, Israel.

The shortage and expense of hand labor for multiple harvest of pickling cucumbers are major factors gearing this crop toward mechanized once-over harvest. Accordingly, breeding of new cucumber cultivars for the pickling industry is focused on improving yield concentration. New cultivars of pickling cucumbers are released annually by public and private breeders, and this new genetic material needs to be evaluated for suitability under different cultural practices and climates before it is recommended to the growers.

Plot size is an important consideration for the evaluation of fruit-set concentration in pickling cucumber cultivars. Plots that are too large result in wasted effort and plots that are too small may not give accurate information. Results of field experiments have indicated that plots as small as 2 or 3 m<sup>2</sup> can give dependable results (1,2). Our objective here was to determine if fruit-set concentration potential under field conditions could be accurately predicted under greenhouse conditions in winter using 6 plants per cultivar.

For this objective 39 pickling cucumber cultivars were grown in a heated greenhouse (minimum night temperature 16°C) at Neve Ya'ar during the winter season of 1982. There were 6 plants per cultivar and the plants were trained to grow on cord hung from stiff wire. The plants were grown on soil mounded on straw bales, distance between plants in the row was 25 cm. A hive of honeybees was provided for pollination. These same cultivars were sown in the open field in April 1982 at 2 locations, Bet haShitta in the Yizre'el Valley and Bet She'an in the Bet She'an Valley. These 2 locations differ in climate and especially soil properties. In the fields, the plants were grown in double rows on raised beds, 2m between bed centers, at a density of 100,000 per hectare. Each cultivar had 4 replications of 6m<sup>2</sup> each. A simulated once-over harvest was conducted in the greenhouse and in the fields when 10% of the fruits were oversized. The number of marketable fruits (20-50 mm diameter) per plant is presented in Table 1. Calculation of correlation coefficients among locations shows no correlation between the Neve Ya'ar greenhouse and Bet haShitta or Bet She'an ( $r=0.15$  and  $r=0.09$ , respectively) but a highly significant ( $P 0.01$ ) correlation ( $r=0.58$ ) exists between Bet haShitta and Bet She'an. The main conclusion is that data obtained under greenhouse conditions using a small number of plants cannot serve to predict fruit-set concentration potential under field conditions.

### Literature Cited

1. Smith, O.S. and R.L. Lower. 1978. Field plot techniques for selecting increased once-over harvest yield in pickling cucumbers. J. Amer. Soc. Hort. Sci. 103:92-94.
2. Wehner, T.C. and W.H. Swallow. 1984. Optimum plot size for once-over harvest of pickling and fresh-market cucumbers. Cucurbit Genet. Coop. Rpt. 7:35-36.

Table 1. Number of fruits produced per plant in a simulated once-over harvest under greenhouse (at Newe Ya'ar) and field (Bet haShitta and Bet She'an) conditions.

| Cultivar          | Sex expression | Number of fruits per plant |              |            |
|-------------------|----------------|----------------------------|--------------|------------|
|                   |                | Newe Ya'ar                 | Bet haShitta | Bet She'an |
| Multipik          | PF             | 3.2                        | 1.1          | 0.8        |
| Trispear          | PF             | 3.2                        | 0.8          | 0.6        |
| Charger           | PF             | 2.7                        | 1.2          | 0.3        |
| Calico            | PF             | 2.7                        | 1.0          | 0.6        |
| Liberty Bell      | PF             | 2.5                        | 1.0          | 0.7        |
| Salvo             | M              | 2.4                        | 1.0          | 0.6        |
| Triplemech        | G              | 2.3                        | 1.2          | 1.1        |
| Poinsett          | M              | 2.2                        | 0.9          | 1.0        |
| Southern Belle    | PF             | 2.2                        | 1.0          | 0.5        |
| Explorer          | M              | 2.0                        | 0.9          | 0.6        |
| Tally             | M              | 2.0                        | 1.0          | 0.6        |
| Bravo             | M              | 1.9                        | 1.1          | 0.7        |
| Crispy            | PF             | 1.8                        | 1.0          | 0.7        |
| Femcap            | G              | 1.8                        | 1.1          | 0.5        |
| Liberty           | M              | 1.8                        | 0.9          | 0.6        |
| Pacer             | M              | 1.8                        | 0.6          | 0.3        |
| Greenpak          | PF             | 1.7                        | 1.3          | 0.6        |
| Triplecrown       | PF             | 1.7                        | 1.0          | 0.7        |
| Greenstar         | PF             | 1.7                        | 1.0          | 0.8        |
| Capir             | PF             | 1.6                        | 1.6          | 0.9        |
| Carolina          | M              | 1.5                        | 0.6          | 0.4        |
| Lucky Strike      | PF             | 1.5                        | 0.8          | 0.9        |
| Calypso           | M              | 1.5                        | 0.9          | 0.8        |
| Pickmore          | M              | 1.4                        | 1.6          | 0.9        |
| Peppi             | PF             | 1.4                        | 1.0          | 0.7        |
| Victory           | PF             | 1.4                        | 0.5          | 0.5        |
| Triplecross       | PF             | 1.3                        | 1.2          | 0.8        |
| Spartan Wonder    | G              | 1.3                        | 1.5          | 0.8        |
| Ashley            | M              | 1.3                        | 0.7          | 0.0        |
| Compass           | PF             | 1.3                        | 1.7          | 0.9        |
| Perfecto Verde 14 | PF             | 1.3                        | 1.3          | 0.5        |
| Fempram           | G              | 1.2                        | 0.9          | 0.8        |
| Sumter            | M              | 1.2                        | 0.5          | 0.5        |
| Tamu Chemset      | G              | 1.2                        | 1.1          | 0.9        |
| Commander         | PF             | 1.2                        | 0.9          | 0.8        |
| Pixie             | M              | 1.2                        | 0.2          | 0.1        |
| Score             | M              | 1.2                        | 0.5          | 0.6        |
| Mariner           | PF             | 1.0                        | 0.7          | 0.5        |
| Galaxy            | M              | 1.0                        | 0.6          | 0.4        |

<sup>z</sup>Sex expression determined in the greenhouse. G=<1 male flower per plant; PF=Ratio of male flowers to female flowers <1.0; M=Ratio of male flowers to female flowers ≥1.0.

Contribution No. 1903-E, 1986 series, from the Agricultural Research Organization, Bet Dagan, Israel.

## Composition of Nuclear DNA in Cucumis species

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R.K.J. Narayan

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The genus Cucumis comprises about thirty species of Asian and African origin. The diploid species are dibasic with haploid chromosome numbers,  $n=7$  or  $n=12$ . About 17 percent of Cucumis species are polyploids and their chromosome numbers vary from  $4x=48$  to  $6x=72$ . The 2C DNA amounts among diploid species range narrowly between 1.37 pg and 2.48 pg, and polyploids between 2.48 pg and 3.38 pg. The quantitative DNA changes associated with speciation in Cucumis has affected all chromosomes within each chromosome complement (5).

The nuclear DNA composition of four Cucumis species viz., C. melo, C. metuliferus, C. anguria and C. sativus were studied by buoyant density gradient analysis and thermal denaturation analysis. The method for DNA extraction was similar to that described by Bendich and Anderson (1). Analytical CsCl density gradient analysis was done in a Centriscan - 75 ultracentrifuge. Purified DNA (1.5 mg from each Cucumis species) was centrifuged to equilibrium in neutral CsCl (density 1.710 g/ml). Micrococcus lysodeikticus DNA of known buoyant density was included as a marker. For thermal denaturation analysis, DNA samples were melted in 0.1 x SSC (SSC = 0.15M NaCl + 0.015M trisodium citrate, pH 7.0) in a fully automated SP 1800 Spectrophotometer equipped with an electronically heated cell block. The increase in absorbance (hyperchromicity) was recorded automatically in a digital printout for every 0.25°C increase in temperature. The absorbance measurements after correction for solvent expansion (4) were plotted as a ratio,  $A_t/A_{25}$  (absorbance at temperature 't' divided by initial absorbance at 25°C) against temperature.

The buoyant density profiles for all species were asymmetric (Figure 1). The asymmetry suggests the presence of satellite DNA sequences with different average buoyant density. Satellite DNA sequences have been identified and characterised previously in the genomes of C. sativus and C. melo (1,2,3). The buoyant densities for the total main band DNA ranged from 1.6909 g/ml for C. anguria to 1.6920 g/ml for C. sativus. C. melo and C. metuliferus have intermediate values (Table 1). The melting profiles when compared under strictly identical ionic conditions gave us information about the base ratio of DNA as well as about heterogeneity, if any, in the dispersion of base pairs.  $T_m$  (the temperature corresponding to half the final increase in relative absorbance), base ratio (G+C content) and base compositional heterogeneity estimated from the melting profile are also given in Table 1.  $T_m$  values ranged between 69.5°C and 70.25°C. Guanine + Cytosine content (G+C<sup>m</sup>) of DNA estimated from  $T_m$  was similar to the values derived from buoyant density (7) and ranged from 38.06 to 39.89%. C. sativus DNA has an entirely different estimate of base compositional heterogeneity (14.67%) compared with the other three Cucumis species. Detailed results of the isolation, characterization and in situ hybridization of satellite DNA sequences from Cucumis species will be published elsewhere (6).

Table 1. Nuclear DNA composition of Cucumis species

| Species               | 2n | Origin | Nuclear DNA amount (pg) | Density of main band (g/ml) | T <sub>m</sub> (°C) | G+C Content (%) | Base compositional heterogeneity (%) |
|-----------------------|----|--------|-------------------------|-----------------------------|---------------------|-----------------|--------------------------------------|
| <u>C. melo</u>        | 24 | Africa | 2.483                   | 1.6911                      | 70.25               | 39.89           | 23.18                                |
| <u>C. metuliferus</u> | 24 | Africa | 2.391                   | 1.6918                      | 69.75               | 38.67           | 21.16                                |
| <u>C. anguria</u>     | 24 | Africa | 1.587                   | 1.6909                      | 70.00               | 39.28           | 22.57                                |
| <u>C. sativus</u>     | 14 | India  | 1.777                   | 1.6920                      | 69.50               | 38.06           | 14.67                                |

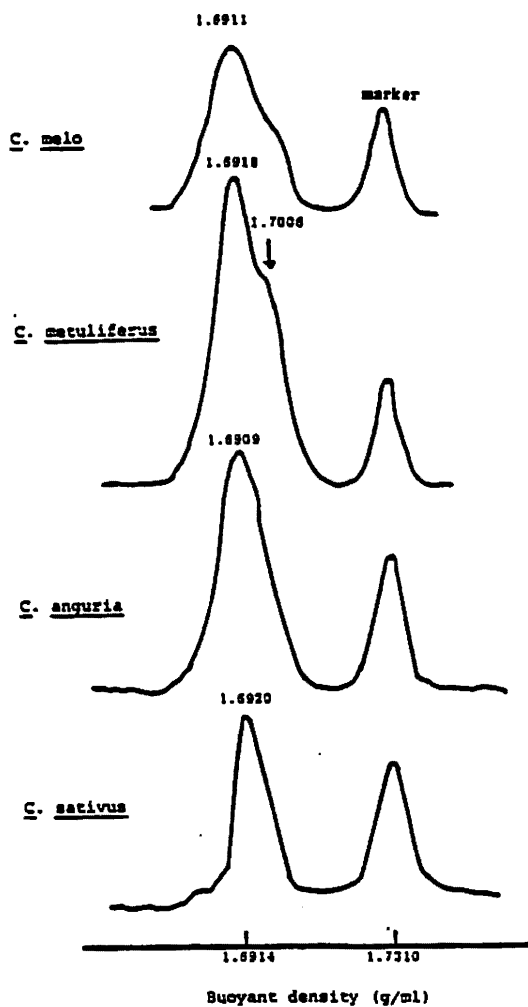


Fig. 1. Buoyant density profiles for nuclear DNA of Cucumis species with marker DNA.

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#### Blunt Leaf Apex, a Cucumber Mutant Induced by a Chemical Mutagen

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Seed of 'Wisconsin SMR 18' was soaked in 1.0% ethyl methane sulfonate for 21 hours. Plants grown from the treated seed were self pollinated, and the next ( $M_2$ ) generation was screened for mutants.

Among the mutants found was one that appears useful as a marker gene since it has a distinctive, easily recognized phenotype at all stages of development and has good vigor and fertility. The leaf apex of the mutant is more obtuse than normal. The gene reduces leaf lobing and serration. Leaves at lower nodes of the main stem are trigonous, and leaves developing later become more elongated. The mutant can be recognized in the early seedling stage, but classification is easier later.  $F_2$  segregation of 74 normal: 23 mutant agreed closely to 3:1. Hence, it is concluded to be due to a single recessive gene, which is designated bla for blunt leaf apex.

Isogenic lines differing for leaf shape can be useful for physiological and other studies. Since bla was induced in 'Wisconsin SMR-18' and self pollinated, it is isogenic to that cultivar.

## Allelism Test with Glabrous Cucumber Mutants

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The radiation-induced glabrous mutant, gl, was found by Inggamer and de Ponti (1) to be allelic to a spontaneous mutant with similar phenotype. Two additional glabrous mutants, derived from different  $M_2$  families after seed treatment with thermal neutron radiation, were crossed with gl and + plants to test for allelism. In each case, the hybrids were normal when crossed with + and glabrous when crossed with gl. Thus, two more mutations have been found to occur at the gl locus.

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## Chlorosis Induced in Glabrous Cucumber by High Temperature

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Glabrous (gl) cucumber plants are sensitive to heat stress. When grown in the field at Geneva, NY, gl plants often grow well early in the season but, as temperatures increase in midsummer, their growth is reduced and their leaves develop a mottling that is not due to a virus.

Glabrous plants grown in a greenhouse at 70°(day)-60°F (night) had good growth and development as long as they remained at that temperature regime. When some of the gl plants were moved to a growth chamber at constant 95°F, however, leaves developing subsequently were yellow. The nature of the chlorosis was not determined, but the symptoms were similar to those induced by iron deficiency. Plants dominant for gl did not develop chlorosis of young leaves in the growth chamber.

Glabrous plants returned to the 60-70° greenhouse, after one week in the 95°F growth chamber, resumed normal growth and development. Their newly developed leaves were the same color as those of gl plants not exposed to heat stress.

The plants for these tests were grown in Hoagland's nutrient solution, facilitating examination of their root systems. The gl plants differed markedly from + plants by having fewer lateral roots and root hairs, suggesting that gl may restrict ion uptake under stress conditions.

## Cordate, a Leaf Shape Gene with Pleiotropic Effects on Flower Structure and Insect Pollination

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Very few leaf shape genes are known for the cucumber. A new cucumber mutant with distinctive leaf shape was induced by thermal neutron radiation of seed of the cultivar Lemon. Leaves of the mutant have a cordate, nearly round shape with revolute margins and no serration. It is easily recognized at the first true leaf stage and at any later stage of development.

The mutant is late to flower and its open pollinated flowers usually have reduced fruit set. The open pollinated fruit are often parthenocarpic, although hand pollinated flowers can have good fruit and seed set. The short calyx segments of the mutant tightly clasp the corolla, preventing it from opening fully, thereby restricting insect pollination and reducing fruit set until the plant develops to the stage that parthenocarpic fruit form.

In view of its induction by radiation, the mutant may represent a small deletion or other chromosomal aberration, but it is inherited as a single gene with disturbed segregation. Cordate-leaved plants breed true when selfed. Hybrids of normal x cordate are nearly normal in appearance, but have reduced leaf lobing. Fewer than 25% cordate individuals occurred in most but not all  $F_2$  populations, the combined segregation data being 616 normal: 155 cordate ( $p$  for 3:1 = 0.1-.001). The symbol cor, for cordate, is assigned to the mutant.

Another cucumber mutant with cordate leaves occurred spontaneously in Russia. The Russian mutant was not available for allelism tests with cor, but they are presumably different since the Russian mutant was reported to have normal flower structure and seed production (1).

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## Genetic Variation in Soluble Solids of Cucumber Fruit

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High soluble solids of cucumber fruit can be detrimental for pickling, due to bloating (1), but may benefit flavor of slicing cucumbers. Genetic variation exists in cucumber for fruit soluble solids, though to a less extent than in some other vegetables, including muskmelon, winter squash, and tomato. McCreight, Lower, and Pharr (3) reported variation in reducing sugar content of 30 cucumber varieties and introductions. McCombs, Sox, and Lower (2) found significant differences in both reducing sugars and soluble solids content of five pickling varieties, but little variation in soluble solids of the more than 200 introductions and 50 varieties tested.

Juice was expressed from thawed transverse slices from the center of uniform sized fruit of 246 varieties and introductions of cucumber, and soluble solids was evaluated with a hand refractometer. Soluble solids content ranged from 3.0 to 5.2%. Entries with the lowest soluble solids content included PI 175686, PI 257486, PI 264668, and PI 283902.

The variety 'Lemon', despite its low yield, odd fruit shape and color, late maturity, and disease susceptibility, is popular with some home gardeners because they prefer its flavor. Interestingly, its fruit were higher in soluble solids than any other variety or introduction tested. 'Crystal Apple', which is similar to and is probably related to 'Lemon', was also high in soluble solids. The possible beneficial effect of high soluble solids on flavor, and the effect of the m gene of 'Lemon' and 'Crystal Apple' on fruit:vine ratio and fruit soluble solids, deserves further investigation.

Fruit pH of 225 introductions and varieties varied from 5.9 (for PI 175697) to 6.9 (for PI 275411 and 'Crystal Apple'), with 95% of the entries having pH of 6.0-6.6.

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Inheritance of Opposite Leaf Arrangement in Cucumis sativus L.

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Cucumber plants normally have alternate leaves, with a single leaf per node at 180° angle from leaves at adjacent nodes. The Lemon cultivar is heterogenous for leaf arrangement, with some plants having alternate leaves and others two opposite leaves per node, borne at 90° angle from the pair of leaves at the next node. In a population of 930 'Lemon' plants, 17% had opposite leaves.

Opposite leaf arrangement is unstable. All plants with opposite leaves at the first nodes of their main stem eventually convert to alternate leaf arrangement. The number of nodes with opposite leaves on 18 opposite-leaved 'Lemon' plants varied from one to ten, with a mean of 5.4. The change from opposite to alternate leaf arrangement is usually abrupt, with all nodes on the main stem above the point of transition having alternate leaves and those below opposite leaves. Occasionally, a plant has a node with a single leaf between nodes with opposite leaves. Internodes are often longer after the change from opposite to alternate leaf arrangement.

Opposite-leaved plants do not breed true for that trait. Progeny of over 100 self-pollinated 'Lemon' plants with opposite leaves all segregated for alternate vs. opposite leaf arrangement.

Opposite leaf arrangement is recessive. All F<sub>1</sub> plants of alternate x opposite or the reciprocal cross had alternate leaves.

There is no evidence of a cytoplasmic factor being involved in the inheritance of opposite leaf arrangement; similar ratios were obtained in the F<sub>2</sub> generation of reciprocal crosses between alternate- and opposite-leaved plants. The proportion of seedlings with opposite leaves was significantly less than 25% in each of 26 F<sub>2</sub> populations. The combined segregation ratio was 875 alternate to 86 opposite.

Tkachenko (1) concluded that at least three genes are required to produce opposite leaves. An alternate explanation is that inheritance is simple, but the single recessive gene has incomplete penetrance. Evidence agreeing with, though not conclusively proving the latter hypothesis, was obtained when linkage was detected between opposite leaf arrangement and two genes known to be on the same chromosome. Genes of 'Lemon' for sex expression (m) and five fruit locules (l) are linked (2), and were associated with opposite leaves in segregating generations. In coupling phase F<sub>2</sub> populations there were 302 alternate +: 51 alternate m: 31 opposite +: 22 opposite m (contingency  $X^2 = 22.9$ ,  $p > .001$ ) and 139 alternate +: 29 alternate l: 21 opposite +: 19 opposite l plants (contingency  $X^2 = 16.6$ ,  $p > .001$ ). It is suggested that the gene for opposite leaves that is linked with m and l be given the symbol opp.

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#### Independence of gl and yc

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Glabrous (gl) and yellow cotyledon (yc) cucumber mutants were originally considered, on the basis of no double recessives being found in a repulsion phase  $F_2$ , to be linked on the same chromosome (1). Subsequently, however, more informative coupling  $F_2$  data from larger populations have been obtained that indicate that the two seedling marker genes are not closely linked. Combined segregation data from four populations (382 ++: 107 + yc: 112 gl +: 35 gl yc) did not differ significantly ( $48.5 \pm 2.0$  % crossing over) from that expected for independent assortment of the two genes.

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## Longevity of Cucumber Seed

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PI 197087, a cucumber introduction from India, is notable for its multiple disease resistance. It has been reported (1,2,4) to be resistant to seven diseases and red spider mites. It is a parent of the disease resistant varieties Pixie and Polaris.

Despite the importance of this introduction, seed was unavailable from U.S.D.A. Plant Introductions when H. M. Munger requested it for powdery mildew tests. Fortunately, however, I was able to provide Dr. Munger with remnant seed from a sample of PI 197087 received in 1971. The seed had been produced at Geneva, NY by Plant Introductions in 1955. Since 1971, the seed had been stored at 40°F and 17% relative humidity. The 31 year-old seed had over 90% germination and produced vigorous seedlings in 1986. Plant Introductions increased their stocks of PI 197087 recently, and perpetuation of this important introduction seems assured.

Several genetic stocks of cucumber also germinated in 1986, although the seed was produced in 1961. Germination of some of the 25 year-old cucumber seed was very slow, but after germination the plants developed normally and segregation ratios in F<sub>2</sub> populations were not altered by aging of the seed.

Bass (2) reported little decline in germination of seed of most varieties of cucumber, muskmelon, and watermelon after nine years storage at 10°C and 50% RH. It is now evident that cucumber seed can retain its viability for more than a quarter of a century if kept cool and dry.

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Imposed Environmental Stresses and Their Relationship to Sex Expression in  
Cucumber (Cucumis sativus L.)

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It has long been clear that environmental factors affect cucumber (Cucumis sativus L.) fruit development (6), and sex expression (1,3,8). In a study by Lower et al. (4) plant density had little effect on the number of pistillate flowers in a series of gynoecious inbreds, but increased density reduced pistillate flowers in several gynoecious (G) x monoecious (M) hybrids made with 'GY 14-1'. Increases in staminate flower production were observed with increasing densities in several G x M F<sub>1</sub> hybrids. Under more controlled environmental conditions no pistillate flowers were present on determinate monoecious plants under a 9 hrs. photoperiod at high temperatures or on indeterminate monoecious plants at longer photoperiods (12 hrs.) and high temperatures (5). In studies by Cantliffe (2) a series of gynoecious processing cucumber cultivars produced more staminate flowers at a light intensity of 17,200 lux than at 8,600, 12,900 or 25,800 lux, while a gynoecious inbred line (MSU 713-5) was unaffected. In contrast to plants grown at a constant temperature of 16°C, those grown at 30°C produced some staminate flowers.

The USDA cucumber breeding program has made use of the hermaphroditic (H) character in cucumber in an attempt to improve gynoecious sex stability. It was concluded that there are no differences in fruit quality or yield among GxG and GxH hybrids produced by genetically similar inbred lines (7). However, because a critical sex expression test has not been devised, it has not been determined whether the hermaphrodite character in these G x H hybrids provides increased sex stability. Therefore, a study was designed to identify environmental stresses which could be used to demonstrate whether sex stability differences exist among these G x G and G x H hybrids.

The imposed stresses included a cool temperature shock, "inadequate" fertilizer concentrations, and sand used as a growing medium. Five genotypically different plants subjected to these conditions and grown under greenhouse conditions were compared to those grown at higher temperatures (25 to 30°C), in a standard greenhouse soil medium, and having received "adequate" fertilization. The factorial set of treatments (5 genotypes x 2 temperatures x 2 fertilizations x 2 growing media) were evaluated in a completely randomized design with 4 replications for plant weight, days to anthesis, number of staminate flowers, and number of pistillate flower abortions in the first 10 nodes. Seven plants in a pot (20 cm in diameter) were considered the experimental unit. The genotypes used to evaluate differences for gynoecious sex stability were two gynoecious inbreds (WI 1379 and WI 1983) and 3 hybrids [1983G x 1379H, 1983G x 1379G, and 1983G x 1379A (andromonoecious)].

Cool shocking of plants was accomplished by subjecting a portion of the plants at the third true leaf stage to 4°C for 8 hrs. under darkened conditions. Plants grown in sand and designated as "adequate" fertilizer, received a standard fertilizer mixture (GEWA porportioner delivering 1.35 kg

Peter's 20-20-20, 0.45 kg urea and 56 gm Peter's micronutrient) to saturation at 4 day intervals beginning 8 days after sowing. Those assigned the "inadequate" fertilizer treatment received the same applications, except for the last 3 watering periods where they received consecutively 50, 25 and 25% of full strength fertilizer. Plants grown in greenhouse mix (1 sand:2 soil:1 peat:1 perlite, by volume) receiving "adequate" fertilizer were treated initially 14 days after sowing and then weekly thereafter, while those designated as "inadequate" fertilizer treatments were given 50, 25 and 25% of full strength during the last 3 waterings. Treatments were chosen such that plants growing under the imposed stress treatments reached anthesis, but were visually stunted.

Significant differences were detected among the main effects: fertilization, media type, and genotype (Table 1). Amount of fertilizer application effected average plant height, and differences in average plant height, days to anthesis, and percentage of staminate flowers and pistillate abortions were produced by the affects of the growing media used. In addition, genotypic differences were observed for all traits measured. Significant 2 and 3-way interactions were not detected, except between soil and genotype for days to anthesis and percentage of staminate flowers.

Means and LSD's for these main effects are provided in Table 2. "Inadequate" fertilization and growth of plants in sand reduced the average plant weight and percentage of pistillate abortions, but increased days to anthesis and the percentage of staminate flowers. The inbred line WI 1379G was on the average smaller, and WI 1983G flowered later than other genotypes. While the percentage of staminate flowers was highest in WI 1983G, the percentage of pistillate abortions was highest in the hybrid 1983G x 1379H. Correlation coefficients (Table 3) indicate that a moderately positive association exists between percentage of pistillate abortions and average plant weight, and a lack of association between average plant weight and days to anthesis. Moreover, a negative association was also detected between days to anthesis and percentage of pistillate abortions.

These data suggest that although the "inadequate" fertilization schedule employed produced smaller plants, it did not affect the sex expression of those plants. In contrast, the sand media produced changes in sex expression, and therefore might be useful treatment in future studies. The hermaphroditic character in the cross 1983G x 1379H provided the most stabilizing effect on sex expression of the crosses using 1983G as the maternal parent. Although these results are equivocal, they suggest that the hermaphrodite character may be potentially useful in stabilizing the gynocious sex expression under environmental stress conditions used in this study.

Table 1. Mean squares for average plant weight, days to anthesis, percentage of staminate flowers, and pistillate flower abortion of 5 cucumber (*Cucumis sativus* L.) genotypes grown in 2 soil media, under 2 types of fertilization after cold shock at the third true leaf stage.

| Source of variation | df | Mean Square             |                  |                       |                          |
|---------------------|----|-------------------------|------------------|-----------------------|--------------------------|
|                     |    | Average plant wt. (gms) | Days to anthesis | Staminate flowers (%) | Pistillate abortions (%) |
| Cool shock (C)      | 1  | 0.16                    | 3.84             | 51.30                 | 3.09                     |
| Fertilization (F)   | 1  | 5.36***                 | 4.39             | 0.01                  | 0.21                     |
| Soil media (S)      | 1  | 109.04***               | 1386.64***       | 353.01***             | 372.71***                |
| Genotype (G)        | 4  | 7.85***                 | 12.85*           | 253.89***             | 56.97***                 |
| C x F               | 1  | 0.07                    | 0.48             | 21.59                 | 0.69                     |
| F x S               | 1  | 0.91                    | 0.44             | 8.22                  | 0.01                     |
| C x S               | 1  | 0.90                    | 0.31             | 33.71                 | 1.63                     |
| F x G               | 4  | 1.72                    | 6.00             | 4.14                  | 6.48                     |
| S x G               | 4  | 2.40                    | 22.99**          | 254.40***             | 6.42                     |
| C x G               | 4  | 2.10                    | 1.26             | 34.90                 | 1.90                     |
| C x F x S           | 1  | 0.14                    | 2.86             | 13.79                 | 10.91                    |
| C x S x G           | 4  | 1.49                    | 3.78             | 39.07                 | 5.60                     |
| F x S x G           | 4  | 0.33                    | 7.64             | 6.70                  | 8.85                     |
| C x F x G           | 4  | 1.09                    | 8.67             | 7.40                  | 11.66                    |

\*,\*\*,\*\*\* Significant at the 5%, 1%, and 0.1%, respectively.

Table 2. Means and LSD's of significant main effects (temperature, fertilization, soil media and genotype) for morphological traits of cucumber (*Cucumis sativus* L.) genotypes grown under several environmental conditions.

| Source of variation              | Trait                   |                  |                       |                          |
|----------------------------------|-------------------------|------------------|-----------------------|--------------------------|
|                                  | Average plant wt. (gms) | Days to anthesis | Staminate flowers (%) | Pistillate abortions (%) |
| <b>Fertilization<sup>z</sup></b> |                         |                  |                       |                          |
| adequate                         | 2.69                    | -                | -                     | -                        |
| inadequate                       | 2.31                    | -                | -                     | -                        |
| <b>Soil media<sup>y</sup></b>    |                         |                  |                       |                          |
| greenhouse mix                   | 3.31                    | 39.5             | 0.3                   | 6.67                     |
| sand                             | 1.61                    | 45.6             | 3.4                   | 3.51                     |
| <b>Genotype<sup>x</sup></b>      |                         |                  |                       |                          |
| 1379G                            | 2.16                    | 42.46            | 0.66                  | 4.40                     |
| 1983G                            | 2.68                    | 43.37            | 6.96                  | 4.23                     |
| 1983G x 1379H                    | 2.56                    | 42.32            | 0.00                  | 7.36                     |
| 1983G x 1379A                    | 2.69                    | 41.90            | 0.59                  | 4.32                     |
| 1983G x 1379G                    | 2.39                    | 42.03            | 0.84                  | 5.45                     |
| LSD <sup>w</sup> (5%)            | 0.32                    | 1.27             | 2.16                  | 1.59                     |

<sup>z</sup> Mixed for GEWA proportioner at 1.35 kilograms Peters 20-20-20, 0.45 kilograms urea & 56 gms Peter's micronutrient.

<sup>y</sup> Greenhouse media contains equal amounts of a 1 sand:2 soil:1 peat:1 perlite (by volume) mix.

<sup>x</sup> G & H indicate the inbred line is gynocious and hermaphroditic, respectively.

<sup>w</sup> Mean comparison for genotype.



Table 3. Phenotypic correlation coefficients of morphological traits of 5 cucumber (Cucumis sativus L.) genotypes grown under several environmental conditions.

| Trait <sup>2</sup>       | Average plant wt. | Staminate flowers (%) | Pistillate abortions (%) |
|--------------------------|-------------------|-----------------------|--------------------------|
| Staminate flowers (%)    | -0.34***          | -                     |                          |
| Pistillate abortions (%) | 0.49***           | -0.34***              | -                        |
| Days to anthesis         | -0.67***          | 0.30***               | -0.56***                 |

<sup>2</sup>Data were merged over growing conditions since scatter plots of data were randomly distributed.

\*\*\* Significant at 0.1% probability level.

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Use of Silver Thiosulfate as a Potential Tool for Testing Gynoecious Sex Stability in Cucumber (Cucumis sativus L.)

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Cucumber (Cucumis sativus L.) plants under stress produce more staminate flowers than those grown under optimal conditions (1,2,4,8). For once-over mechanical harvest, processing cucumbers with uniform flowering and concentrated fruit set are essential (5). Among the genetic manipulations which has shown potential for increasing sex stability and uniform flowering is the characterization and introduction of the hermaphroditic character (3). Hybrids made using hermaphrodites have been hypothesized to be sexually more stable under conditions of environmental stress (7).

Recently improved pollen induction on gynoecious inbreds achieved by using foliar applications of silver nitrate (9) or aminoethoxyvinylglycine (6) instead of gibberellic acid, has resulted in increased interest in using gynoecious (G) x (G) hybrids. If hermaphroditic (H) pollen parents could be developed which conferred adequate yield and quality, then a less-costly pollen supply could be used in hybrid seed production. This and the fact that G x H hybrids may be sexually more stable makes this an attractive methodology.

However, a consistent, rapid test for gynoecious sex stability has not been developed. This has made identification of sex stable genotypes difficult, and has been at least partially responsible for the lack of widespread acceptance of the hermaphroditic character in plant breeding programs. Determining whether silver thiosulfate could be used to identify plants which possessed a more stable gynoecious character is thought to be desirable. If a dosage could be identified which would provide a consistent stress threshold, then rapid procedures might be developed for seedling screening for sex stability. Naturally a prerequisite for such a procedure would necessitate that the action of any chemical be unaffected by minor changes in the test environment (photoperiod, temperature, relative humidity, etc.).

A series of experiments were designed to determine at what threshold concentration and dosage silver thiosulfate would cause gynoecious genotypes to produce staminate flowers. In the first experimental sequence, a dosage of either 10 or 20  $\mu$ l silver thiosulfate was administered at concentrations of 6, 3, 1.5, and 0.75 mM to cotyledons of 7-day-old seedlings of the gynoecious processing inbred line WI 1701 which had been germinated in pots 10 cm in diameter. Treatments were arranged in a randomized complete block design with 4 replications having 2 subsamples. Plants were grown to 10 flowering nodes in a standard growing medium (2 soil: 1 sand: 1 peat: 1 perlite by volume) in a greenhouse under a 16 hr. photoperiod at 25 to 28°C and 40 to 60% RH. The average quanta per day from solar radiation over 16 hrs. during this experimental period ranged from 423 to 843  $\mu$ mol s<sup>-1</sup> m<sup>-2</sup> and plants were supplemented by fluorescent lights (Sylvania - F96T12/CW/VHO) providing approximately 40  $\mu$ mol s<sup>-1</sup> m<sup>-2</sup> at the shoot apices to extend the photoperiod. Number of nodes with staminate flowers were recorded. Based on these data, potentially effective concentrations were isolated and used in 2 subsequent experiments where plants of WI 1701 were grown under similar

environmental conditions as those described above, except that the average solar radiation ranged from 240 to 281  $\mu\text{mol s}^{-1}\text{m}^{-2}$ .

Data suggest that 20  $\mu\text{l}$  was a consistent, effective dosage for the conversion of nodes to the staminate condition, except at 6mM (table 1). The 20  $\mu\text{l}$  dosage at 6 mM was apparently above the threshold concentration (Table 1, exp. 1), but elicited a response in a second series of experiments (Table 2). Moreover, in the second series of experiments 1.5 mM at 20  $\mu\text{l}$  was not as effective as 6 mM. The lowest effective concentration was 0.75 mM (Table 1, exp. 2).

One explanation which might account for disparities observed between experiments was the difference in average quanta of solar radiation received by the plants during experimentation. If this hypothesis is correct, then effective threshold silver thiosulfate concentrations may vary and implementation of this procedure for sex stability testing will require careful control of environmental conditions.

Table 1. Mean percentage of staminate flowers after treatment of the USDA processing cucumber inbred line WI 1701 with  $\text{Ag}(\text{S}_2\text{O}_3)_2$  at the cotyledon stage.

| Conc.<br>(mM) | $\text{Ag}(\text{S}_2\text{O}_3)_2$ <sup>z</sup> | Dosage<br>( $\mu\text{l}$ ) | Percentage of staminate flowers |      |              |      |
|---------------|--------------------------------------------------|-----------------------------|---------------------------------|------|--------------|------|
|               |                                                  |                             | Experiment 1 <sup>y</sup>       |      | Experiment 2 |      |
|               |                                                  |                             | node                            |      | node         |      |
|               |                                                  |                             | 1-5                             | 1-10 | 1-5          | 1-10 |
| 6             |                                                  | 10                          | 65                              | 33   | -            | -    |
|               |                                                  | 20                          | 5                               | 3    | -            | -    |
| 3             |                                                  | 10                          | 40                              | 20   | 20           | 10   |
|               |                                                  | 20                          | 75                              | 38   | 80           | 35   |
| 1.5           |                                                  | 10                          | -                               | -    | 15           | 15   |
|               |                                                  | 20                          | -                               | -    | 80           | 35   |
| 0.75          |                                                  | 10                          | -                               | -    | 0            | 0    |
|               |                                                  | 20                          | -                               | -    | 70           | 50   |
| Control       |                                                  |                             | 5                               | 3    | 13           | 7    |

<sup>z</sup>Plants treated at the cotyledons stage; approximately 7 days after sowing.

<sup>y</sup>4 replications.

Table 2. Mean percentage of staminate flowers after treatment of the USDA processing cucumber inbred line WI 1701 with  $\text{Ag}(\text{S}_2\text{O}_3)_2$  at the cotyledon stage.

| Ag(S <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> <sup>z</sup><br>Conc.<br>(mM) | Percentage of staminate flowers |      |              |      |
|-------------------------------------------------------------------------------|---------------------------------|------|--------------|------|
|                                                                               | Experiment 1 <sup>y</sup>       |      | Experiment 2 |      |
|                                                                               | node                            |      | node         |      |
|                                                                               | 1-5                             | 1-10 | 1-5          | 1-10 |
| 1.5                                                                           | 10                              | 5    | 5            | 3    |
| 3                                                                             | 18                              | 9    | 10           | 5    |
| 6                                                                             | 30                              | 15   | 23           | 13   |
| Control                                                                       | 0                               | 0    | 0            | 0    |

<sup>z</sup>Plants treated at the cotyledon stage with 20 µl AgNO<sub>3</sub>.

<sup>y</sup>4 replications with 2 subsamples.

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Resistance to Downy Mildew [Pseudoperonospora cubensis (Berk. & Curt.) Rostow.] and Scab (Spot Rot) [Cladosporium cucumerinum Ellis & Arthur] in Cucumis spp.

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Many important centers of origin are facing immediate threats to their native plant populations due to man's encroachments. Resources are often inadequate to provide for all the expeditions needed to salvage critically needed germplasm from these areas and therefore efforts should be made to study existing collections. There are about 1131 documented accessions of the Cucumis species, excluding melo and sativus (1). Undoubtedly some of these are duplications. Nevertheless, given the available information, approximately 22, 17, 15, 11, and 12% of the Cucumis spp. accessions are held by the Philippines, German Democratic Republic, United States, Netherlands, and Japan, respectively. The reported African collections are held by Nigeria and South Africa and these are only 1 and 4% of the world collection, respectively. It becomes clear that countries which are in the major center of origin of these wild species do not retain large working collections. Therefore, research which will lead to a determination of the potential usefulness of these species must occur in countries which have relatively large collections, expertise, and adequate financial resources.

The existing collection of wild species houses resistances to several pests which are not currently found in C. sativus germplasm (eg. nematode, and green mottle mosaic). With the advent of workable recombinant DNA techniques, these resistances may in fact be transferred between cross-incompatible species. However, the accessions frequently lack information on essential characteristics which the plant breeder and/or molecular biologist will need for effective selection of initial germplasm. Resistance to economically important pathogens of commercial cucumber is of primary importance. Therefore, we felt it useful to obtain preliminary information on the susceptibility of 9 Cucumis spp. to downy mildew [Pseudoperonospora cubensis (Berk. & Curt.) Rostow.], and scab (spot rot) [Cladosporium cucumerinum Ellis & Arthur].

Materials. Seeds were sown in steam sterilized coarse grade vermiculite placed in wooden flats (52 x 36 x 7 cm.). Separate plantings were made for each pathogen. Resistance checks (GY-14 for downy mildew and 'Wisconsin SMR-18' for scab) and susceptible checks ('Wisconsin SMR-18' for downy mildew and 'Straight 8' for scab) were seeded in the middle row of each flat. Plants which received no fertilization were grown in a greenhouse (22-35°C day, 20-26°C night) under supplemental lighting (16 day) provided by Sylvania VHO fluorescent lamps at about 80  $\mu\text{mol s}^{-1}\text{m}^{-2}$ . Plants were inoculated when cotyledons had expanded (approximately 7-10 days after sowing).

Since P. cubensis is an obligate parasite, it was maintained on infected 'Wisconsin SMR-18' seedlings placed in a 16°C growth chamber. Two to three weeks after inoculation, sporulation was induced by incubating the 'SMR-18' at 100% RH, 20°C for 24 hr. Cotyledons were placed in distilled water and rubbed with a finger to dislodge the sporangia and the concentration adjusted to  $1.2 \times 10^7$  sporangia/ml. Inoculum was maintained at 20°C for 1-2 hr. to induce zoospores. A 0.01-0.03 ml. droplet of inoculum was placed on the center of the adaxial surface of one cotyledon using a Pasteur pipet.

Seedlings were incubated in the dark at 100% RH, 20°C for 48 hr., and returned to the greenhouse. Seven to eight days after inoculation plants were rated as resistant (R), intermediate (I), or susceptible (S) (Table 1).

Three to six days before inoculation C. cucumerinum spores were streaked onto potato dextrose agar slants and incubated at 20°C. Inoculum was prepared by scraping the spores into distilled water and adjusting the concentration to  $4 \times 10^5$  spores/ml. The inoculum was sprayed on the hypocotyl using a Badger (TM) air brush model 100XF. Plants were incubated in the dark at 100% RH, 20°C for 48 hr., and then were maintained in a greenhouse at 20°C. Six to nine days after inoculation plants were rated as resistant (R), intermediate (I), or susceptible (S) (Table 1).

Results. Of the 3 C. africanus accessions surveyed, all possessed some individuals which showed an intermediate (I) response to downy mildew, but were susceptible to scab. The C. anguria accessions PIs 386037 and 386051 possessed a relatively high frequency of individuals resistant to downy mildew. However, recent data (2,3) suggests that these accessions from Iran have been misclassified and are in reality C. melo. Only the PI's 196477, 438570, and 438678 have resistance to downy mildew. Likewise, only PI 282442 provides resistance to scab. Collections of C. dipsaceus, C. ficifolius, and C. myriocarpus possesses individuals with intermediate resistance to downy mildew and resistance to scab. The C. hardwickii, C. longipes, and C. zeyheri had resistance to both pathogens. Although the germination of C. pustulatus was low, an individual was observed to be resistant to downy mildew. These data indicate that inter- and intraaccession variation for these diseases exist in the wild species studied. Using this type of information, selected Cucumis spp. with disease resistance can be used for transfer of other genes into commercial C. sativus germplasm.

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Table 1. Response of nine Cucumis spp. to downy mildew and scab.

| Cucumis spp.   | PI No.              | Downy mildew <sup>a</sup> |    |    | Scab <sup>b</sup> |            |    |
|----------------|---------------------|---------------------------|----|----|-------------------|------------|----|
|                |                     | R                         | I  | S  | R                 | I          | S  |
| C. africanus   | 299571              |                           | 3  | 9  |                   |            | 24 |
|                | 299572              |                           | 12 | 14 |                   |            | 25 |
|                | 374151              |                           | 15 | 3  |                   |            | 10 |
| C. anguria     | 147065              |                           | 1  | 23 |                   |            | 22 |
|                | 196477              | 1                         | 2  | 18 |                   |            | 17 |
|                | 233646              |                           | 1  | 5  |                   | late germ. |    |
|                | 282442              |                           | 2  | 21 | 23                |            | 1  |
|                | 320052              |                           |    | 12 |                   |            | 11 |
|                | 386029              |                           | 20 | 3  |                   |            | 23 |
|                | 386031              |                           | 4  | 15 |                   |            | 16 |
|                | 386033              | 1                         | 2  | 15 |                   |            | 12 |
|                | 386034              |                           | 23 |    |                   |            | 23 |
|                | 386035              |                           | 7  |    |                   |            | 8  |
|                | 386036              | 1                         | 13 |    |                   |            | 16 |
|                | 386037              | 7                         | 9  |    |                   |            | 13 |
|                | 386039              |                           | 9  |    |                   |            | 17 |
|                | 386044              |                           | 15 |    |                   |            | 32 |
|                | 386046              |                           | 27 |    |                   |            | 22 |
|                | 386048              |                           | 11 |    |                   |            | 21 |
|                | 386050              | 3                         | 18 |    |                   |            | 28 |
|                | 386051              | 12                        | 11 |    |                   |            | 30 |
|                | 386052              |                           | 5  |    |                   |            | 20 |
|                | 386053              | 1                         | 25 |    |                   |            | 31 |
|                | 386054              | 5                         | 2  | 2  |                   |            | 10 |
|                | 386055              |                           | 8  |    |                   |            | 10 |
|                | 386062              |                           | 16 |    |                   |            | 19 |
|                | 386064              | 3                         | 11 |    |                   |            | 28 |
|                | 386066              |                           | 6  |    |                   |            | 14 |
|                | 386067              | 1                         | 7  |    |                   |            | 26 |
|                | 386070              |                           | 12 | 4  |                   |            | 26 |
|                | 386071              | 1                         | 10 |    |                   |            | 21 |
|                | 386075              |                           | 8  | 1  |                   |            | 13 |
|                | 386076              |                           | 3  |    |                   |            | 6  |
|                | 386080              |                           | 17 |    |                   |            | 19 |
|                | 386082              |                           | 33 |    |                   |            | 29 |
|                | 386085              |                           | 9  |    |                   |            | 15 |
| 386086         | 1                   | 6                         |    |    |                   | 12         |    |
| 390449         | 1                   |                           | 20 |    | 1                 | 25         |    |
| 438570         | 1                   | 2                         | 13 |    | 1                 | 15         |    |
| 438678         | 2                   | 3                         | 13 |    | 1                 | 14         |    |
| 438679         |                     | 3                         | 19 |    |                   | 26         |    |
| 442176         |                     |                           | 1  |    |                   | 6          |    |
| C. dipsaceus   | 236468              |                           | 6  | 2  |                   | 9          | 4  |
| C. ficifolius  | 196844              |                           | 9  | 8  |                   | 2          | 16 |
| C. hardwickii  | 462369              | 1                         | 3  | 20 |                   | 1          | 24 |
| C. longipes    | 249894              | 5                         | 9  | 5  |                   | 17         |    |
| C. myriocarpus | 282449              |                           | 2  | 19 |                   | 15         | 4  |
|                | 374153              |                           | 6  | 11 |                   | 3          | 1  |
| C. pustulatus  | 343699              | 1                         |    | 1  |                   |            | 8  |
| C. zeyheri     | 282450              | 7                         | 4  | 12 |                   | 1          | 22 |
|                | 315212              | 2                         | 15 | 7  |                   |            | 22 |
| C. sativus     | GY 14               |                           | 32 |    |                   |            |    |
|                | Straight 8          |                           |    |    |                   |            | 38 |
|                | Wisconsin<br>SMR 18 |                           | 6  | 32 |                   | 37         |    |

<sup>a</sup>R=no symptoms or faint spot

I=necrotic spot or pale yellow spot with or without necrosis

S=yellow to bright yellow spot with or without necrosis

<sup>b</sup>R=no symptoms or blisters on hypocotyl

I=restricted tan lesions on hypocotyl

S=range from small tan lesions on hypocotyl to dead

## Linkage Relationships of Watermelon Mosaic Virus-1 Resistance in Cucumber

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'Wisconsin 2757' is a valuable source of multiple disease resistance for cucumber (1). It is resistant to nine diseases, but is susceptible to watermelon mosaic virus-1 (WMV-1). A source of resistance to WMV-1, due to a single recessive gene, is 'Surinam' (2). 'WI 2757', 'Surinam, and the F<sub>2</sub> of this cross were tested for WMV-1 resistance (Wmv-1-1), scab resistance (Ccu), powdery mildew resistance, spine color (B), gynocious sex expression (F), and bitterness (bi).

There was no significant association of wmv-1-1 with Ccu, B, F, or powdery mildew resistance in the F<sub>2</sub>. Close linkage was observed, however, between wmv-1-1 and bi. There were 86 susceptible, bitter F<sub>2</sub> plants: 40 susceptible, nonbitter: 43 resistant, bitter: 1 resistant, nonbitter.

Classification for the nonbitter gene can be easily accomplished in the seedling stage. Thus, bi may be a useful seedling marker for resistance to watermelon mosaic virus-1.

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## Genotype-Environment Interaction for Cucumber Yield in 23 North Carolina Environments

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Pickling and fresh-market cucumber cultivars are regularly tested for fruit yield to determine their usefulness for growers in particular areas of North Carolina. Plant breeders developing new cultivars for the state need to know how many environments should be used in order to assure adequate performance. Genotype-environment interaction provides an indication of whether testing should be carried out at several different environments, or if one is sufficient to represent the region (1).

Yield in a multiple-harvest trial can be estimated efficiently by counting number of fruits per plot in a single harvest at the stage when 10% oversized fruits are present (3, 4). Two or 3 replications of each entry provided the most information for the resources expended.

The objective of this study was to determine the importance of genotype-environment interaction for fruit yield using 23 combinations of years, seasons and locations in North Carolina.

Methods. A random set of hybrids, inbreds, cultivars and experimentals (referred to as genotypes hereafter) was tested in North Carolina for yield and quality. The 44 genotypes were chosen to represent new and old, tall and dwarf, resistant and susceptible (to southern foliar diseases), and pickling and fresh-market types (22 genotypes each).

Plots were 25-plant rows 1.5 m long and 1.5 m apart seeded on raised beds. The environments consisted of 2 years (1984, 1985), 3 seasons (spring, summer, fall), 4 locations (Clayton, Clinton, Castle Hayne and a stress field in Clinton), and 2 replications. The stress environment consisted of heavier soil and only half the irrigation, fertilization and pesticide applications given the main Clinton location. Data were analyzed for only 23 of the 24 environments tested, because the Fall-1984-Castle Hayne trial was destroyed by a hurricane. Yield was measured as fruit number in a single-harvest trial. Harvest was made when 10% of the fruits were oversized in the check plots ('Calypso' for pickling and 'Poinsett 76' for fresh-market genotypes).

Results. Analysis of variance indicated significant mean squares for genotype and environment, and for the interaction (GxE) of the two, with the largest variances for environment. The GxE component was only 32 to 52% as large as the genotype component (Table 1). In a 2-season study run at 1 location and year, Strefeler and Wehner estimated additive genetic genotype and its interaction with environment in 3 fresh-market cucumber populations. The ratio of GxE interaction to genotype ranged from 58 to 112% depending on population.

Since the ranking of cucumber genotypes changed significantly over the North Carolina environments sampled in this study, plant breeders must test in more than one environment for development of cultivars adapted to the area. The next step is to determine how many environments are needed for proper representation of the performance of North Carolina conditions.

Table 1. Variance components for yield in pickling and fresh-market cucumbers.<sup>2</sup>

| Source of variation           | Degrees of freedom | Mean square | Expected mean square <sup>y</sup>          | Variance component estimate |
|-------------------------------|--------------------|-------------|--------------------------------------------|-----------------------------|
| <b>Pickling cucumbers</b>     |                    |             |                                            |                             |
| Genotype                      | 21                 | 3250**      | $\sigma^2 + r\sigma_{GE}^2 + re\sigma_G^2$ | 67                          |
| Environment                   | 22                 | 7618**      | $\sigma^2 + r\sigma_{GE}^2 + rg\sigma_E^2$ | 170                         |
| G x E                         | 458                | 137**       | $\sigma^2 + r\sigma_{GE}^2$                | 35                          |
| Error                         | 477                | 67          | $\sigma^2$                                 | 67                          |
| <b>Fresh-market cucumbers</b> |                    |             |                                            |                             |
| Genotype                      | 21                 | 4665**      | $\sigma^2 + r\sigma_{GE}^2 + re\sigma_G^2$ | 99                          |
| Environment                   | 22                 | 7190**      | $\sigma^2 + r\sigma_{GE}^2 + rg\sigma_E^2$ | 160                         |
| G x E                         | 460                | 128**       | $\sigma^2 + r\sigma_{GE}^2$                | 31                          |
| Error                         | 478                | 65          | $\sigma^2$                                 | 65                          |

\*\* Indicates significant mean square at the 1% level according to F test.

<sup>2</sup> Estimates are from a test with 23 environments and 22 genotypes of cucumbers.

<sup>y</sup> Constants for estimation of variance component mean squares are  $g=22$ ,  $e=23$ , and  $r=2$ .

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## Value of 12 Season-Location Combinations for Cucumber Yield Trials in North Carolina

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New experimental breeding lines of pickling and fresh-market cucumbers are tested in many North Carolina environments before release as cultivars for use in the state. The early stages of testing are most efficiently done using 2 to 3 replications per entry harvested once-over when the check cultivar has 10% oversized fruits (3, 4). The optimum plot size for such trials is approximately 1.5 x 1.5m (2).

The most efficient component of environment to use as the factor for repetition is years, followed by seasons, locations, and, finally, replications (5). However, additional years of testing add greatly to the time required to complete a cycle of selection and pollination. Therefore, it may be best to use season-location combinations as the method of sampling the environment before releasing an experimental line as a new cultivar. A number of methods have been proposed for choosing the ideal environment (1). The best environments for testing new lines for use in a region are representative of the region, and provide maximum separation of the lines involved. In other words, a good environment should be correlated with the mean of all environments, and have a large standard deviation among line means.

The objective of this study was to determine which season-location combinations make the best testing environments for use in initial testing of experimental lines in North Carolina.

Methods. A random set of hybrids, inbreds, cultivars and experimentals was tested in North Carolina for yield and quality. The 44 lines were chosen to represent new and old, tall and dwarf, resistant and susceptible (to southern foliar diseases), and pickling and fresh-market types (22 lines each).

Plots were 25-plant rows 1.5 m long and 1.5 m apart seeded on raised beds. The environments consisted of 2 years (1984, 1985), 3 seasons (spring, summer, fall), 4 locations (Clayton, Clinton, Castle Hayne and a stress field in Clinton), and 2 replications. The stress environment consisted of heavier soil and only half the irrigation, fertilization and pesticide applications given the main Clinton location. Yield was measured as fruit number in a single-harvest trial. Harvest was made when 10% of the fruits were oversized in the check plots ('Calypso' for pickling and 'Poinsett 76' for fresh-market genotypes).

Results. The correlations for line performance at each of the 12 season-location environments with line performance over all environments were all significant at the 1% level (data not shown). However, coefficients of determination ( $r^2$ ) and standard deviations for line means ( $\sigma_L$ ) were highest at the Clinton and Stress locations for all seasons tested (Table 1). No other season-location combinations had both high  $r^2$  and high  $\sigma_L$ .

Therefore, a test involving 2 plots per line could be run efficiently using a spring season at the Clinton and Stress locations (in May and June), leaving enough time to intercross or self-pollinate the best lines before the end of the growing season (in July through September).

Table 1. Coefficients of determination ( $r^2$ ) and standard deviations of line means ( $\sigma_L$ ) for 16 location-season combinations evaluated for usefulness as yield trial environments in North Carolina.

| Environment         |        | 2-year mean <sup>z</sup> |                          |
|---------------------|--------|--------------------------|--------------------------|
| Location            | Season | $r^2$ (%)                | $\sigma_L$ (fruits/plot) |
| Clayton             | Spring | 56                       | 11                       |
|                     | Summer | 42                       | 10                       |
|                     | Fall   | 56                       | 14                       |
| Clinton             | Spring | 65                       | 13                       |
|                     | Summer | 64                       | 12                       |
|                     | Fall   | 66                       | 11                       |
| Stress <sup>y</sup> | Spring | 60                       | 18                       |
|                     | Summer | 60                       | 14                       |
|                     | Fall   | 62                       | 11                       |
| Castle Hayne        | Spring | 53                       | 13                       |
|                     | Summer | 37                       | 8                        |
|                     | Fall   | 61                       | 9                        |

<sup>z</sup>Data are from 1984 and 1985 tests of 22 pickling and 22 fresh-market cucumber lines.

<sup>y</sup>Stress location was at Clinton, with low inputs of fertilizer, pesticides and irrigation.

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Optimum plant density for multiple-harvest yield of determinate and indeterminate cucumbers

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The determinate plant type is conditioned by a recessive, single-gene mutant (1). It causes the main stem to terminate in flower buds, and is often associated with shorter vine length as well. Our observations indicated that plants with the determinate gene have higher, more concentrated yield than the normal plants. The objective of this study was to evaluate the optimum plant density for determinate and normal cucumbers.

Methods. The experiment was run in 1981 through 1983, although we will report only the 1983 data here. Treatments included 2 crops (pickle, fresh-market), 4 determinate lines (M21, Castlepik, Spacemaster, Bush Champion), 4 normal lines of similar type, (Clinton, Calypso, Tablegreen 65, Marketmore 76), 4 densities (65, 129, 258 and 516 thousand plants per hectare), and 4 replications.

Plots were 3 x 3 m with 4 rows 0.75 m apart in each. Alleys 1.5 m wide separated plot ends. Vine length was measured at early fruiting stage, sampling 3 plants per plot at random. Plots were harvested when fruits around 51 mm diameter (60 mm for the fresh-market lines) were observed in the check plots. The center 2 rows of each plot were harvested, leaving the border rows to provide the proper amount of competition for each density. Harvests were made twice weekly for 3 weeks, making a total of 6 for the pickling and 5 for the fresh-market cucumbers.

Results. The normal vines had 115cm-long main stem, and the determinates had 77cm-long stems. There was no difference in length between the pickling and fresh-market types.

The determinates generally had higher yield than indeterminate types, but since the lines were not isogenic, that comparison is not valid. Highest yield (as fruit weight) occurred at 65 thousand plants per hectare in both the pickling and fresh-market cucumbers for both determinate and indeterminate types (Table 1). Highest fruit value for the pickling cucumbers occurred at 129 thousand plants per hectare. In 1981, only fresh-market cucumbers were tested and densities of 37 to 143 thousand plants per hectare were evaluated. However, the optimum still occurred at 64 thousand plants per hectare (data not shown).

Therefore, determinate plant types have the same optimum plant density as normals for yield in multiple-harvest production systems in North Carolina. That is similar to the results obtained by Munger for multiple-harvest of fresh-market cucumbers in New York (2). Even though the vines of determinate types are shorter, the plants require as much space as normal plant types for optimum yield. Perhaps the roots are the limiting factor in space requirement.

Table 1. Yield of determinate and normal pickling and fresh-market cucumbers in multiple-harvest<sup>2</sup>.

| Plant type  | Density (th pl /ha) | Pickling yield |         | Fresh-market yield (Mg/ha) |
|-------------|---------------------|----------------|---------|----------------------------|
|             |                     | (\$/ha)        | (Mg/ha) |                            |
| Determinate | 65                  | 4967           | 55      | 40                         |
|             | 129                 | 5038           | 53      | 30                         |
|             | 258                 | 5008           | 45      | 26                         |
|             | 516                 | 4250           | 35      | 21                         |
| Normal      | 65                  | 3418           | 41      | 27                         |
|             | 129                 | 3560           | 38      | 24                         |
|             | 258                 | 4082           | 35      | 15                         |
|             | 516                 | 3532           | 27      | 12                         |
| LSD (5%)    |                     | 509            | 4       | 9                          |

<sup>2</sup>Data are means of 4 replications and are summed over 6 harvests (5 for the fresh-market cucumbers).

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A Seedling Test for Resistance of Cucumber Lines to Fruit Rot Caused by Rhizoctonia solani

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Fruit rot caused by Rhizoctonia solani Kuhn. is one of the 3 most important cucumber diseases in North Carolina. Significant differences among lines were found for resistance to the disease in both field and detached-fruit (lab) tests (2). Differences were significant and heritable, and there are commercially acceptable lines that are at least moderately resistant, such as M21 and Marketmore 76.

Plant breeding programs would be able to incorporate fruit rot resistance into new cultivars more efficiently if an easy test could be used for preliminary selection work. The field test is useful for final selection trials, but involves much work to inoculate and evaluate plants. The detached-fruit test is useful for isolating particular factors for evaluation, but we are able to handle more lines with the field test.

A seedling test for Rhizoctonia resistance like the one now used for scab resistance would be ideal if it were correlated with the field test. Previous efforts to develop a correlated test using a damping-off test were not successful, since it was not correlated with fruit rot resistance (1).

The objective of this study was to develop a seedling test that would be easy to run and significantly correlated with the field test for fruit rot resistance.

Methods. Plants were grown in the greenhouse at the University of Wisconsin, Madison. A randomized complete block experimental design was used with 2 stages (cotyledon and 2nd leaf), 5 lines (M21, Marketmore 76, Poinsett 76, Sumter and Supergreen), 4 inoculation methods (water spray control, soil drench, spray, and agar blocks), and 4 replications of 10 plants each. The lines were chosen to represent a range of resistance. Inoculation methods involved spraying the plants with water, drenching the base of each plant with 1 ml of inoculum, spraying inoculum on the leaves until droplets formed, or placing 4 agar blocks on one leaf per plant.

Inoculum was prepared by transferring inoculum (Rhizoctonia-infested soil, isolate R5-H-2) to petri plates containing potato dextrose agar. After 2 days at room temperature, 10 ml of distilled water was added to each plate and the culture rubbed with a rubber policeman. The resulting liquid was ground in a blender for 1 min., and a concentration of  $8.5 \times 10^4$  fragments/ml was produced for the spray or drench treatments. Agar blocks were produced by placing a 2 x 2 mm piece of inoculated potato dextrose agar onto petri plates containing water agar. After 6 days at room temperature, 5 mm diameter

disks were punched out of the water agar using a cork bore. The disks were then placed, inoculum side down, on the leaves.

**Results.** The best treatment was the agar block method at the 2nd leaf stage (Table 1). The spray and drench methods did not work, possibly because of the grinding of the mycelium which apparently reduces the virulence of the fungus (Dr. E. Echandi, personal communication, 1986).

The cotyledon stage treatments did not work, but the plants were left in the chamber an extra day, so that may have been the cause (data not shown). The agar block test of plants at the 2nd leaf was correlated with previous field and detached-fruit (lab) tests, but not with the damping-off test (Table 2). Additional work is needed to refine the method, but it will undoubtedly be useful in preliminary screening work to incorporate moderate resistance into new cultivars.

Table 1. *Rhizoctonia* resistance in the seedling test for 2nd leaf stage compared with previous field, laboratory and damping-off tests.

| Cultivar<br>or line | Seed<br>source | Seed-<br>ling <sup>z</sup> | Field | Lab | Damping<br>-off |
|---------------------|----------------|----------------------------|-------|-----|-----------------|
| Marketmore 76       | Asgrow Seed    | 6                          | 1     | 1   | 5.4             |
| M21                 | NC State Univ  | 4                          | 2     | 1   | 4.4             |
| Poinsett 76         | PetoSeed       | 6                          | 2     | 1   | 4.4             |
| Sumter              | Asgrow Seed    | 4                          | 3     | 4   | 3.1             |
| Supergreen          | Harris-Moran   | 9                          | 10    | 34  | 3.2             |
| LSD (5%)            |                | 3                          | -     | -   | -               |

<sup>z</sup>Data are means over 4 replications of 10 plants each.

Table 2. Correlations among 4 *rhizoctonia* resistance tests.

| Test     | Field | Lab   | Damping<br>-off |
|----------|-------|-------|-----------------|
| Seedling | 0.77+ | 0.83+ | -0.23           |
| Field    | -     | 0.99* | -0.60           |
| Lab      | -     | -     | -0.51           |

\*,+ Indicates significance at the 1 or 10% levels, respectively.

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## Inheritance of Littleleaf and Multi-branched Plant Type in Cucumber

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Little-leaf is a plant type discovered in an inbred selection in Arkansas. Goode *et al.* (1) reported that the  $F_1$  of several crosses was normal, and the  $F_2$  (resulting from self-pollination of the  $F_1$ ) segregated in a ratio which suggested a 3:1. It has leaves, stems and flowers smaller than normal, and multi-branched vines. The multi-branched habit is similar to the *Cucumis sativus* var. *hardwickii* line, LJ 90430. The Goode *et al.* little-leaf line ('Little John') holds up better than normal leaf lines such as 'Calypso' under the rough treatment they receive during multiple harvest. It also seems to grow better than the normal cultivars under dry or windy conditions. That is at least partly due to the small, tough leaves on the plant.

Breeders are using the new plant type to incorporate the small leaf habit and/or the multi-branched habit into commercially acceptable lines. Although it appears to be a single-gene mutant, and the symbol ll has been used as a symbol to represent the trait (2), no inheritance data have been published. The objective of this study was to determine the inheritance of the two most important traits of 'Littleleaf', small leaves, and multi-branched plant habit.

**Methods.** 'Little John' was crossed as the paternal parent to 'Wisconsin 2757' and the  $F_1$  were self-pollinated and backcrossed to each parent to form 6 generations for testing: Pa (W2757), Pb (Little John),  $F_1$ ,  $F_2$ ,  $BC_1$ Pa, and  $BC_1$ Pb. The 6 generations were tested in the field at Hancock, Wisconsin in the summer, 1986 using randomized, replicated and coded treatments to prevent bias during evaluation. Data were checked for fit to the expected 3:1 ratio using a Chi-square analysis. The multi-branched data were coded such that 0 to 2 = few, and 3 to 5 = many branches. Branching was also evaluated using generation variances where phenotypic =  $F_2$  variance, and additive =  $2(F_2) - (BC_1Pa) - (BC_1Pb)$  variances. Narrow-sense heritability was calculated as additive/phenotypic variance.

**Results.** Multi-branched habit did not follow any single-gene inheritance pattern we could determine. It must, therefore, be considered a quantitative trait. Unfortunately, heritability of multi-branch was approximately 0 using generation variances. In the cross of two other inbreds (data not shown) the heritability was 0.61, but the data from several crosses we have studied is extremely variable.

On the other hand, the littleleaf trait of 'Little John' is controlled by a single recessive gene (Table 1). Some of the plants were misclassified (see, for example, the  $F_1$  data) due to environmental variability for leaf size. Thus, littleleaf can be considered a good marker, already named ll, where 'Little John' carries the recessive mutant allele, and W2757 (as well as most other cucumber lines) carries the dominant, wild-type allele.

Table 1. Inheritance of littleleaf trait (ll) in the cross of 'W2757' (Pa) x 'Little John' (Pb).

| Group       | Leaf size | Allele    | Number of plants per type per generation |    |    |       |       |     |
|-------------|-----------|-----------|------------------------------------------|----|----|-------|-------|-----|
|             |           |           | Pa                                       | Pb | F1 | BC1Pa | BC1Pb | F2  |
| Observed    | Normal    | <u>Ll</u> | 45                                       | 0  | 50 | 79    | 38    | 116 |
|             | Small     | <u>ll</u> | 0                                        | 45 | 4  | 1     | 40    | 38  |
| Expected    | Normal    | <u>Ll</u> | 45                                       | 0  | 54 | 80    | 39    | 38  |
|             | Small     | <u>ll</u> | 0                                        | 45 | 0  | 0     | 39    | 38  |
| $\chi^2$    |           |           | -                                        | -  | -  | -     | 0.05  | 0   |
| Probability |           |           | -                                        | -  | -  | -     | 0.8   | 0.9 |

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Autonomous Apomictic Propagation of Cucumis ficifolius A. Rich and C. anguria L.

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The incompatibility barrier of C. ficifolius with C. sativus was studied in 1985 under greenhouse conditions. Treatments included sib- and self-pollination and unpollinated female flowers to check apomictic seed production (Table 1.). The reason for this check was the in vitro investigation regarding pollen tube behavior observed under ultraviolet microscope of diallel crosses between wild species of Cucumis and C. sativus. After self-pollination of C. ficifolius, the pollen tubes either widened or branched in an unusual way just above the embryo sac.

The results obtained were unexpected, showing both in C. ficifolius and C. anguria that 100% of the isolated female flowers produced fruits and 100% of their fruits had numerous seeds (122 to 270 in C. ficifolius compared to 249 in C. anguria). The high percentage of fruits with seeds following hybridization of C. ficifolius and C. anguria with C. sativus (Table 1) confirms also the presence of apomictic propagation in these species. Obviously, both C. ficifolius and C. anguria are species with autonomous apomictic propagation, i.e. propagation independent of pollination and the effect of pollen tubes.

To establish the apomictic propagation type in these species, we investigated the way the embryo sac and the embryo are formed. It was found that C. ficifolius and C. anguria form two female flower types: embryo sacs of the Polygonum type are formed in the first type, and diploid generative apospory embryo sacs (prevailing) (Fig. 1) and embryo sacs of the Polygonum type are formed in the second type. The generative apospory embryo sacs have 8 nuclei. Their egg-apparatus is three-celled, but their synergids have no vacuoles and their nuclei are located at the chalazal end near the egg nucleus. The behavior of one of the synergids of the generative apospory embryo sacs is quite impressive. It grows fast and leaves behind the other synergid and egg (Fig. 2). It is evident that it will divide and form an embryo. This kind of behavior of some embryo sac cells is characteristic of automixis. We think that automixis is one of the mechanisms of apomictic propagation with C. ficifolius and C. anguria.

Table 1. Results of the identification of apomictic propagation.

| Cross <sup>z</sup> | Gen-eration <sup>y</sup> | No. of crosses made | Fruits produced |     | Fruits with seeds |     | No. seeds per fruit |
|--------------------|--------------------------|---------------------|-----------------|-----|-------------------|-----|---------------------|
|                    |                          |                     | No.             | %   | No.               | %   |                     |
| fic x sat          | F <sub>1</sub>           | 92                  | 10              | 11  | 10                | 100 | 153                 |
| fic x ang          | F <sub>1</sub>           | 6                   | 5               | 83  | 5                 | 100 | 109                 |
| fic                | sib                      | 7                   | 2               | 29  | 2                 | 100 | 46                  |
| fic                | self                     | 22                  | 2               | 9   | 2                 | 100 | 78                  |
| fic                | iso                      | 13                  | 13              | 100 | 13                | 100 | 270                 |
| ang x sat          | F <sub>1</sub>           | 19                  | 4               | 21  | 4                 | 100 | 193                 |
| ang x fic          | F <sub>1</sub>           | 2                   | 1               | 50  | 1                 | 100 | 101                 |
| ang                | sib                      | 4                   | 0               | 0   | 0                 | 0   | 0                   |
| ang                | self                     | 1                   | 1               | 100 | 1                 | 100 | 141                 |
| ang                | iso                      | 2                   | 2               | 100 | 2                 | 100 | 249                 |

<sup>z</sup>

fic = C. ficifolius

sat = C. sativus

ang = C. anguria

<sup>y</sup>sib = intra cultivar (sib) mating

self = self-pollination

iso = isolated, unpollinated flowers

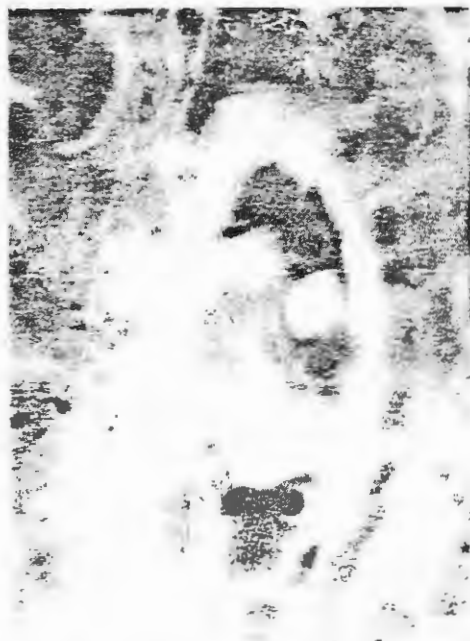


Fig. 1. Generative apospory embryo sacs in C. ficifolius.



Fig. 2. Beginning of automixis in C. ficifolius.

Pollen Mother Cell Meiosis in the Haploid of Cucumis ficifolius A. Rich

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Haploid forms in the genus Cucumis ficifolius have not been reported previously in the literature. Tetraploid ( $2n=48$ ) (1,2) and hexaploid ( $2n=72$ ) forms (1) have been established. With C. ficifolius ( $2n=24$ ) autonomous apomictic propagation has been found (3), both after the isolation of non-fertilized female flowers and when hybridized with C. sativus, C. melo var. flexuosus, etc. Progenies resulting from crosses of C. ficifolius with C. sativus and C. melo var. flexuosus appeared to be matromorphic. In the progeny of isolated non-fertilized female flowers of C. ficifolius, one out of eight plants had a chromosome number of  $2n=12$ . It is evident that besides the unreduced egg cells, egg cells with reduced chromosome number in C. ficifolius embryo sacs are capable of apomictic development as well.

These haploids develop slowly, but preserve the species traits. Plants are as tall as the original diploids, but its leaves are smaller and deeper cut. Staminate and pistillate flowers are smaller, with shorter petioles, and have petals that are not joined at the base.

Meiosis in pollen mother cells of the haploids is characterized by disturbances. During diakinesis, 12 non-spiralized chromosomes are established. During Metaphase I they are strongly spiralized and in 92.9% of pollen mother cells, they are scattered along the divisional spindle that is most often considerably elongated and twisted (Fig. 1). It is followed by mitosis and diad formation of microspores (Fig. 2). It is evident that the haploid has inherited from the parent plant the ability to form unreduced gametes. In 7% of pollen mother cells chromosomes are incorporated into the metaphase plate, and from Metaphase I through Anaphase I, 1 to 10 chromosomes are divided into chromatids. Through Anaphase I different numbers of chromosomes move in the direction of the divisional spindle poles, but most often it is 6+6. In Metaphase II and Anaphase II, the location and division of chromosomes is irregular. In a part of the pollen mother cells, three-pole divisional spindles are formed. Microspore types consisted of diads (66.7%), tetrads (18.6%), polyads (11.0%) and triads (3.7%). Only 28% of the pollen is fertile.

When self-pollinated, the haploid did not set fruits. When crossed with C. sativus, 80% of the pollinated flowers set fruits with seeds. The average seed number per fruit was 3.5. The embryo takes up half of the volume of the seed. On an artificial medium in culture, the white part of the seed formed plants that died of secondary infections. The haploid can be maintained through in vitro propagation from apical meristems.

This is the first case we know of where the incompatibility barrier between the wild species of Cucumis in the melo group and Cucumis sativus L. has been overcome.

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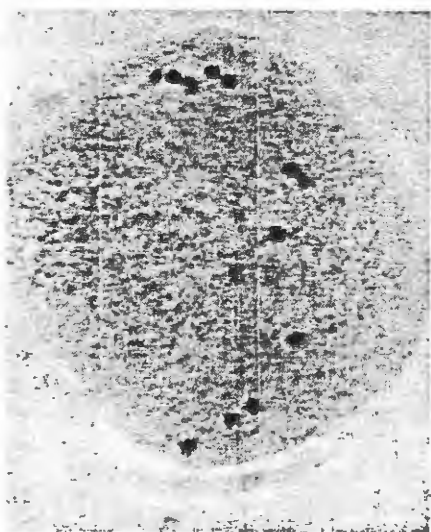


Fig. 1. Pollen mother cells with 12 chromosomes (MI-AI).

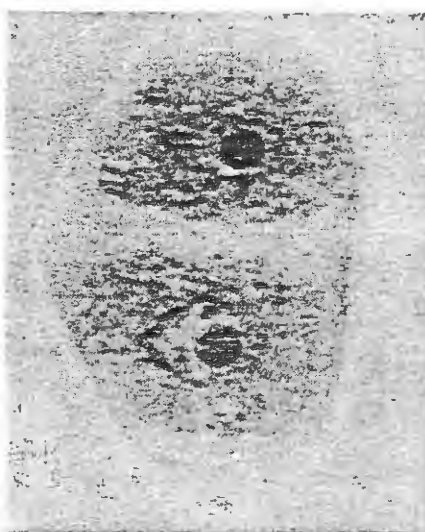


Fig. 2. Dyad from microspores.

Further linkage studies in Cucumis sativus L.

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In 1986 linkage studies for seven combinations of two different marker genes have been continued at the IVT. Involved were the recessive marker genes bi (bitterfree), cp (compact), ccu (susceptibility to scab), lh (long hypocotyl), ro (rosette), gl (glabrous), ul (umbrella leaf) and dvl (divided leaf).

Independent segregation was found in the  $F_2$ 's from the crosses bi x cp, ccu x lh, ccu x ro, ccu x cp and gl x lh, as can be seen from the low and nonsignificant  $X^2$  values (Table 1). It was not possible to detect the umbrella leaf (ul) plants reliably in the  $F_2$ 's of the crosses ro x ul and ul x dvl. The expression of this character in the parent line was also very poor, presumably because the climate was not suitable. In the  $F_2$  of the cross ul x dvl the divided leaf genotypes could not be clearly distinguished, in contrast to the leaves from the dvl parent line. It is conceivable that the two leaf shape mutants interact.

Until now, 15 combinations of marker genes have been analysed at the IVT (1, 2, 3) in which 10 genes were involved. Linkage was detected only for the genes dvl and gl (1), sp and ul (3) and for sp and lh (3).

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Table 1. Segregation and size (N) of F<sub>2</sub> populations for linkage tests, recombination percentage (%) and calculated chi-square ratio for the 9:3:3:1 ratio ( $\chi^2$ ) for 5 pairs of genes.

| Cross                  | ++  | +- | -+ | -- | N   | %  | $\chi^2$ |
|------------------------|-----|----|----|----|-----|----|----------|
| <u>bi</u> x <u>cp</u>  | 158 | 40 | 37 | 14 | 249 | 50 | 5.42     |
| <u>ccu</u> x <u>lh</u> | 137 | 54 | 54 | 12 | 257 | 50 | 2.80     |
| <u>ccu</u> x <u>ro</u> | 143 | 53 | 53 | 11 | 260 | 50 | 2.51     |
| <u>ccu</u> x <u>cp</u> | 156 | 50 | 37 | 16 | 259 | 50 | 3.54     |
| <u>gl</u> x <u>lh</u>  | 116 | 30 | 27 | 11 | 184 | 50 | 3.75     |

++ indicates dominant allele was observed for both loci involved in the cross, +- indicates dominant allele for the first locus and 2 recessive alleles for the second, etc.



## Salt Tolerance among Spanish Cultivars of Cucumis melo

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Coastal areas of the South East yield the main part of the vegetable crops grown in Spain but they are under the threat of soil salinization due to intensive irrigation with brackish waters and some parts are already unsuitable for some crops. A search for more tolerant crops is of great priority.

As we have at our disposal a wide germplasm collection of Spanish melons (1,2), the present work is focused on the selection of melon cultivars showing tolerance to high salinity conditions.

A pre-selection based on the soil, climate and problems of salinity of the origin areas of the cultivars was made.

Forty accessions of the local types: 5 'Rochet'(R), 12 'Piel de sapo'(PS), 10 'Amarillo'(A) and 13 'Tendral'(T) were chosen and planted in a highly salinized soil ( $EC_e=35.0$  dS/m;  $SAR=21.6$ ) irrigated with brackish water (C3S1) (3) by via-flow system and at the same time in a control experiment. Both were carried out during the spring-summer of 1986 in Murcia in open air conditions sowing 14 small plots per accession with 2-3 seeds per plot.

The majority of the accessions initiated germination in the salinity experiment although only 5 grew out of the seedling stage and set fruits (Table 1).

Table 1. Survival data at several stages in the plant life cycle under saline conditions.

| Type | Number of accessions |             |                                        |                                                    |              |                                    |
|------|----------------------|-------------|----------------------------------------|----------------------------------------------------|--------------|------------------------------------|
|      | Sowing               | Germination | True leaves<br>80 days<br>after sowing | Developed<br>plants at<br>110 days<br>after sowing | Fruit<br>set | No. of<br>plants<br>with<br>fruits |
| R    | 5                    | 5           | 5                                      | 5                                                  | 2            | 1+1                                |
| PS   | 12                   | 6           | 3                                      | 0                                                  | 0            | 0                                  |
| A    | 10                   | 10          | 10                                     | 3                                                  | 1            | 6                                  |
| T    | 13                   | 13          | 13                                     | 2                                                  | 2            | 1+1                                |

A strong reduction in the leaf surface was noted in every accession in comparison with the control, as well as a shortened internode length with reductions of about 80% and between 12 and 50% in the thickness of the shoot (Table 2).

The weight and size of the fruit were also strongly diminished in the experiment, the weight being more affected (between 58 and 84%) (Table 3).

The extreme conditions of salinity present in the experiment led to a lack of normal growth in the plants. Nevertheless, a number of accessions with some degree of tolerance useful for further breeding programs, especially that showed by 00073117, has been possible under natural conditions of high salinity.

Table 2. Vegetative traits. Length (cm) between 1st and 4th internode (IL) and shoot diameters (cm) (SD) at 1st and 4th internode in both experiments.

| Accession | Type | IL       |         | SD 1st inter. |         | SD 4th inter. |         |
|-----------|------|----------|---------|---------------|---------|---------------|---------|
|           |      | Control  | Saline  | Control       | Saline  | Control       | Saline  |
| 00073050  | R    | 22.4+4.5 | 3.3+0.6 | 2.1+0.2       | 1.3+0.2 | 1.5+0.2       | 1.5+0.3 |
| 00073270  | R    | 19.7+4.0 | 3.3+0.2 | 2.4+0.4       | 1.2+0.2 | 1.9+0.2       | 1.3+0.0 |
| 00073117  | A    | 16.5+3.9 | 3.3+0.8 | 2.3+0.4       | 1.3+0.1 | 1.7+0.2       | 1.4+0.1 |
| 00073254  | T    | 12.2+2.8 | 2.5+0.0 | 2.6+0.4       | 1.4+0.0 | 2.3+0.2       | 1.6+0.0 |
| 00073296  | T    | 14.4+5.1 | 3.5+0.0 | 2.2+0.3       | 1.4+0.0 | 1.8+0.3       | 1.6+0.0 |

Table 3. Characteristics of the fruit in both experiments.

| Accession | Type | Weight(gr) |         | Length(cm) |          | Width(cm) |         |
|-----------|------|------------|---------|------------|----------|-----------|---------|
|           |      | Control    | Saline  | Control    | Saline   | Control   | Saline  |
| 00073050  | R    | 1293+406   | 270+ 0  | 17.0+1.8   | 9.0+0.0  | 12.3+1.2  | 7.5+0.0 |
| 00073270  | R    | 1681+320   | 464+ 0  | 17.8+1.7   | 11.0+0.0 | 12.7+1.1  | 8.4+0.0 |
| 00073117  | A    | 1075+316   | 446+102 | 14.2+2.3   | 9.1+0.7  | 11.9+1.2  | 9.3+0.9 |
| 00073254  | T    | 1800+482   | 286+ 0  | 16.0+1.9   | 8.5+0.0  | 15.1+1.8  | 8.4+0.0 |
| 00073296  | T    | 1740+419   | 370+ 0  | 18.5+2.4   | 9.0+0.0  | 12.8+1.3  | 8.7+0.0 |

#### Acknowledgments.

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## Improving Artificial Pollination Techniques for Muskmelon

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The combination of mechanical techniques of stamen removal and pollination with the growth regulator BA (6-Benzyladenine), increases fruit set in muskmelon (Cucumis melo L.).

Stamen removal and pollination of hermaphrodite flowers have been carried out by two different methods,  $M_1$  and  $M_2$ :

$M_1$  - stamen removal-pollination method:

One longitudinal incision is made with a lancet on the top of the calyx (from the bottom of the sepals and upwards) and the stamens are removed.

Pollination is achieved by rubbing a brush impregnated with pollen from three male flowers on the stigma.

$M_2$  - stamen removal-pollination method:

The corolla, calyx, and stamens are removed with the fingers. Pollination is achieved by rubbing stamens on the stigma. Although the highest percentage fruit-set was obtained with the  $M_2$  method, the differences were not significant ( $P = 0.05$ ) between the  $M_1$  and  $M_2$  methods. The  $M_2$  method is recommended to simplify work.

Jones using a BA/lanoline paste obtained a fruit-set percentage of about 37.5%, while the percentage of fruit-set for other treatments was only 0.83% (2). Melon plants treated with 6-BA yielded up to 70% more fruit than control plants (1).

Four different treatments have been used in this work: ( $M_1$ ,  $M_2$ ,  $M_1 + BA$ , and  $M_2 + BA$ ).

The BA is applied with a brush to the base of the ovary of the hermaphrodite flowers after pollination. The paste is obtained by mixing 0.5 g of BA, 10 ml of 70% ETOH, 9 g of stearic acid, 123.18 ml of distilled water, and 48 g of lanolin. The formula is a modification of the one used by Munger et al. (3).

The percentage fruit-set for each treatment were:

| TREATMENT:    | $M_1$ | $M_2$ | $M_1 + BA$ | $M_2 + BA$ |
|---------------|-------|-------|------------|------------|
| FRUIT-SET (%) | 49.5  | 57.3  | 91.2       | 96.2       |

Application of BA increased fruit set.

Variation due to BA treatments was significant, but differences in mechanical techniques were not significant at a level of 95%.

There was no interaction between the mechanical techniques and BA treatment.

The four cultivars of muskmelon used in this work were 'Ogen', 'Amarillo Oro', 'Honey Dew', and 'Tendral'.

There were no significant differences among genotypes. Neither were there interactions between treatments and genotypes.

We suggest the utilization of the  $M_2$  + BA technique for improving the fruit-set in muskmelon breeding programs.

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## Multiple-Flowering Character in Muskmelon (Cucumis melo L.)

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The expression of multiple flower per node has potential for concentration of fruit set with ultimate value in once-over harvest and thus reduced harvest costs. Yield potential and inheritance of multi-pistillate flowering in cucumber has been thoroughly investigated (recessive major gene, multi-pistillate, symbol mp), with the goal of circumventing "first-set fruit" inhibition (2,7). Multiple hermaphroditic flowering evident in Capsicum sp. has been transferred by interspecific hybridization with inheritance subsequently characterized as quantitative with some epistatic interactions (5,6). Muskmelon has several mutations for flower-related traits, with 13 such mutants listed in the 1986 gene list for muskmelon (1). We report here the spontaneous occurrence of multiple flowers per node.

The multiple flowering pattern was observed on a single plant of PI 414723, a monoecious introduction from India. This entry is one of several land races grown in the Indian subcontinent and locally termed "Phutt," which means literally "to split," referring to the extreme longitudinal splitting of fruits at maturity. We are interested in PI 414723 as a source of virus resistance. Provvidenti (4) has determined that plants within PI 414723 are immune to strains of zucchini yellow mosaic virus, and Pitrat and Lecoq (3) report it to be a source of multiple insect and disease resistance. Preliminary indications are that this material may resist egg mass survival for root knot nematodes (Mankau, personal communication).

During the process of screening shoot-tip derived plants of PI 414723 in the greenhouse for ZYMV resistance in December 1985, we observed one plant with multiple pistillate flowers at every node. The number of flowers per node varied from 2 to 5. Cuttings from the original plant maintained the multiple-flower trait. However, some branches interrupt the multiple-flowering expression with nodes of only single flowers. Cutting-derived plants were grown at 2 field locations in California in 1986. Multiple fruits were set per node and these were borne to maturity, with a noticeable concentration of set and maturity.

Reciprocal crosses have been made using both the original plant and derived cuttings with an andromonoecious commercial cv. Topmark. All  $F_1$ 's expressed monoecy, as expected, with nodes containing multiple flowers dispersed along the runners. A series of greenhouse-grown  $F_1$  plants showed multiple flowering on main runners at early nodes (5-7), at intermediate nodes (12-14), and again at later nodes (20-24). The remaining nodes either failed to form flowers or exhibited only single pistillate flowers. Individual flowers in the multi-flowered nodes appeared both axially and terminally. In Fig. 1, the 3 pistillate flowers are seen terminating a lateral branch of one of the  $F_1$ 's. The central, terminal flower is the youngest, and is smaller in size and later in development compared to the 2 lateral flowers. Hybrid vigor was evident in fruit size. Additionally, some other 'Topmark' expression such as fruit netting and total soluble solids were strongly transmitted.

Seeds for F<sub>1</sub>, F<sub>2</sub>, and BC generations are available for field testing in 1987 to permit additional data on expression and inheritance of multiple flowering in muskmelon.

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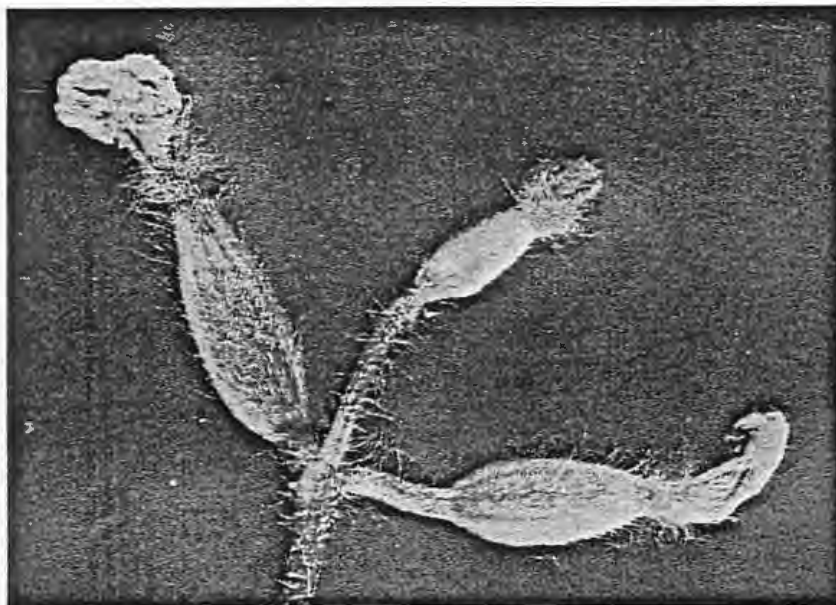


Figure 1. Multiple flowering in lateral branch F<sub>1</sub> (PI 414723 X Topmark) muskmelon.

## Inheritance of Gynoecious Sex Type in Muskmelon

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Rosa (6) in his earliest experiments on the inheritance of flower type in muskmelon, showed that monoecism is dominant to andromonoecism by a single pair of alleles. Poole and Grienball (5) proposed a two gene explanation of sex expression in muskmelon: AG is monoecious, Ag is gynomonoecious, aG is andromonoecious, and ag is hermaphrodite. These authors assumed that the gynoecious sex type in their F<sub>2</sub> population (in the cross hermaphrodite x monoecious) was a transitory phenotype caused by environmental conditions and therefore considered it gynomonoecious. Rowe (7) suggested that gynoecious sex expression was controlled by modifying genes in addition to the major genotype A-gg. Peterson *et al* (4) reported on WI 998 muskmelon population in which stable, homozygous, gynoecious plants have been identified. This paper reports on the inheritance of gynoecism in the crosses gynoecious x monoecious and gynoecious x andromonoecious under greenhouse conditions.

Seeds of WI 998, obtained from C.E. Peterson, USDA, ARS, University of Madison, WI, were grown in the greenhouse. Plants were sprayed with AgNO<sub>3</sub> (3) to induce the formation of hermaphrodite flowers, and then self-pollinated. The 200 seeds collected from a single fruit produced all gynoecious plants in the field. A single plant was reproduced vegetatively in the greenhouse and used as a female or a male (following AgNO<sub>3</sub> treatments) parent in reciprocal crosses with Cucumis melo PI 124111 (monoecious) or with the breeding line '36' (andromonoecious, homozygous, resistant to powdery and downy mildews derived from PI 124111 x Hemed x Perlita, see (1)).

F<sub>1</sub> plants were all monoecious (Table 1) thus confirming earlier reports (2, 8). F<sub>2</sub> populations segregated for sex type giving rise to 21 gynoecious (out of 293) plants (1/16) in the cross WI 998 x PI 124111, and 7 gynoecious (out of 312) plants (1/64) in the cross WI 998 x '36' (Table 1), indicating the possibility that gynoecism is recessive to monoecism by 2 genes, and recessive to andromonoecism by 3 genes. In the population derived from the BC WI 998 x (WI 998 x PI 124111) 1/4 of the plants were gynoecious. Gynoecious plants produced only pistillated flowers, until senescence.

Based on the data presented we propose that gynoecism in WI 998 is conferred by a recessive gene nn in addition to the major genotype A-gg. This gene is homozygous NN or heterozygous Nn in other sex types of muskmelon. The double recessive gene difference in the cross WI 998 x PI 124111 suggests A-G-N as monoecious. The triple recessive gene difference in the cross WI 998 x '36' suggests aa G-N- as andromonoecious. We assume that gynomonoecious is A-gg N- and hermaphrodite is aagg N-. More data are currently collected to verify these assumptions. Gynoecious plants, resistant to powdery mildew were identified in the F<sub>2</sub> population of the cross WI 998 x '36'. This, and the fact that WI 998 carries Fom 3 for resistance against races 0 and 2 of Fusarium wilt (8) make it important for hybrid production.

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Table 1. Frequency distribution of the F<sub>1</sub> and the F<sub>2</sub> populations in muskmelon from the crosses WI 998 x PI 124111, and WI 998 x '36', for sex expression.

| Cross                        | No. of plants with sex expression |           |            |       |          |        | Total |
|------------------------------|-----------------------------------|-----------|------------|-------|----------|--------|-------|
|                              | mono.                             | gynomono. | andromono. | gyno. | trimono. | herma. |       |
| WI 998x124111 F <sub>1</sub> | 53                                |           |            |       |          |        | 53    |
| 124111xWI 998 F <sub>1</sub> | 56                                |           |            |       |          |        | 56    |
| WI 998x124111 F <sub>2</sub> | 204                               | 38        | 20         | 21*   | 10       | 0      | 293   |
| WI 998x'36' F <sub>1</sub>   | 44                                |           |            |       |          |        | 44    |
| WI 998x'36' F <sub>2</sub>   | 187                               | 11        | 79         | 7**   | 12       | 16     | 312   |

WI 998 - gynoecious; PI 124111 - monoecious; '36' - andromonoecious.

\*  $21/293 = 1/16$  with  $\chi^2 = 0.422$

\*\*  $7/312 = 1/64$  with  $\chi^2 = 0.941$



## Development of Gynoecious Lines in Muskmelon

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Development of true breeding gynoecious lines has led to phenomenal exploitation of heterosis and development of hybrids in cucumber. Research on this aspect in muskmelon has been in progress and Peterson et al. (1983) developed a stable gynoecious line, Wisconsin 998 (WI 998).

At the Indian Agricultural Research Institute, New Delhi, three gynoecious lines namely 86-104, 86-105, and 86-118 have been bred for true breeding gynoecious sex. The first two lines came from a cross between a gynomonocious line and hermaphrodite-2 (Ac. No. 433), two of the parents were introduced from Bulgaria and the third one came from a cross between Monoecious-1 (developed at this Institute) and hermaphrodite-1 (EC 70674 i.e. AC No. 354). Original crosses were made by Magdum in 1979-80 and reported in 1982. All the gynoecious lines in this report are in  $F_6$  generation. From  $F_2$  onwards, efforts were made to select only the pure gynoecious plant from the segregating population and plants were maintained by layering followed by 400 ppm silver-nitrate spray. Perfect flowers were induced after three months of scoring for gynoecism and were increased either by selfing or sibbing. For advancing the generations from  $F_4$  onwards, the plants were scored for gynoecious stability for 8-10 weeks at which stage they were sprayed with 400 ppm silver-thiosulphate (STS). This treatment induced perfect flowers and seeds were produced. Thus, the parents were maintained in pure gynoecious state. By this way the scoring for gynoecious stability and maintenance was achieved in the same growing season. In later generations the selected lines were sprayed with 400 ppm STS at two true leaf stage and the perfect flowers were induced, which were used for perpetuation of gynoecious stock. In this method, it was assumed that a genetically true breeding gynoecious plant, after temporary induction of perfect phase, should revert back to gynoecious phase again, once the effect of exogenously applied STS has been lost.

F:C ratio and number of seeds per fruit were higher in I.A.R.I. gynoecious lines compared with WI 998 (Table 1). All the four gynoecious lines have to be improved for T.S.S. content, keeping in mind the consumers' preference for sweet fruits in India. For the remaining characters, WI 998 was comparatively better than the gynoecious lines under report.

Summing up the results, it is reported that these gynoecious lines need further improvement for horticultural characters especially fruit weight, shape index (preferably round) and T.S.S. content. These characters can be improved to the desired level by further selections. It is worthy of mention here that these lines were always scored for gynoecism from the end of March to the middle of June when the temperatures were  $40^{\circ}/25^{\circ}\text{C}$  (day/night) and above with the prevailing long photoperiod, under field conditions. This report brings out the possibility of developing a stable gynoecious line in muskmelon under high temperature and long photoperiodic conditions compared to the earlier report of Peterson et al. (1983) who developed a stable gynoecious line under comparatively moderate environmental glasshouse conditions.

Table 1. Mean performance of different gynoecious lines.

| Lines        | Average fruit weight (g) | Shape index | F:C ratio | T.S.S. (%) | No. of seeds/fruit | Seed weight/fruit (g) | 100 seed weight (g) |
|--------------|--------------------------|-------------|-----------|------------|--------------------|-----------------------|---------------------|
| IARI 86-104  | 440                      | 1.29        | 1.31      | 7.95       | 264                | 6.14                  | 2.33                |
| -105         | 325                      | 1.32        | 1.26      | 7.60       | 355                | 7.94                  | 2.24                |
| -118         | 387                      | 1.35        | 1.10      | 7.34       | 239                | 5.95                  | 2.44                |
| Range        | 200-600                  | 0.87-1.61   | 0.75-1.93 | 4.80-10.00 | 115-355            | 1.56-12.50            | 1.11-3.88           |
| General mean | 384                      | 1.32        | 1.22      | 7.63       | 286                | 6.68                  | 2.34                |
| S.E. $\pm$   | 0.0141                   | 0.0316      | 0.0469    | 0.2419     | 13.5572            | 0.4147                | 0.5622              |
| WI 998       | 473                      | 0.94        | 0.82      | 8.00       | 153                | 4.33                  | 2.87                |
| S.E. $\pm$   | 0.0469                   | 0.0224      | 0.0520    | 0.3742     | 14.3206            | 0.4151                | 0.1952              |
| Range        | 340-900                  | 0.77-1.11   | 0.55-1.23 | 5.0-10.4   | 103-205            | 3.33-6.46             | 2.25-3.80           |

Average fruit weight and number of seeds/fruit were equated to the nearest whole number.

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Localization of genes v, ms-3, f, lmi and powdery mildew resistance in muskmelon

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Until now 9 independent gene groups have been described in muskmelon. We continue to make systematic studies to improve our knowledge of melon genetics. The names and symbols of the genes used in this paper are these of the reference list (1).

Virescent (v) has previously been found independent from genes belonging to groups 1,2,3,4,6,7,8,9 (3). In a F2 progeny with a line with Papaya ringspot virus resistance (Prv in group 5) we found 137 Resistant/47 Susceptible ( $\chi^2 = .15084$ ; Prob = 69.8 %) among the  $v^+/-$  plants (v/v are more or less lethal). We conclude that v is not in group 5 and is alone in group 10.

Linkage of Pm-3 (Powdery mildew resistance in PI 124111 or VA 435) with short internode-1 (si-1 in group 1), necrotic spot virus resistance (nsv in group 7), cut leaf (cl in group 9) and long mainstem internode (lmi) has been tested (table 1). We found it independent from si-1, cl and lmi but linked with nsv (distance = 33.9 according to the maximum likelihood method).

A gene controlling powdery mildew resistance in WMR 29 (2) provisionally named Pm-w and which is different from Pm-3 (about 1/16 susceptible in F2) has been found linked with Virus aphid transmission resistance (Vat) and Flaccida necrosis (Fn) in group 2 (Table 2). We have tested the backcrosses with the double recessives. In (Vat Pm-w<sup>+</sup>/Vat<sup>+</sup> Pm-w) x (Vat<sup>+</sup> Pm-w<sup>+</sup>) progeny we found 4 [Vat Pm-w] : 114 [Vat<sup>+</sup> Pm-w] : 106 [Vat Pm-w<sup>+</sup>] : 5 [Vat<sup>+</sup> Pm-w<sup>+</sup>]  $\chi^2 = 194.98$  percentage of recombination = 3.9. In (Fn Pm-w/Fn<sup>+</sup> Pm-w) x (Fn<sup>+</sup> Pm-w<sup>+</sup>) progeny we found 0 [Fn Pm-w] : 96 [Fn Pm-w<sup>+</sup>] : 93 [Fn<sup>+</sup> Pm-w] : 3 [Fn<sup>+</sup> Pm-w<sup>+</sup>]  $\chi^2 = 180.37$  percentage of recombination = 1.6. As the distance between Fn and Vat has been estimated to 11.6 we can conclude that Pm-w is between Fn and Vat.

Long mainstem internode (lmi) has been found independent from genes belonging to groups 2,3,4,5,6,7 and 9 (Table 3) and flava (f) from groups 3,8 and 10 but linked with lmi (Table 4). The group f-lmi must still be tested with group 1.

Male sterile -3 (ms-3) is independent from groups 2,3,4,6,10 and also from f-lmi (Table 5).

In conclusion the linkage groups in muskmelon may be summarized :

group 1 : si-1 yv  
group 2 : Vat Pm-w Fn  
group 3 : ms-1 r gl Pa

group 4 : Zym a h Pm-X (order unknown)  
group 5 : Prv Fom-1  
group 6 : ms-2 yg Fom-2  
group 7 : nsv Pm-3  
group 8 : ms-4  
group 9 : cl  
group 10 : v  
group 11 : f lmi (independent from groups 2 to 10 but must be tested  
with group 1)

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Table 1 : Segregation data observed in F2 progenies between powdery mildew resistance (Pm-3) and si-1, nsv, cl, and lmi

| Linkage group | Genes                | Phase <sup>2</sup> | [Resistant] | [Susceptible]                        | chi-square (9:3:3:1) |             |
|---------------|----------------------|--------------------|-------------|--------------------------------------|----------------------|-------------|
|               |                      |                    | Pm-3/-      | Pm-3 <sup>+</sup> /Pm-3 <sup>+</sup> | Value                | Probability |
| 1             | si-1 <sup>+</sup> /- | R                  | 176         | 44                                   | 8.1865               | 4.2 %       |
|               | si-1/si-1            |                    | 37          | 17                                   |                      |             |
| 7             | nsv <sup>+</sup> /-  | C                  | 138         | 52                                   | 16.414               | .09 %       |
|               | nsv/nsv              |                    | 75          | 9                                    |                      |             |
| 9             | cl <sup>+</sup> /-   | R                  | 87          | 31                                   | .98234               | 80.6 %      |
|               | cl/cl                |                    | 24          | 9                                    |                      |             |
|               | lmi <sup>+</sup> /-  | R                  | 86          | 25                                   | 4.0670               | 25.4 %      |
|               | lmi/lmi              |                    | 25          | 15                                   |                      |             |

<sup>2</sup> C : Coupling R : Repulsion

Table 2 : Segregation data observed in F2 progenies between powdery mildew resistance of WMR 29 (Pm-w) and Vat, Fn, Fom-1, Pw and Fom-2

| Linkage group | Genes                                  | Phase <sup>2</sup> | [Resistant] | [Susceptible]                        | chi-square (9:3:3:1) |             |
|---------------|----------------------------------------|--------------------|-------------|--------------------------------------|----------------------|-------------|
|               |                                        |                    | Pm-w/-      | Pm-w <sup>+</sup> /Pm-w <sup>+</sup> | Value                | Probability |
| 2             | Vat/-                                  | R                  | 105         | 45                                   | 30.137               | <.01 %      |
|               | Vat <sup>+</sup> /Vat <sup>+</sup>     |                    | 64          | 0                                    |                      |             |
| 2             | Fn/-                                   | R                  | 105         | 42                                   | 7.3731               | 6.09 %      |
|               | Fn <sup>+</sup> /Fn <sup>+</sup>       |                    | 42          | 4                                    |                      |             |
| 5             | Fom-1/-                                | R                  | 116         | 32                                   | 1.1022               | 77.7 %      |
|               | Fom-1 <sup>+</sup> /Fom-1 <sup>+</sup> |                    | 40          | 12                                   |                      |             |
| 5             | Prv/-                                  | C                  | 126         | 31                                   | 5.2064               | 15.7 %      |
|               | Prv <sup>+</sup> /Prv <sup>+</sup>     |                    | 32          | 8                                    |                      |             |
| 6             | Fom-2/-                                | R                  | 114         | 27                                   | 3.1111               | 37.5 %      |
|               | Fom-2 <sup>+</sup> /Fom-2 <sup>+</sup> |                    | 40          | 11                                   |                      |             |

<sup>2</sup> C : Coupling R : Repulsion

Table 3 : Segregation data observed in F2 progenies between long mainstem internode (lmi) and Vat, gl, Zym, Fom-1, Fom-2, yg, nsv, Pm-3 and cl (all in repulsion phase)

| Linkage group | Genes                                  | [Normal]<br>lmi <sup>+</sup> /- | [long internode]<br>lmi/lmi | chi-square (9:3:3:1) |             |
|---------------|----------------------------------------|---------------------------------|-----------------------------|----------------------|-------------|
|               |                                        |                                 |                             | Value                | Probability |
| 2             | Vat/-                                  | 116                             | 33                          | .89288               | 82.7 %      |
|               | Vat <sup>+</sup> /Vat <sup>+</sup>     | 34                              | 12                          |                      |             |
| 3             | gl <sup>+</sup> /-                     | 151                             | 45                          | 3.8371               | 28.0 %      |
|               | gl/gl                                  | 44                              | 9                           |                      |             |
| 4             | Zym/-                                  | 109                             | 34                          | .83818               | 84.0 %      |
|               | Zym <sup>+</sup> /Zym <sup>+</sup>     | 41                              | 11                          |                      |             |
| 5             | Fom-1/-                                | 129                             | 47                          | 7.3375               | 6.19 %      |
|               | Fom-1 <sup>+</sup> /Fom-1 <sup>+</sup> | 56                              | 7                           |                      |             |
| 6             | Fom-2/-                                | 169                             | 79                          | 6.6995               | 8.21 %      |
|               | Fom-2 <sup>+</sup> /Fom-2 <sup>+</sup> | 69                              | 21                          |                      |             |
| 6             | yg <sup>+</sup> /-                     | 134                             | 44                          | .23352               | 97.2 %      |
|               | yg/yg                                  | 45                              | 13                          |                      |             |
| 7             | nsv <sup>+</sup> /-                    | 242                             | 92                          | 2.5350               | 46.9 %      |
|               | nsv/nsv                                | 75                              | 23                          |                      |             |
| 7             | Pm-3/-                                 | 86                              | 25                          | 4.0670               | 25.4 %      |
|               | Pm-3 <sup>+</sup> /Pm-3 <sup>+</sup>   | 25                              | 15                          |                      |             |
| 9             | cl <sup>+</sup> /-                     | 197                             | 52                          | 2.3704               | 49.9 %      |
|               | cl/cl                                  | 65                              | 22                          |                      |             |

Table 4 : Segregation data observed in F2 progenies between flava (f) and gl, ms-4, v and lmi (all in repulsion phase)

| Linkage group | Genes                | [green]           | [yellow] | chi-square (9:3:3:1) |             |
|---------------|----------------------|-------------------|----------|----------------------|-------------|
|               |                      | f <sup>+</sup> /- | f/f      | Value                | Probability |
| 3             | gl <sup>+</sup> /-   | 206               | 55       | 2.7974               | 42.4 %      |
|               | gl/gl                | 60                | 19       |                      |             |
| 8             | ms-4 <sup>+</sup> /- | 119               | 35       | 4.8627               | 18.2 %      |
|               | ms-4/ms-4            | 31                | 6        |                      |             |
| 10            | v <sup>+</sup> /-    | 175               | 53       | .37427 <sup>z</sup>  | 54.1 %      |
|               | lmi <sup>+</sup> /-  | 345               | 159      |                      |             |
|               | lmi/lmi              | 163               | 0        |                      |             |

<sup>z</sup> tested for 3 : 1 segregation among green plants (v<sup>+</sup>/-) because v/v are more or less lethal

Table 5 : Segregation data observed in F2 progenies between male sterile-3 (ms-3) and Vat, gl, Zym, yg, v, and f (all in repulsion phase)

| Linkage group | Genes                              | [Fertile]            | [Sterile] | chi-square (9:3:3:1) |             |
|---------------|------------------------------------|----------------------|-----------|----------------------|-------------|
|               |                                    | ms-3 <sup>+</sup> /- | ms-3/ms-3 | Value                | Probability |
| 2             | Vat/-                              | 90                   | 40        | 3.4100               | 33.3 %      |
|               | Vat <sup>+</sup> /Vat <sup>+</sup> | 36                   | 8         |                      |             |
| 3             | gl <sup>+</sup> /-                 | 149                  | 53        | .34450               | 94.9 %      |
|               | gl/gl                              | 54                   | 17        |                      |             |
| 4             | Zym/-                              | 95                   | 33        | 1.7344               | 62.9 %      |
|               | Zym <sup>+</sup> /Zym <sup>+</sup> | 31                   | 15        |                      |             |
| 6             | yg <sup>+</sup> /-                 | 146                  | 49        | 2.0346               | 56.5 %      |
|               | yg/yg                              | 57                   | 21        |                      |             |
| 10            | v <sup>+</sup> /-                  | 204                  | 76        | .68571 <sup>z</sup>  | 40.8 %      |
|               | f <sup>+</sup> /-                  | 95                   | 20        |                      |             |
|               | f/f                                | 21                   | 6         |                      |             |

<sup>z</sup> tested for 3 : 1 segregation among green plants (v<sup>+</sup>/-) because v/v are more or less lethal

The Search for Sources of Resistance to Squash Mosaic Virus In Melon: A Preliminary Report.

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In nature, squash mosaic virus (SqMV) is confined to Cucurbitaceae. Squash (Cucurbita spp.) and melons (Cucumis melo) are particularly affected and most of the outbreaks of this virus can be traced to their infected seeds. Seed transmission may range from 1 to 10%, but a much higher percentage has been also recorded (1,5,7). From plant to plant, SqMV is efficiently spread by the striped and spotted cucumber beetles (Acalymma spp. and Diabrotica spp.), or less frequently by contact (2).

Through the years, seed producers have succeeded in limiting the presence of SqMV from their seed stocks, but lately, some well known melon cultivars have been found to be carriers, with alarming consequences. Thus, the development of resistant cultivars would be of great benefit to the seed industry.

Since all domestic and foreign cultivars are susceptible to SqMV, a major effort is under way to locate sources of resistance in foreign introductions, primitive cultivars or land races of C. melo and its botanical varieties. Resistance is being sought against the two known pathotypes, the melon strain (SqMV-I) and the squash strain (SqMV-II) (3). Both occur in melons, and can be differentiated by host reaction and serology (3). Generally, SqMV-I causes more severe symptoms in melons than the SqMV-II. However, this distinction is not absolute, since some cultivars appear to react similarly to both pathotypes.

Sixty accessions of C. melo from several areas of the world have already been screened with both pathotypes, using from 15 to 20 plants of each line for each strain. None has been found resistant or tolerant to the melon strain, but a few lines have yielded plants tolerant to the squash strain. This tolerance is expressed as a mild foliar mottle with limited plant stunting. Particularly promising appears PI 157080 (China), which was previously reported to be tolerant to an isolate of SqMV (9).

The results with the melon strain have been disappointing, but the search for resistance to both pathotypes is continuing. The plant introduction collection of C. melo is very rich in numbers and in genetic diversity, hence, it may eventually reveal valuable SqMV-resistant germplasm. In breeding for resistance or tolerance, any genetic factor(s) that will preclude seed transmission and prevent or minimize systemic infection of the virus can be considered very useful.



Several years ago, Provvidenti and Robinson (6) reported resistance to SqMV in Cucumis metuliferus, commonly known as 'jelly melon' or 'horned cucumber'. This feral species reacts to both pathotypes of the virus with chlorotic lesions on inoculated leaves, but is systemically resistant (6). Both C. metuliferus and C. melo possess the same number of chromosomes ( $n=12$ ) (5), but it is very difficult to obtain interspecific crosses. The only successful attempt was reported by Norton, but progenies of that cross (4) have not been made available. Thus, it is not known whether they still retain the resistance to SqMV and watermelon mosaic virus 1 possessed by their wild parent (6).

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## Resistance to Cucumber Green Mottle Mosaic Virus (CGMMV) in Muskmelon

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A sap transmissible virus causing mild mosaic of muskmelon was identified as CGMMV (2) which is one of the few viruses with a natural host range restricted to Cucurbitaceae (1). In sub-tropical parts of India around Delhi, a strain of virus from muskmelon was isolated (3) and identified as a minor variant of CGMMV.

A project on breeding for virus resistance has been initiated at the Indian Agricultural Research Institute, New Delhi. About 152 collections of muskmelon from 10 countries comprised of 124 dessert varieties, 10 non-dessert forms and 18 wild species of Cucumis were screened against CGMMV in three stages, twice under natural field conditions (summer and rainy season of 1985) and once under artificial transmission by inoculating with a purified strain of CGMMV adopting a sap-inoculation technique (3) in an insect proof nethouse. Plants were rated on a 0-5 scale, 0 being immune/symptomless and 5 being highly susceptible.

All the dessert varieties of muskmelon tested were found susceptible to CGMMV both under natural field condition and artificial inoculation. Among the wild species, C. myriocarpus (disectifolium) Naud., C. myriocarpus (normal type) Naud., C. africanus L. f. (two collections), C. figarei Naud., C. meeusii C. Jeffrey, C. zeyheri Sond. and C. ficifolius A. Rich. were found to be resistant to CGMMV under all the screening tests.

It was observed that the two non-dessert types "phoot" (C. melo var. momordica) and "Kachri" grown mostly in north India and Cornell breeding line No. 83-273-6R (Mon. MR. 328) of Dr. H. M. Munger, Cornell University, Ithaca, U.S.A. were found to be resistant (Score = 1) to CGMMV. These three resistant genotypes are crossable with all the cultivated dessert varieties of muskmelon and could be directly used in virus resistance breeding program. It is pointed out that "phoot" and "kachri" (non-dessert forms) can possibly serve also as bridge species to transfer resistance from wild species to the cultivated ones after studying their crossability with the wild species.

Cucumis metuliferus Naud. and C. anguria var. longipes A. Maeuse showed moderate susceptibility (Score = 3) to CGMMV. Nevertheless resistance reaction to CGMMV showed by some of the above wild species conformed to the reports from IVT (4).

Preliminary studies were conducted on the confirmation of the nature of resistance by back inoculation technique. Taking the sap of resistant genotypes 150 days after inoculation with pure isolate of CGMMV and inoculating it on the susceptible stock (uninfected) in virus free nethouse, indicated that C. figarei was immune to CGMMV while all the other resistant types, "phoot",

"kachri", Cornell No. 83-273-6R and other Cucumis sp., were found to show symptomless carrier reaction. Further investigation is planned on this latter type of reaction.

Studies on the interspecific hybridization between CGMMV resistant wild species and cultivated forms of muskmelon indicated that C. figarei crosses with Pusa Madhuras and Monoecious-4 (cultivated forms) with 11 to 25 per cent fruit set. The F<sub>1</sub> generation and succeeding generations will be used to evaluate the nature of resistance.

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Controversy on Resistance to Fusarium Wilt in 'Perlita' (Cucumis melo L)

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In 1985, Zink and Gubler (3) reported that 'Perlita' resistance to Fusarium wilt (Fusarium oxysporum f. sp. melonis) races 0 and 2 is conferred by a dominant gene Fom-3 different from the gene Fom-1 that controls resistance to races 0 and 2 in 'Doublon' (2).

Actually, resistance of 'Perlita' appears different from that of 'Doublon' in our experience. When inoculated by our usual method (1) 'Perlita' exhibits a very fluctuating reaction : usually, cotyledons are yellowing, then sometimes, young leaves are yellowing too and seldom apex dies ; so, in one experiment, we can observe resistant and susceptible seedlings and reaction of the same line is very fluctuating according to the experiment. This heterogeneity is observed in every accession of 'Perlita' that we have received and is carried on after several self pollinations, so we cannot attribute it to genetic heterogeneity. We cannot control heterogeneity of 'Perlita' reaction by using different inoculum concentrations or seedling stages at inoculation but using growth chamber with more light than usual allows 'Perlita' to exhibit a more regular resistance.

If expression of 'Perlita' resistance appears different from that of 'Doublon', observed segregations in progenies from crosses between 'Perlita' and a line with gene Fom-1 from 'Doublon' do not agree with the hypothesis of two different genes if we select experiments where no plants of 'Perlita' are noted as susceptible (table 1).

So our results do not agree with those of Zink and Gubler (3) but 2 hypothesis can explain them : 'Perlita' has an allele of Fom-1 different of the allele of 'Doublon' and 'Charentais Fom-1' or the genetic background of 'Perlita' does not allow Fom-1 to act well. Studies are going on to decide which of the hypothesis is the good one.

I should be happy to receive results of other breeders which are using 'Perlita' as parent.

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Table 1. Ségrégation in progenies from crosses between Perlita and Charentais T or Charentais Fom-1 after inoculation with races 0 or 2 of Fusarium oxysporum f. sp melenis

| Parents and crosses                         | test <sup>z</sup> | Expected plant numbers according hypothesis |                |                     |     | Observed plant numbers |     |
|---------------------------------------------|-------------------|---------------------------------------------|----------------|---------------------|-----|------------------------|-----|
|                                             |                   | 2 different genes                           |                | 2 different alleles |     | R + D                  | S   |
|                                             |                   | R <sup>y</sup> + D <sup>x</sup>             | S <sup>w</sup> | R + D               | S   |                        |     |
| Charentais T <sup>v</sup>                   | A                 | 0                                           | 128            | 0                   | 128 | 0                      | 128 |
|                                             | B                 | 0                                           | 116            | 0                   | 116 | 0                      | 116 |
|                                             | C                 | 0                                           | 90             | 0                   | 90  | 0                      | 90  |
| Perlita                                     | A                 | 59                                          | 0              | 59                  | 0   | 59                     | 0   |
|                                             | B                 | 27                                          | 0              | 27                  | 0   | 27                     | 0   |
|                                             | C                 | 93                                          | 0              | 93                  | 0   | 93                     | 0   |
| Charentais Fom-1 <sup>u</sup>               | A                 | 62                                          | 0              | 62                  | 0   | 62                     | 0   |
|                                             | C                 | 93                                          | 0              | 93                  | 0   | 93                     | 0   |
| F1 Charentais T x Charentais Fom-1          | A                 | 50                                          | 0              | 50                  | 0   | 50                     | 0   |
|                                             | C                 | 26                                          | 0              | 26                  | 0   | 26                     | 0   |
| F1 Charentais Fom-1 x Perlita               | A                 | 67                                          | 0              | 67                  | 0   | 67                     | 0   |
|                                             | B                 | 29                                          | 0              | 29                  | 0   | 29                     | 0   |
|                                             | C                 | 30                                          | 0              | 30                  | 0   | 30                     | 0   |
| F1 Charentais Fom-1 x Perlita               | A                 | 63                                          | 0              | 63                  | 0   | 63                     | 0   |
|                                             | C                 | 57                                          | 0              | 57                  | 0   | 56                     | 0   |
| F2 (Charentais Fom-1 x Perlita)             | A                 | 112                                         | 7              | 119                 | 0   | 119                    | 0   |
|                                             | B                 | 56                                          | 4              | 60                  | 0   | 60                     | 0   |
|                                             | C                 | 119                                         | 8              | 127                 | 0   | 127                    | 0   |
| (Charentais Fom-1 x Perlita) x Charentais T | A                 | 45                                          | 15             | 60                  | 0   | 60                     | 0   |
|                                             | B                 | 22.5                                        | 7.5            | 30                  | 0   | 29                     | 1   |
|                                             | C                 | 23                                          | 8              | 31                  | 0   | 30                     | 1   |

z. Test A plantlets inoculated with race 0

Tests B and C plantlets inoculated with race 2

y. R : resistant plant without symptoms

x. D : doubtful plant with some yellowing but without wilt or collapse

w. S : susceptible plant : wilting or died

v. Charentais T is a line susceptible to races 0,1,2 and 1-2 of Fom

u. Charentais Fom-1 is a quasi isolate of Charentais T, bred by backcrossing Doublon to Charentais T

Obtention of embryos and plants from in vitro culture of fertilized ovules of Cucumis melo 5 days after pollination.

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In Cucurbitaceae, failure of interspecific hybridization may be due to an early abortion of the hybrid embryos (3). Young Cucumis embryos when they are extracted at globular or heart shape stages are sometimes able to continue their differentiation in vitro (1). However it is often difficult to isolate embryos at these stages.

The results reported here involved the development of a new in vitro culture technique to obtain embryos from very young fertilized ovules of Cucumis melo.

Plants of melon commercial F1 hybrid ('Alfa') were grown in a greenhouse under normal light and culture conditions. Plants were self pollinated. The ovaries were harvested 5 days after pollination.

They were surface sterilized by dipping for 10 min. in a solution of 10 % calcium hypochlorite with a few drops of tween 20 emulsifier. Ovaries were then rinsed three times with sterile distilled water and aseptically transferred to moistened sterile filter paper in Petri dishes. Ovules were excised under binocular, placenta tissue was carefully removed.

Twenty naked ovules were placed in a 5 cm Petri dish filled with the following culture medium :

- macronutrients and micronutrients as described for pepper anthers culture (2) but used half strength.
- Na<sub>2</sub>EDTA 18.65 mg/l and FeSO<sub>4</sub> 13.9 mg/l
- Fuji and Morel vitamins as detailed for pepper (2)
- sucrose 20 g/l; Agar 10 g/l.

pH of the medium was adjusted to 5.9 before autoclaving at 115°C for 20 min. Culture was made at 25°C with 12 hrs light per day.

Germination of the embryos occurred within 3 or 4 weeks. One ovule gives always only one embryo. Embryos grew very fast and developed into complete plants ; in about four weeks they reached a developmental stage suitable for transplanting to soil.

This technique presents 2 advantages :

- the artificial culture can start 5 days only after pollination. That may be important in the case of early embryo abortion ;

- isolation of young ovules is easier than extraction of embryos at the globular stages.

So its application to interspecific crosses in this genus can be envisaged.

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Germplasm Resources of Citrullus lanatus [(Thunb.) Matsum and Nakai]

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During 1984 and 1985, a collection of vegetable crop species seeds was carried out in Spain. Watermelon was enclosed in this project which was partially supported by I.B.P.G.R./F.A.O. In addition to many other crops, 67 accessions of watermelon were collected. Samples of all of them have been sent to the National Seed Storage Laboratory, U.S.D.A., Fort Collins (U.S.A.).

Samples were collected from the following areas: Valencia (V), Andalucía (AN), Castilla-La Mancha (CM), Extremadura (E), Cataluña (C), and Aragón (A). Table 1 shows the items collected.

Table 1. Accessions collected.

| Label   | Local name            | Locality             | Observations                         |
|---------|-----------------------|----------------------|--------------------------------------|
| V-CI-1  | Sandía                | Villar del Arzobispo | very dark skin colour                |
| V-CI-2  | Sandía                | Villar del Arzobispo | pale green skin colour               |
| V-CI-3  | Sandía piel blanca    | Villar del Arzobispo | white skin colour                    |
| V-CI-4  | -                     | Jalón                | very big size                        |
| V-CI-5  | Melona                | Lliber               | dry farming                          |
| V-CI-6  | Melo' d'alger         | Lliber               | dry farming                          |
| V-CI-7  | Melo' d'alger         | Lliber               | from Egypt, dry farming              |
| V-CI-8  | Sandía                | Benisa               | long cycle                           |
| V-CI-9  | Melón de agua         | Novelda              | -                                    |
| V-CI-10 | Melón de agua         | Novelda              | -                                    |
| V-CI-11 | Sandía                | Bensia               | long cycle                           |
| V-CI-12 | Melona                | Algar                | -                                    |
| V-CI-13 | Melón de sang de Rosi | Algar                | -                                    |
| V-CI-14 | Melona                | Algar                | pale-dark skin, striped              |
| V-CI-15 | Sandía                | Vinaroz              | long cycle                           |
| V-CI-16 | Sandía                | Albocácer            | long cycle, dry farming              |
| V-CI-17 | Sandía                | Ademuz               | -                                    |
| V-CI-18 | Sandía                | Ademuz               | -                                    |
| V-CI-19 | Sandía                | Ademuz               | long, yellow flesh colour, bad taste |
| V-CI-20 | Sandía                | Ademuz               | -                                    |
| V-CI-21 | Sandía                | Ademuz               | -                                    |
| V-CI-22 | Sandía                | Casas Altas          | grown at 900m                        |
| V-CI-23 | Sandía                | Chulilla             | -                                    |
| V-CI-24 | Sandía                | Fontanares.          | dry farming, striped                 |
| V-CI-25 | Sangre de toro        | Játiva               | -                                    |
| V-CI-26 | -                     | Onda                 | -                                    |
| V-CI-27 | Sandía                | Fanzara              | long cycle                           |



| Label    | Local name          | Locality                    | Observations              |
|----------|---------------------|-----------------------------|---------------------------|
| V-CI-28  | Melona              | Fanzara                     | long cycle                |
| V-CI-29  | Melón del agua      | Venta del Moro              | -                         |
| V-CI-30  | Melón del agua      | Venta del Moro              | -                         |
| AN-CI-1  | Del terreno         | Puente Genil                | -                         |
| AN-CI-2  | Sandía              | Portugós                    | late crop                 |
| AN-CI-3  | Sandía larga rayada | Ugijar                      | late crop                 |
| AN-CI-4  | Sandía              | Igualaja                    | dry farming               |
| AN-CI-5  | Sandía              | Los Barrios                 | -                         |
| AN-CI-6  | Sandía              | Ubriquez                    | late crop                 |
| AN-CI-7  | Sandía blanca       | Benaocaz                    | late crop                 |
| AN-CI-8  | Sandía larga        | Tarifa                      | late crop                 |
| AN-CI-9  | Verde redonda       | Tarifa                      | late crop                 |
| AN-CI-10 | Verde rayada        | Tarifa                      | late crop                 |
| AN-CI-11 | Sandía de Rota      | Rota                        | late crop                 |
| CM-CI-1  | Sandía              | Retamoso de la Jara         | dry farming, not sweet    |
| CM-CI-2  | Sandía              | Retamoso de la Jara         | dry farming, sweet, small |
| CM-CI-3  | Sandía              | Retamoso de la Jara         | dary farming, big size    |
| CM-CI-4  | Sandía              | Retamoso de la Jara         | dry farming, big size     |
| CM-CI-5  | Sandía              | Retamoso de la Jara         | dry farming, short cycle  |
| CM-CI-6  | Sandía              | Retamoso de la Jara         | dry farming, short cycle  |
| CM-CI-7  | Sandía              | Oropesa                     | -                         |
| CM-CI-8  | -                   | Aldeanueva de San Bartolomé | -                         |
| E-CI-1   | Sandía rayada       | Hervás                      | long cycle                |
| E-CI-2   | Del terreno         | Hoyos                       | -                         |
| E-CI-3   | Inverniza           | Alagon del Caudillo         | long-term conservation    |
| E-CI-4   | Del terreno         | Alagón del Caudillo         | -                         |
| E-CI-5   | Grande americana    | Jaraiz de la Vera           | -                         |
| E-CI-8   | Común               | Garganta de la Olla         | -                         |
| E-CI-9   | Sandía              | Navaconcejo                 | -                         |
| C-CI-1   | Sandía              | Tortosa                     | -                         |
| C-CI-2   | Sandía              | Tortosa                     | -                         |
| C-CI-3   | Sandía grosa        | Falset                      | -                         |
| C-CI-4   | Sandía mediana      | Falset                      | -                         |
| C-CI-5   | Sandía pequeña      | Falset                      | -                         |
| A-CI-1   | Sandía              | Quicena                     | -                         |
| A-CI-2   | Sandía alargada     | Quicena                     | -                         |
| A-CI-3   | Sandía              | Lumpiaque                   | -                         |
| A-CI-4   | Sandía              | Lumpiaque                   | -                         |
| A-CI-5   | Sandía              | Lumpiaque                   | -                         |
| A-CI-6   | Sandía              | Magallón                    | -                         |

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## OUTCROSSING IN WATERMELONS

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The minimum distance to prevent pollen transfer between watermelon plants is reported to be 0.25 mile (1). This distance is difficult to achieve when a large number of isolations are required on a small research station. This study attempted to estimate amount of pollen transfer by indigenous bees at distances less than 0.25 mile.

Studies of natural pollen transfer among watermelon plants were made in 1983, 1984 and 1985 at Blackville, SC and Experiment, GA. A dominant gene, Spotted (Sp) (2) was used to measure how much pollen was transferred and how far. From open pollinated fruit selected from specific field sites, seed were removed for scoring the dominant seedling marker. Usually more than 100 seedlings from each melon were scored at the third or fourth true leaf stage.

We attempted to design studies to address the following questions: (1) How far is pollen transferred? (2) What is the effect of interplanting muskmelons between the pollen source and the female parent? The following studies were conducted:

1983. At Blackville, 16-hill blocks of 13 watermelon varieties were alternated with 32-hill blocks of muskmelons in four replicates. This planting was actually a watermelon and muskmelon trial that lost a number of seedlings in several blocks after a late freeze. Missing hills were replaced by Sp seedlings. The location of each Sp plant was mapped. A watermelon block was isolated on two sides by muskmelons and on two sides by an unplanted strip 25 feet wide.

1984. At Experiment, eight plant introductions were replicated four times in blocks of eight watermelon plants buffered on each side by a 'Butternut' squash plant. Hills of watermelons were equidistant from a row of Sp plants alternating with 'Butternut' squash plants. After fruit set, the nearest distance from the melon to a pollen source was measured.

1985. Two studies were done - one at Blackville, SC and one at Experiment, GA.

At Blackville, four hills of the Sp line were planted inside a large block of 'Crimson Sweet', planted two feet apart on the row, with rows ten feet apart. The nearest distance from Sp hills to 'Crimson Sweet' plants was 15 feet and the greatest distance was 125 feet. Seed were saved from fruit from each quadrant: 21, 32, 19 and 25 for quadrants NW, NE, SW and SE, respectively.

At Experiment, the study employed two blocks with 5 Sp plants in the center and watermelon PI's planted at 12 and 48 feet from Sp in one block and at 12, 36 and 48 feet in the other block. Spaces between blocks were 15 feet apart within the row and 12 feet apart between rows.

The same four PI's were planted at 12, 36 and 48 feet. There was only one set of plants at 12 feet, four sets of the PI's at 36 feet and 8 sets of the PI's at 48 feet.

To determine the effect of distance on per cent outcrossing, distances were first bracketed into 12 groups and means from each of the four data sets were considered replicates (Table 1). All four studies indicate that outcrossing is rare beyond 90 feet. The presence of a nearby bee hive could increase outcrossing.

To determine the effect of the muskmelon "barrier" in the 1985 Blackville field, outcrossing was compared within the block where the pollinator was located, in another block of watermelons separated only by a 25 foot unplanted area and in a watermelon block isolated from the pollinator by a block of muskmelons (Table 2). It is interesting that outcrossing occurred in only one case where watermelons were isolated by muskmelons. However, outcrossing percentages of 0.72, 0.72 and 1.01 were found in blocks of watermelons separated by two blocks of muskmelons. Thus, these small barriers are not sufficient to prevent outcrossing.

Where only natural bees are present, outcrossing beyond 80 feet appears to be rare. A final observation made with an entirely different genetic system supports this idea. In 1986, a block of several hundred male sterile watermelon plants were sited 450 feet from several hundred male fertile watermelon plants. Adjacent rows of male fertile plants were removed after the initial fruit set. Every fruit was then removed from male sterile plants. The male fertile plants produced pollen for three more weeks but none of the thousands of female blossoms on the male sterile plants were pollinated. One-fourth mile (1320 feet) is a generous isolation and, in most cases, half this distance is sufficient.

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Table 1. Effect of distance from pollen source on per cent outcrossing in watermelon.

| Distance (feet)<br>from pollen source | Per Cent Outcrossing <sup>z</sup> |        |        |        | Mean |
|---------------------------------------|-----------------------------------|--------|--------|--------|------|
|                                       | '83(B)                            | '85(B) | '84(E) | '85(E) |      |
| <10                                   | 0.2                               | -      | -      | -      |      |
| 10-20                                 | 0.7                               | 2.4    | 7.6    | 5.5    | 4.1  |
| 20-30                                 | <0.1                              | <0.1   | 3.0    | 0      | 0.8  |
| 30-40                                 | 0.2                               | <0.1   | 2.5    | 0.3    | 0.7  |
| 40-50                                 | 0                                 | 0      | 0      | 0.6    | 0.1  |
| 50-60                                 | <0.1                              | <0.1   | 0      | 0.1    | <0.1 |
| 60-70                                 | <0.1                              | <0.1   | 0      | 0.1    | <0.1 |
| 70-80                                 | <0.1                              | <0.1   | 0      | 0.2    | 0.1  |
| 80-90                                 | 0                                 | 0      | 0      | 0      | 0    |
| 90-100                                | 0                                 | 0      | 0      | 0      | 0    |
| 100-110                               | <0.1                              | <0.1   | 0      | 0      | 0    |
| >110                                  | 0                                 | 0      | 0      | 0      | 0    |

<sup>z</sup>Per cent of seedlings showing dominant Sp trait.  
(B) is Blackville, S.C. location and (E) is Experiment, GA. location.

Table 2. Per cent outcrossing within blocks of watermelons and among blocks isolated by unplanted blocks or muskmelon blocks.

| Watermelon<br>Block <sup>z</sup> | Type of Isolation |                              |                         |
|----------------------------------|-------------------|------------------------------|-------------------------|
|                                  | None              | Unplanted Block <sup>y</sup> | Muskmelons <sup>x</sup> |
| Per Cent Outcrossing             |                   |                              |                         |
| 1                                | 0.4               | 0                            | 0.7                     |
| 2                                | -                 | 0.3                          | 0                       |
| 3                                | -                 | 0                            | 0                       |
| 4                                | <0.1              | 0.1                          | 0                       |
| 5                                | 1.3               | 0                            | 0                       |
| 6                                | -                 | 0.4                          | 0                       |
| 7                                | -                 | 0                            | 0                       |
| 8                                | -                 | 0                            | 0                       |
|                                  | 0.6               | 0.1                          | 0.1                     |

<sup>z</sup>Four X 4-hill block of watermelons spaced 5 feet apart.

<sup>y</sup>Twenty-five feet wide.

<sup>x</sup>Eight X 4-hill block of muskmelons spaced 2.5 feet apart on the row with 5 feet between rows.

Cucurbita fraterna, the Closest Wild Relative and Progenitor of C. pepo

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Cucurbita fraterna Bailey is one of the least known species of Cucurbita. Our knowledge has been based entirely on the holotype, a single herbarium specimen collected by C.L. Lundell in 1937 that contains no pistillate flowers, fruits, seeds or roots. Recently, T.C. Andres, M. Nee, and J.J. Wyland collected the first complete specimens of C. fraterna, including seeds for germplasm, from two populations in northeastern Mexico. One of these populations represents the topotype (collected at the type locality near Llera, Tamaulipas). The other population was found 50 km northeast of this site. Some previously unidentified Cucurbita specimens collected in 1971 by J.V.A. Dieterle (nos. 3800 and 3804, deposited at the University of Michigan Herbarium), now appear to represent two additional populations of C. fraterna in Nuevo Leon, ca. 325 km north-northwest of the type locality.

When Bailey (3) described C. fraterna, he believed it was closely related to C. texana (Scheele) Gray, hence the specific epithet from the Latin 'frater' (brother) of C. texana. (Another less closely related species, C. sororia Bailey, had already been named the 'soror' (sister) of C. texana). C. fraterna is similar to C. texana but differs in leaf shape and lobing, trichome morphology, and natural distribution.

C. texana has stimulated a considerable number of biosystematic investigations (5), since it has been considered the progenitor of C. pepo L. (1,2,3,4,6,11). This hypothesis is flawed because C. texana has a fairly restricted range and does not occur in Mexico, where the oldest archaeological records of C. pepo have been found (7,10) and where primitive landraces of C. pepo are still being grown today (12). Furthermore, C. texana contains only a small, atypical subset of the isozymes found in various C. pepo cultivars (9; Andres, unpub.). Ancestral species typically exhibit greater genetic diversity than recently derived species, although this may not necessarily be true in outcrossing species under artificial selection (8). Thus, no alternative hypothesis existed regarding the origin of C. pepo.

C. fraterna has fibrous roots and the plants are annual like the related taxa. The round fruits are similar in coloration and shape to C. texana, although most populations of C. texana also contain pyriform-shaped fruits. The round and pyriform fruit types resemble 'Miniature Ball Gourd' and 'Striped Pear Gourd', respectively, both being types of ornamental gourds, C. pepo var. ovifera (L.) Alef.

The fruits of all wild Cucurbita species and most ornamental gourds are extremely bitter, but a non-bitter form of C. fraterna was described on a herbarium label (Dieterle, no. 3804). Similarly, non-bitter C. sororia fruits have been found (R.A. Bye, personal communication).

The fruits of C. sororia differ from C. fraterna and C. texana by lacking very definite carpellary stripes (3). C. sororia appears to represent the wild progenitor of C. mixta Pang. (L.C. Merrick, personal communication). A population of C. sororia (Andres and Nee, collection no.177) was found growing near the type locality of C. fraterna. This species has not been previously reported in the state of Tamaulipas.

C. fraterna generally occurs in upland, seasonably dry thornscrub habitat whereas C. texana is restricted to river bottomland. C. texana fruits readily abscise from the peduncles earlier in development than those of C. fraterna and C. sororia. This may represent an adaptation for dispersal by floating during spring floods.

Accessions of C. fraterna, C. texana, C. pepo, C. sororia, and other Cucurbita species were grown in 1986 at Geneva, NY. Crosses were made and isozymes compared using horizontal starch gel electrophoresis. A total of approximately 25 loci representing ten enzymes was examined. The isozyme data indicate that considerably more allelic variation exists among the two sampled populations of C. fraterna than among 20 sampled populations of C. texana. Unlike C. texana, the alleles of C. fraterna are all commonly found in cultivars of C. pepo. A landrace of C. pepo with elongated, medium size Jack-O-Lantern like fruit, found near the populations of C. fraterna, was indistinguishable from C. fraterna in the enzymes sampled and in its vegetative morphology. This suggests that C. pepo pumpkins may represent an early domesticated form of C. fraterna. The sympatric C. sororia population did share many alleles with C. fraterna, but also contained several novel alleles not found in C. pepo.

Based on this information, pollinations were made primarily between C. fraterna and various accessions of C. pepo, C. texana, C. sororia and, to a lesser extent, other less closely related species. C. fraterna was readily crossable with all types of C. pepo, including C. texana, but was much less compatible with other species of Cucurbita. C. sororia can be crossed with C. fraterna, but with poor seed set. The crossing data are therefore congruent with the isozyme data.

C. fraterna appears to be the original wild progenitor of C. pepo, based on the genetic evidence, occasional occurrence of non-bitter fruits, proximity to archaeological sites of C. pepo, and similarity to traditional landraces of C. pepo. C. fraterna and C. texana may have originally been incipient species or ecotypes. But secondary contact with C. texana may have occurred when C. pepo (i.e., domesticated C. fraterna) spread northward by humans into eastern U.S., introducing new sources of genetic variability. If future evidence proves this to be the case, C. pepo would represent a compilospecies composed of the originally separate taxa, C. fraterna and C. texana. Furthermore, introgression may have occurred with populations of C. sororia. The astonishing range of cultivars present today in C. pepo may have arisen over a period of 10,000 years by a complex pattern of (A) multiple incipient domestications among the small, semi-isolated populations of C. fraterna, (B) dispersal to new areas, resulting in various genetic-environmental interactions, and (C) a generally reticulated pattern of occasional hybridization both among landraces

and between these taxa and related wild taxa. Additional germplasm collecting and molecular analysis will help clarify these presumptive patterns of domestication.

Based on the evidence given, C. pepo should be classified as containing three subspecies: C. pepo ssp. pepo, C. pepo ssp. fraterna, and C. pepo ssp. texana.

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**Hybridization of Cucurbita foetidissima with C. pedatifolia, C. radicans, and C. ficifolia**

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Cucurbita foetidissima HBK has been regarded as an aberrant species, showing no affinity toward any other Cucurbita species, based on phenetic relationships (4), pollinator species relationships (6), and compatibility relationships (3). Many attempts have been made to hybridize C. foetidissima with other Cucurbita species, but with only limited success. Fruit set was reported in crosses between C. foetidissima and several species, but only by using C. moschata (Duch. ex Lam.) Duch. ex Poir. as the pollen parent were partially developed embryos obtained (3,5). These embryos were cultured and produced vigorous, but sterile plants. Female fertility, however, was restored in the C. foetidissima x C. moschata hybrids by producing amphidiploids (1).

As part of a biosystematic investigation of the genus Cucurbita, two wild xerophytic species, C. pedatifolia Bailey (syn. C. moorei Bailey) and C. radicans Naud. (syn. C. gracilior Bailey), plus one domesticated ombrophilic species, C. ficifolia Bouché, were successfully hybridized with C. foetidissima (Table 1). This is the first report of fully developed interspecific F<sub>1</sub> seeds produced by C. foetidissima. Although none of these species were completely cross-compatible with C. foetidissima, F<sub>1</sub>, F<sub>2</sub> and backcross to C. foetidissima progeny were produced in interspecific crosses of C. foetidissima with C. pedatifolia. C. radicans, a species closely related to C. pedatifolia, showed less compatibility with C. foetidissima. All 18 pollinations made between C. radicans and C. foetidissima set fruit, but only a few seeds were fully developed. These latter pollinations, however, were performed at Geneva, NY during the unusually cool, wet summer of 1986 and therefore may be misleading. Most of the other pollinations listed in Table 1 were made during the previous more normal summers.

C. ficifolia hybridized with C. foetidissima only when used as the female parent. The F<sub>1</sub> plants were male sterile and possibly also female sterile, since they did not develop any embryos. C. ficifolia may also be hybridized with C. pedatifolia, but again, no subsequent generations were obtained.

C. ficifolia differs from the aforementioned species in having a fibrous, rather than storage-type, root system. C. radicans and C. pedatifolia share the unique ability to produce stolons, or nearly leafless stems, and bare multiple tubers per rooted node; each tuber is 3- 4 cm long in C. radicans and 10- 15 cm long in C. pedatifolia. Although C. ficifolia, like C. foetidissima, is considered an aberrant species in the genus, it does share a unique morphological character with C. foetidissima, C. pedatifolia, and C. radicans in having pubescent filaments. The remaining species of Cucurbita all have glabrous filaments.

C. foetidissima is of particular interest as a potentially new agronomic crop for arid and semi-arid lands, and a multi-disciplinary team has developed a breeding program to domesticate this species as a source of oil from the seeds, starch from the roots, and forage from the vines (2). Additional sources of germplasm available through interspecific hybridization of C.



foetidissima with C. pedatifolia, C. radicans, and C. ficifolia may provide useful genetic traits for this project. For example, heterosis was observed in hybrids between C. foetidissima and C. pedatifolia. C. pedatifolia also has the ability to survive under more extreme xerophytic conditions than C. foetidissima. The potato-size tubers of C. pedatifolia may be more suitable for mechanical harvest than the single huge taproots produced by C. foetidissima.

Table 1. Experimental crosses between C. foetidissima and C. pedatifolia, C. radicans, and C. ficifolia.

| Cross                        | Fruit set/<br>pollinations | Fruit with fully<br>developed seeds | Fruit with under<br>developed embryos | Fruit with no<br>embryo development | No. developed<br>seeds/fruit <sup>2</sup> |
|------------------------------|----------------------------|-------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------------|
| FOET. x PED.                 | 1/2                        | 1                                   | 0                                     | 0                                   | I                                         |
| PED. x FOET.                 | 2/2                        | 1                                   | 0                                     | 1                                   | F                                         |
| (FOET. x PED.) selfed        | 3/8                        | 3                                   | 0                                     | 0                                   | M                                         |
| (PED. x FOET.) selfed        | 1/4                        | 0                                   | 1                                     | 0                                   | -                                         |
| (FOET. x PED.) FOET.         | 4/4                        | 4                                   | 0                                     | 0                                   | F-M                                       |
| FOET. (FOET. x PED.)         | 0/1                        | -                                   | -                                     | -                                   | -                                         |
| FOET. x RAD.                 | 6/6                        | 0                                   | 1                                     | 5                                   | -                                         |
| RAD. x FOET.                 | 12/12                      | 3                                   | 6                                     | 3                                   | F                                         |
| FOET. x FIC.                 | 0/10                       | -                                   | -                                     | -                                   | -                                         |
| FIC. x FOET.                 | 4/25                       | 4                                   | 0                                     | 0                                   | I                                         |
| FIC. (FOET. x PED.)          | 1/10                       | 1                                   | 0                                     | 0                                   | F                                         |
| (FOET. x PED.) FIC.          | 0/46                       | -                                   | -                                     | -                                   | -                                         |
| (PED. x FOET.) FIC.          | 0/5                        | -                                   | -                                     | -                                   | -                                         |
| (FIC. x FOET.) FOET.         | 0/2                        | -                                   | -                                     | -                                   | -                                         |
| (FIC. x FOET.) FIC.          | 0/25                       | -                                   | -                                     | -                                   | -                                         |
| (FIC. x PED.) FOET.          | 1/3                        | 0                                   | 1                                     | 0                                   | -                                         |
| (FIC. x PED.) FIC.           | 4/13                       | 0                                   | 2                                     | 2                                   | -                                         |
| (FIC. x FOET.)(FOET. x PED.) | 0/2                        | -                                   | -                                     | -                                   | -                                         |
| (FIC. x FOET.)(PED. x FOET.) | 0/1                        | -                                   | -                                     | -                                   | -                                         |

<sup>2</sup> F= few (25-50), I= intermediate (51-200), M= many (>200).

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**Relationship of Cucurbita scabridifolia to C. foetidissima and C. pedatifolia: a case of natural interspecific hybridization**

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Cucurbita scabridifolia Bailey is a poorly known wild perennial gourd from southern Tamaulipas, Mexico (2). Recently T.C. Andres, J.J. Wyland, and M. Nee collected several populations of C. scabridifolia-like plants near the type locality. Based on field observations and an examination of herbarium specimens, C. scabridifolia appears to be one of a gradient of biotypes occurring between two other wild perennial gourd species, C. foetidissima HBK and C. pedatifolia Bailey. Table 1 lists five morphological characters which are distinct between C. foetidissima and C. pedatifolia, and shows the generally intermediate position of C. scabridifolia. These three taxa are similar in other characters, such as in their flower and fruit morphology.

The various intermediate forms between C. foetidissima, C. scabridifolia, and C. pedatifolia has led to considerable taxonomic confusion. For example, Bailey (2) described a lobed-leaf form of C. foetidissima which "may or may not belong to this species".

The distribution of these intermediate types, including C. scabridifolia, occurs in north-central Mexico, an area where C. foetidissima and C. pedatifolia overlap in range. C. pedatifolia, however, generally inhabits more arid regions south of the large range of C. foetidissima, which extends northward into the U.S.

An experimental hybridization study was conducted to demonstrate the genetic compatibility and thus, potential for natural hybridization to occur between C. foetidissima and C. pedatifolia. Fully developed seeds, although in somewhat limited numbers, were successfully obtained in the F<sub>1</sub>, F<sub>2</sub> and backcross generations (1). The F<sub>1</sub> plants showed hybrid vigor and bore numerous fruits. The plants were intermediate in morphology between the two parent species, but had generally more deeply lobed-leaves than typical of C. scabridifolia. The phenotypes of the F<sub>2</sub> plants were extremely variable, due to Mendelian segregation of the genetic factors responsible for the interspecific differences. Some plants resembled the lobed-leaf forms of C. foetidissima that Bailey originally described, *op. cit.*, others were extremely stunted bush types, while still others contained deformed "virus-like syndromes" similar to those described for other interspecific Cucurbita crosses by Whitaker and Bemis (4). Backcrosses of the F<sub>1</sub> to C. foetidissima produced plants indistinguishable from the type specimen and the original description of C. scabridifolia.

Apparently there are no pre-zygotic barriers to natural hybridization between C. foetidissima and C. pedatifolia. The two species occur within pollination range of each other, flower during the same period, and may be pollinated by the same species of bees.

Therefore, C. foetidissima evidently naturally hybridizes with C. pedatifolia, and C. scabridifolia represents one of the hybrid derived biotypes. C. foetidissima and C. pedatifolia seem to be maintaining the essential integrity of their separate gene pools, despite hybridization between them, because of

sterility barriers preventing extensive gene flow and also possibly due to natural selection working against inferior F<sub>2</sub> and backcross combinations. Therefore, unlike C. scabridifolia, C. foetidissima and C. pedatifolia are legitimate species. Although a numerical taxonomic study on Cucurbita phenotypic relationships (3) grouped C. foetidissima and C. pedatifolia into separate groups, unrelated to any other species, they are genetically related.

Table 1. Morphological comparison between C. foetidissima, C. scabridifolia, and C. pedatifolia.

|                         | Leaf shape       | Leaf-lobing           | Leaf trichomes | Seed margin        | Storage roots     |
|-------------------------|------------------|-----------------------|----------------|--------------------|-------------------|
| <u>C. foetidissima</u>  | sagitate         | unlobed               | scabrid        | immarginate        | fusiform taproot  |
| <u>C. scabridifolia</u> | broadly sagitate | lobed halfway to base | scabrid        | somewhat marginate | shortened taproot |
| <u>C. pedatifolia</u>   | reniform-cordate | lobed nearly to base  | soft pubescent | marginate          | multiple tubers   |

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## Inheritance of Bitterness in Cucurbita pepo L.

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Cucurbitacins are very bitter and toxic secondary plant substances which can be found in roots, cotyledons and fruits of many cucurbit species. Synthesis of the bitter principles is initiated with the onset of seed germination. It increases rapidly in roots and cotyledons of up to 6 day old seedlings after which it decreases (Rehm, 1960). Jaworski et al. (1985) found a decrease in the cotyledons but not in the roots of Cucurbita pepo 'Blackjack'. The inheritance of bitterness in C. pepo was thought to be controlled by one major dominant gene (Grebenscikov 1954). Nonbitter (NB) fruits were found on bitter (B) seedlings, however, which pointed to the action of a suppressor gene (Rehm, 1960). In later studies Rehm (1968) found the gene for fruit bitterness to be independent from the gene for seedling bitterness.

The occurrence of bitter fruit on 'Zucchini' -type summer squash in commercial plantings prompted our genetic studies. Our aim was to determine the inter-relationships of genes controlling bitterness in roots, cotyledons and fruits and to develop effective seedling screening techniques for the selection of NB genotypes. Bitterness of the roots, cotyledons and fruits was evaluated organoleptically; roots 4 days or more and cotyledons 6 days or more after germination. Thin layer chromatography techniques (Gorski et al., 1968) could not be used to distinguish B from NB roots and cotyledons on an individual plant basis.

Bitterness was detected in the seedlings of several C. pepo cultivars. The following combinations of B and/or NB roots and cotyledons were found:

| Cultivar                                | root | cot | fruit | No. plants tested |
|-----------------------------------------|------|-----|-------|-------------------|
| 'Early Summer Crook Neck' ('ESCN')      | NB   | NB  | NB    | 10                |
| 'Yellow Summer Crookneck Improved'      | NB   | NB  | NB    | 15                |
| 'Early Prolific Straight Neck' ('EPSN') | B    | NB  | NB    | 20                |
| 'Zucchini Dark Green'                   | B    | B   | NB    | 10                |
| 'Black Zucchini'                        | B    | B   | NB    | 10                |
| 'Cocozelle'                             | B    | B   | NB    | 13                |

With the exception of 'Cocozelle', a decline in root and cotyledon bitterness could be detected in these cultivars and in bitter fruited 'Zucchini' -type squash. Crosses between 'ESCN' and 'Black Zucchini' or 'Cocozelle' and backcrosses to 'ESCN' showed the expected monogenic B:NB ratios in the roots and cotyledons of 'ESCN' x 'Cocozelle', but not in 'ESCN' x 'Black Zucchini'. The lower than expected degree of bitterness found in 'ESCN' x 'Black Zucchini' may be attributed to the low quantity of cucurbitacins present in the B parent.

Crosses between 'Zucchini' -type squash and 'EPSN' differing in cotyledon bitterness only showed the expected 3B:1NB ratio for cotyledon bitterness.

Our genetic studies with F4 and F5 selections of bitter fruited 'Zucchini' -type squash were in general agreement with Rehm's (1968) studies. Fruit bitterness was found to be controlled by one dominant gene (Table 1). It segregated

independently from genes controlling bitterness in other plant parts. The low degree of bitterness found in the roots and cotyledon points to a low concentration of cucurbitacins in these plant parts.

The results indicate that bitterness in the root, cotyledons and fruit of Cucurbita pepo appears to be controlled in each plant part by a monofactorial dominant gene. Seedlings with nonbitter roots generally had nonbitter cotyledons, while seedlings with bitter roots had non-bitter or bitter cotyledons. The gene for cotyledon bitterness may be dependent on the gene for root bitterness. Since fruit bitterness segregates independently from seedling bitterness, selection against bitterness of the fruit can not be done in the seedling stage.

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Table 1. Chi-square test results of F2 and BC data from crosses between NB and B 'Zucchini' type summer squash.

| Plant part | Generation | Observed B:NB ratio | Expected B:NB ratio | x <sup>2</sup> | P      |
|------------|------------|---------------------|---------------------|----------------|--------|
| root       | F2         | 124:67              | 3:1                 | 10.53          | < .005 |
|            | BC         | 31:52               | 1:1                 | 5.32           | < .05  |
| cotyledon  | F2         | 65:126              | 3:1                 | 170.99         | < .005 |
|            | BC         | 15:68               | 1:1                 | 33.84          | < .005 |
| fruit      | F2         | 140:50              | 3:1                 | .18            | > .50  |
|            | BC         | 37:44               | 1:1                 | .30            | > .50  |

## Early Vegetative Development of Spaghetti Squash is Unaffected by Seed Size

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Spaghetti squash (Cucurbita pepo) has experienced a surge of popularity during the past few years. Since its introduction into North America from Japan in 1936 (2), the sole cultivar has been 'Vegetable Spaghetti', a vine-type, open-pollinated, pale-fruited cultivar. We introduced for 1986 the first hybrid spaghetti squash cultivars, 'Orangetti' and 'Go-Getti' (1). Both hybrids are improved over 'Vegetable Spaghetti' by having semi-bush habit and attractive, intense fruit color.

As with many other commercial C. pepo hybrids, 'Orangetti' and 'Go-Getti' are produced by treatment of the female parent with ethephon and use of honeybees for transferring pollen from the male parent to the female. It was readily noticeable that ethephon treatment of squash resulted in smaller plants which in turn produced smaller fruits with seeds about one-third smaller than normal. Although germinability was excellent, no comparison had been made between plants developing from small seeds with plants developing from large seeds. The aim of the present work was to compare the vegetative development of plants developing from large and from small seeds.

Two experiments were conducted for this comparison, each with 2 treatments, "large" seeds (average seed weight 161 mg) and "small" seeds (average seed weight 108 mg). Seeds of both treatments were derived from a commercial stock of 'Vegetable Spaghetti' obtained from Sakata Seeds. The largest and smallest seeds from this commercial stock were selected for the treatments.

The first experiment was sown in a heated greenhouse on 16 October 1985 at Newe Ya'ar (Yizre'el Valley, northern Israel) in 5-liter plastic pots. The medium was grumusol-peat-vermiculite (1:1:1, v:v:v). There were 4 plants per treatment. The plants were taken 3 weeks after emergence for measurement of leaf blade fresh weight, stem and petiole fresh weight, and stem length.

The second experiment was sown in the field on raised beds, 2 m between bed centers and 2 plants every 50 cm in the row, on 3 April 1986 at Newe Ya'ar. There were 6 plants per treatment, which were taken from the center of a 24 m<sup>2</sup> plot 40 days after emergence for measurement of the same variables as above and for counting the number of internodes.

The results, presented in Tables 1 and 2, show that in spaghetti squash early vegetative development is unaffected by seed size. Seeds produced following ethephon treatment can be expected to develop into plants of the same vegetative vigor as seeds produced without ethephon treatment.

Table 1. Influence of seed size on early vegetative development of spaghetti squash. Greenhouse, Autumn 1985.

| Seed size | Leaf blade<br>fresh weight (g) | Stem+petiole<br>fresh weight (g) | Stem length<br>(cm) |
|-----------|--------------------------------|----------------------------------|---------------------|
| Large     | 9.58                           | 9.67                             | 27.6                |
| Small     | 9.12                           | 9.25                             | 31.6                |
| t         | 0.60                           | 0.41                             | 0.67                |
| P         | >0.50                          | >0.50                            | >0.50               |

Table 2. Influence of seed size on early vegetative development of spaghetti squash. Field, Spring 1986.

| Seed size | Leaf blade<br>fresh weight<br>(g) | Stem+petiole<br>fresh weight<br>(g) | Stem length<br>(cm) | Number of<br>internodes |
|-----------|-----------------------------------|-------------------------------------|---------------------|-------------------------|
| Large     | 217.3                             | 437.9                               | 364.8               | 47.3                    |
| Small     | 254.9                             | 409.2                               | 392.3               | 53.7                    |
| t         | 0.33                              | 0.27                                | 0.10                | 0.24                    |
| P         | >0.50                             | >0.50                               | >0.50               | >0.50                   |

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Inheritance of Resistance to Zucchini Yellow Mosaic Virus in Cucurbita moschata

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Since Provvidenti et al (1) reported resistance to zucchini yellow mosaic virus (ZYMV) in a C. moschata from Nigeria ('Nigerian Local'), work has been underway to transfer resistance to Butternut squash, also C. moschata. Butternut has never shown the high susceptibility to cucumber mosaic virus that we see regularly in C. pepo, but it is extremely susceptible to ZYMV. Butternut seedlings in greenhouse tests are frequently killed by ZYMV, and plants infected naturally at flowering time in the field in 1985 produced almost no fruit. Consequently there seems to be some urgency to breed a resistant Butternut.

The F<sub>1</sub> of Nigerian Local X Waltham Butternut (WBN) showed clear symptoms of ZYMV, much less severe than on WBN but far greater than on Nigerian, suggesting partial dominance that might permit selection during successive backcrossing. The F<sub>1</sub> of the BC<sub>1</sub> was started in the greenhouse and inoculated and selected there, with 24 classified as resistant and 23 susceptible. These were transplanted to the field, alternating individual resistant and susceptible plants. Differences between the 2 groups were maintained throughout the season, but differences in extent of growth appeared within the resistant group, suggesting modifier genes for a single basic resistance gene. As the resistant plants did not set fruit well, most backcrosses were made with their pollen and only a few obtained because of severe ZYM on uninoculated Butternut growing in another field to serve as female parent.

Six BC<sub>2</sub> F<sub>1</sub> progenies were grown in the greenhouse in the early months of 1986. All had approximately 50% resistant plants for a total of 73 resistant to 66 susceptible. Once again, little fruit set on the resistant plants and their male flowers were used to pollinate Waltham Butternut and Puritan Butternut. Their BC<sub>3</sub> F<sub>1</sub> progenies were started and inoculated in the greenhouse for the 1986 field planting, with 17 resistant plants represented in the parentage. Of these, only 2 failed to give approximately 50% resistant plants. Exact counts were not made because over 500 plants were grown and some discarded before it was clear how they should be classified. A better site than that of 1985 was chosen for growing the resistant segregates and there was no problem in getting self-pollinated fruit on them. Nine selfs giving the BC<sub>3</sub> F<sub>2</sub> generation planted in the greenhouse in December 1986 were chosen on the basis of their similarity to Waltham BN and Puritan in size, shape, color, and quality of cooked flesh. When symptoms appeared after ZYMV inoculation, plants were classified as follows:



- 40 homozygous resistant (slight mottling of older leaves but symptomless young leaves)
- 91 heterozygous resistant (definite mottling of young leaves but little stunting)
- 37 susceptible (strong mottling and stunting or death).

The 9 progenies all had some apparently homozygous resistant plants and were traceable to 5 different resistant segregates in the BC2. These results indicate that a single gene when homozygous in C. moschata confers a high level of resistance to ZYMV and cast doubts on the original thought that additional modifiers were needed.

We have attempted to transfer resistance from Nigerian Local to various C. pepo summer squashes. We have found in BC2 F<sub>2</sub> progenies infected segregates with vastly better growth than the C. pepo parents and presumably carrying the resistance gene from C. moschata. However the best of these do not approach the growth or freedom from mottling found in F<sub>2</sub> plants of the third backcross to Butternut.

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Lack of Seed Transmission in Squash and Melon Plants Infected with Zucchini Yellow Mosaic Virus.

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The rapid spread of zucchini yellow mosaic virus (ZYMV) to cucurbits grown in different areas of the world strongly suggests its transmission through seed. Early attempts to demonstrate this important avenue of dissemination in squash and melon seeds were unsuccessful (2,3,4). More recently, Davis and Mizuki (1) reported that ZYMV was transmitted in seed of the Black Beauty cultivar of Cucurbita pepo. However, though the virus recovered from infected seeds appeared to be serologically related to ZYMV, it caused only a limited systemic infection and inconspicuous symptoms in 'Black Beauty' and 'Multipik' squash. Since this seedborne virus incited symptoms that were radically different from those usually attributed to ZYMV, their claim of seed transmission must be considered inconclusive.

In the last two years, we have attempted to demonstrate transmission of ZYMV in summer squash and melon seeds. In the summer of 1985, 4266 squash plants were grown from three seed lots of Cucurbita pepo deriving from ZYMV-infected plants that were kindly provided by F. Angell (A. L. Castle, Inc. Hollister, CA). None of these plants exhibited symptoms associated with ZYMV infection. In 1986, a second attempt utilized locally grown 'Ambassador' seeds harvested in 1985 from severely malformed ZYMV-infected fruits. Of 2475 plants, none exhibited symptoms caused by this virus. In the same year, a third attempt involved 'Iroquois' melon seeds, which were harvested in 1985 from ZYMV infected plants. Although these seeds were small and malformed, germination was about 90%. None of the 100 plants from these seeds, that were grown in the greenhouse for about two months, was found infected by ZYMV. A field trial with 334 'Iroquois' plants deriving from the original seed lot was also free of ZYMV infection.

Except for the melon plants grown in the greenhouse, no assays were attempted to detect ZYMV infection. However, field grown plants were kept under constant observation until the end of the season, and none exhibited symptoms of ZYMV infection. The squash and melon plants eventually became infected with other viruses that are prevalent in the region, such as watermelon mosaic virus 2, cucumber mosaic virus, and clover yellow vein virus (= the severe strain of bean yellow mosaic virus).

Both here and abroad, circumstantial evidence has strongly suggested seed transmission of ZYMV in squash, melon, and watermelon. Though recent trials have been unsuccessful or inconclusive, more research is needed to clarify this very important point.

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## Electrophoretic Classification of Cucurbita Cultivars

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Cucurbita mixta was not recognized as a distinct species until 1930. Previously, members of this species were considered to be C. moschata. Although separated by partial sterility barriers, the two species are very similar in morphology. The taxonomic key feature, of the peduncle of C. mixta being corky and not flared at its attachment to the fruit, is not always reliable for distinguishing the two species.

A more reliable method of classification is by electrophoresis, since C. moschata and C. mixta have distinct patterns for esterase, peroxidase, peptidase, acid phosphatase, and other isozymes. By means of isozyme analysis, we determined that some cultivars previously considered to be C. mixta are actually C. moschata.

The term cushaw, like that of pumpkin and squash, refers to fruit type and usage rather than to a taxonomic entity. Isozyme analysis revealed that each of these terms has been used for cultivars of more than one species of Cucurbita. 'Green Striped Cushaw' has isozymes of C. mixta, agreeing with the previous morphological classification by Cutler and Whitaker (2). 'White Cushaw' also was previously classified as C. mixta (2), but it had isozymes of C. moschata. 'Golden Cushaw' also had isozyme banding patterns characteristic of C. moschata. 'Tennessee Sweet Potato' has been reported (2) to be C. mixta, but the isozymes of the accession of this cultivar we tested were typical of C. moschata.

The Seminole Pumpkin, which has been cultivated for centuries by Indians in Florida (3), was identified by Bailey (1) and Erwin (3) as C. moschata, despite its peduncle being more typical of C. mixta than C. moschata (3). Its isozyme phenotype, although distinctive from other C. moschata cultivars tested, is in agreement with the Seminole Pumpkin being C. moschata. The isozyme evidence does not support the theory (4) that 'Seminole' is derived from a cross between C. moschata and C. okeechobeensis. Fertile hybrids were easily obtained of 'Seminole' x 'Butternut', confirming that 'Seminole' is C. moschata.

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#### Inheritance of Fruit Skin Color in Cucurbita moschata

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Mature fruit of C. moschata cv. Long Neopolitan (PI 287531) have mottled dark and lighter green skin, in contrast to the buff color of 'Butternut'. The  $F_1$  of 'Butternut' x 'Long Neopolitan' had green fruit, and the  $F_2$  segregated 90 green: 32 buff. Thus, green skin color is due to a single dominant gene, designated G.

$F_2$  fruit of 'Butternut' x 'Long Neopolitan' were weighed, measured for length and maximum diameter, analyzed for soluble solids content with a hand refractometer, and subjectively rated for flesh color. G segregated independently of genes for fruit size and weight, soluble solids, and flesh color.

## Novel Variation in an Interspecific Cross

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An unusually large number of different chlorophyll deficient mutants was observed in breeding lines derived from the interspecific cross, Cucurbita maxima x C. ecuadorensis. One F<sub>2</sub> population segregated 147 normal : 18 albino seedlings that died in the cotyledon stage. Eight other F<sub>2</sub> populations, derived from different F<sub>1</sub> plants, did not segregate for the albino mutant. When the F<sub>1</sub> plant that produced albino progeny was backcrossed, to different plants of both species than used in the original cross, none of the backcross plants was albino. Thus, the albino mutant is recessive and probably due to a single gene with disturbed segregation ratio. It could have been present in heterozygous form in only one of the parental plants, or it may have occurred as a spontaneous mutation in a gamete of one of the parental plants and was therefore transmitted to only one of the F<sub>1</sub> plants.

Other chlorophyll deficiency mutants occurred, but not until subsequent generations of the same interspecific cross. Eleven advanced breeding lines segregated for seedlings with chlorotic cotyledons. Each of the 11 lines was derived from a different F<sub>2</sub> plant with normal phenotype, and the mutants are therefore probably the result of 11 different mutations. Mutants of one of the lines had cream colored cotyledons, and mutants of the other lines had cotyledons of varying degrees of yellow or light green. Each of the 11 mutants is recessive and probably monogenic. Mutants of one of the lines were lethal in the seedling stage. The others survived, although they remained chlorotic; they were fertile and produced seed under field conditions and are useful as seedling marker genes.

Cutler and Whitaker (1) also found novel variation in progeny of this interspecific cross. They reported finding various patterns of chlorophyll deficiency in F<sub>2</sub> and BC generations of C. ecuadorensis x C. maxima. Wall and Whitaker (4) reported F<sub>2</sub> segregation of 3:1 for one of these mutants, which had chlorotic leaves, stems, and petioles.

The disturbed segregation ratio of the albino mutant that segregated in one of the F<sub>2</sub> populations is not unique. Weeden and Robinson (5) reported significant deviations from Mendelian segregation ratios for 14% of the data for allozyme segregation in progeny of C. maxima x C. ecuadorensis.

Novel variation has been reported previously in progeny of species hybrids. Rick (2) concluded that the most likely source of the unusual variants he found in progeny of interspecific Lycopersicon crosses was heterozygosity in the self incompatible, wild species used as parents. Cucurbita ecuadorensis is self compatible and thus less likely to accumulate heterozygotes for deleterious, recessive genes than are the obligate

outcrossing Lycopersicon species. No chlorophyll deficient or other mutants were found in the self pollinated progeny of several C. ecuadorensis plants, indicating that heterozygosity for deleterious recessive genes is not common in that species. The original albino mutant found in the F<sub>2</sub> of C. maxima x C. ecuadorensis could have resulted from heterozygosity of one of the parental plants, but the 11 other mutants found subsequently have a different origin since none of these 11 mutants segregated in the F<sub>2</sub> generation of the interspecific cross. It wasn't until one or more additional generations of pedigree selection, or backcrossing to C. maxima followed by selfing, that the mutants were observed. Genome-cytoplasm interaction or complementary interaction of genes of the two parental species are also unlikely causes for the 11 mutants, since they did not occur in the F<sub>2</sub> of the interspecific cross. The relatively large number of different mutants found is probably not due to chance, and may reflect a high rate of mutation induced by hybridization such as that suggested by Sturtevant (3).

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## Downy Mildew Resistance in Cucurbita

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Cucurbita ecuadorensis and C. okeechobeensis are generally considered to be not very closely related. They were placed in different groups, when Cucurbita species were classified for similarity by numerical taxonomy (1), isozymes (4), and gourd bee relationships (3). Thus, it is surprising that they can be crossed easily. The cross was first made by Cutler and Whitaker (2). We obtained good fruit set for the interspecific cross, and each fruit had many plump seed with good germination.

Both species are late to flower, but they produced both pistillate and staminate flowers by September at Geneva, NY. The interspecific hybrid, however, was still vegetative and had no floral buds when frost occurred a month later. Cuttings were therefore sent to Homestead, Florida, where the F<sub>1</sub> plants finally flowered in April, 11 months after the seed was sown.

Downy mildew occurred in the planting at Florida, devastating all squash plants in the field, except those of C. ecuadorensis x C. okeechobeensis. Since the parents of this cross were not included in the planting, it was not possible to determine which species contributed resistance to Pseudoperonospora cubensis. If only one of the parental species is resistant, then downy mildew resistance must be dominant.

The interspecific F<sub>1</sub> was crossed with C. maxima cv. Buttercup, C. moschata cv Calabaza, and with breeding lines of C. pepo. Each 3-way cross produced viable seed that germinated without embryo culture, although the seed was thin, due to poorly developed cotyledonary tissue. There was a high degree of sterility in the next generation of each cross, but selfs, sibs, and backcrosses to the cultivated species were obtained, producing germplasm useful for breeding mildew and virus resistant squash.

<sup>1/</sup> Deceased

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## Genetic Variability for Compatibility of an Interspecific Cross

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The cross Cucurbita pepo x C. ecuadorensis, which is of interest for breeding squash for multiple disease resistance, was first made by Washek (2). The cross is usually difficult to make, even with embryo culture.

Our repeated attempts to cross C. ecuadorensis with C. pepo cultivars 'Early Prolific Straightneck', 'Caserta', and 'Scallop' were unsuccessful. Another C. pepo cultivar, 'Black Jack', proved to be much more compatible in this interspecific cross (Table 1). Fruit set was much better when 'Black Jack' was the C. pepo parent, and hybrids with C. ecuadorensis were obtained for 'Black Jack' but not for the other C. pepo cultivars.

'Black Jack' is a zucchini-type  $F_1$  hybrid. Washek (2) also succeeded in crossing C. ecuadorensis with a zucchini-type cultivar of C. pepo. Thus, a 'Zucchini' gene background may be helpful for accomplishing the interspecific cross, but more zucchini-type cultivars need to be tested to verify this.

Even though 'Black Jack' crossed more easily with C. ecuadorensis than did the other C. pepo cultivars tested, the cross is still encumbered with sterility barriers. Embryo culture was required in the initial cross, and many fruit were without embryos that could be cultured (Table 1). Fertility of the interspecific hybrid was low, but backcross seed was produced without embryo culture by using 'Black Jack' as the pollen parent. The interspecific hybrid was also successfully used as the maternal parent in crosses with C. moschata.

Both parental species are monoecious but the C. pepo x C. ecuadorensis hybrid was gynoeocious, agreeing with Washek's observation (2). A similar phenomenon was reported (1) for the cross C. maxima x C. moschata, which also produced a gynoeocious hybrid. Staminate flowers were induced to develop on the C. pepo x C. ecuadorensis  $F_1$  by multiple applications of GA<sub>4/7</sub> or Ag(NO<sub>3</sub>)<sub>2</sub>. However, pollen production was scanty and no  $F_2$  seed was produced when the treated interspecific hybrid was self- or sib-pollinated. The  $F_1$  plants did produce open-pollinated seed (Table 1), and the progeny indicated it was the result of outcrossing with C. pepo.

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Table 1. Fruit set and seed production of Cucurbita crosses.

| Cross                                      | No. crosses | No. set | % set | No. fruit with |           |                        | Seed with no embryos | No. progeny obtained |
|--------------------------------------------|-------------|---------|-------|----------------|-----------|------------------------|----------------------|----------------------|
|                                            |             |         |       | Plump seed     | Flat seed | Poor seed with embryos |                      |                      |
| 'Caserta' x <u>C. ecuadorensis</u>         | 190         | 15      | 7.8   | 0              | 0         | 10                     | 5                    | 0                    |
| 'Straightneck' x <u>C. ecuadorensis</u>    | 114         | 14      | 12.3  | 0              | 0         | 14                     | 0                    | 0                    |
| 'Scallop' x <u>C. ecuadorensis</u>         | 249         | 19      | 7.8   | 0              | 0         | 13                     | 6                    | 0                    |
| 'Black Jack' x <u>C. ecuadorensis</u>      | 145         | 95      | 65.5  | 1              | 2         | 23                     | 69                   | 30                   |
| <u>C. ecuadorensis</u> x 'Black Jack'      | 2           | 0       | 0.0   | -              | -         | -                      | -                    | 0                    |
| <u>C. ecuadorensis</u> x 'Straightneck'    | 21          | 0       | 0.0   | -              | -         | -                      | -                    | 0                    |
| <u>C. ecuadorensis</u> x 'Caserta'         | 20          | 5       | 25.0  | 0              | 0         | 5                      | 0                    | 0                    |
| F <sub>1</sub> <sup>z</sup> x 'Black Jack' | 83          | 10      | 12.0  | 0              | 4         | 6                      | 0                    | 14                   |
| F <sub>1</sub> x 'Straightneck'            | 3           | 1       | 33.3  | 0              | 0         | 1                      | 0                    | 0                    |
| F <sub>1</sub> x <u>C. ecuadorensis</u>    | 38          | 13      | 34.2  | 0              | 0         | 4                      | 9                    | 0                    |
| F <sub>1</sub> x self                      | 104         | 5       | 4.8   | 0              | 0         | 2                      | 3                    | 0                    |
| F <sub>1</sub> x OP                        | -           | 138     | -     | 0              | 0         | 29                     | 111                  | 45                   |

<sup>z</sup> F<sub>1</sub> = C. pepo cv. Black Jack x C. ecuadorensis

## Inheritance of Internal Fruit Color in an Interspecific Cross

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Cucurbita ecuadorensis, which has white flesh color, was crossed with C. maxima cv. Buttercup, which has deep orange flesh due primarily to  $\beta$ -carotene. Fruit of the interspecific hybrid had yellow flesh.

Acetone extracts of samples from fruit of the parents,  $F_1$ , and  $F_2$  generations of this cross were scanned with a Beckman DB spectrophotometer at  $440\mu$ , the wave length absorbing  $\beta$ -carotene. 'Buttercup' was high in carotenoids, with readings of 0.98 and above, whereas C. ecuadorensis showed absorbance readings of 0.03 or less. The  $F_1$  was intermediate, ranging from 0.22 to 0.30. Low concentration of  $\beta$ -carotene appears to be incompletely dominant.

Transgressive segregation occurred in the  $F_2$ . Two of 69  $F_2$  plants had readings of 1.50, higher than any parental plant. Continuous variation for flesh color occurred in the  $F_2$ , not only within the ranges of the parents and  $F_1$ , but also in the intervening ranges and beyond. Approximately three-fourths of the  $F_2$  population (49 of 69 plants) had color intensity similar to the  $F_1$  or less, and very few plants had as much  $\beta$ -carotene as 'Buttercup'. Thus, large populations in segregating generations will be required to combine the high level of  $\beta$ -carotene of C. maxima with the multiple virus resistance of C. ecuadorensis.

A source of Genes for Improved Fruit Color and Large Fruit Size in  
Cucurbita moschata

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PI 287531, an introduction from Naples, Italy of the cultivar Long Neopolitan, was included in the 1980 planting of Cucurbita maxima at the North East Regional Plant Introduction Station at Geneva, NY. Although originally identified as C. maxima, its fruit shape, peduncle, and other traits were more typical of C. moschata. It crossed readily with C. moschata cv. Butternut but not with C. maxima, confirming its identity as C. moschata. It did have two features, however, more characteristic of C. maxima than of C. moschata: extremely large fruit size and very deep orange flesh color, presumably due to  $\beta$ -carotene. Fruit of such large size is seldom encountered in any Cucurbita species other than maxima, and the bright, intense orange flesh color is unusual for C. moschata.

'Butternut' and 'Long Neopolitan' have similar phenotype but different genotypes for fruit shape. The  $F_1$  had large, oval fruit, and the  $F_2$  segregated for round, oval, and intermediate types as well as for the elongated neck type that is characteristic of both parents.

'Long Neopolitan' is of interest as a parent, due to its fruit flesh having such an attractive appearance, both in color and texture, and to its very long, thick neck that is devoid of seeds. Its appearance of good fruit quality, however, is misleading; the flavor, alas, is insipid and disappointing. The poor flavor is attributed to the low soluble solids content, only about a third of that of 'Butternut' fruit. The usefulness of 'Long Neopolitan' as a parent would be jeopardized if its large fruit size were genetically associated with low soluble solids content, with solids content being diluted in large fruited segregants. Fortunately, however, it was possible to recover  $F_2$  plants with large fruit and acceptable soluble solids content. Soluble solids content of fruit from 122  $F_2$  plants ranged from 3.5 to 10.5%, and was not closely correlated with fruit weight ( $r = 0.098$ ) or fruit length ( $r = -0.034$ ).

## Anther and Ovule Culture of Cucurbita

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Previous attempts to grow plants from cultured anthers have been successful for cucumber (3) and muskmelon (2) but not for squash (1). We found that squash pollen did not respond, other than to form pollen tubes, but longitudinal anther segments developed callus growth in vitro. Best results were achieved with anthers 10 to 20 mm in length. Murashige and Skoog medium with 1 or 2 ppm 2,4-D was more successful than the other media tested, and NAA (2 ppm) plus benzyl adenine (3 ppm) encouraged differentiation. Explants on solid media (0.8% agar) developed better than those in liquid on a rotary shaker.

Squash varieties differed in callus formation and differentiation on this medium. 'Gold Nugget' anthers developed a moderate amount of callus, more than the other C. maxima cultivars tested (Buttercup, Emerald, Oregold, and NK 530). C. moschata cv. Butternut, unlike 'Waltham Butternut' and 'Ponca', consistently developed callus from cultured anthers. All C. pepo cultivars tested (Straight Neck, Caserta, Lady Godiva, Scallop, Table Queen, and Cinderella) produced callus, but the nature of the callus and tissue formation differed for each cultivar. Best differentiation occurred with 'Scallop' and 'Cinderella'. Root formation occurred, particularly with 'Buttercup' and 'Scallop'. Shoot formation also occasionally occurred with these cultivars, but plantlets were not obtained. It was not determined if the cultured cells were haploid and of pollen origin, or diploid and derived from the anther wall.

Excised ovules of C. pepo cultivars Scallop and Early Prolific Straightneck did not respond well to tissue culture, but 'Black Jack' ovules developed prolific callus growth. Organized growth developed subsequently from the callus, but plantlets were not obtained.

Ovules excised from C. pepo cv. Black Jack, 24 to 72 hrs after pollination with C. ecuadorensis, produced callus and roots but not plants when cultured. The interspecific hybrid was obtained, however, by culturing embryos from mature fruit.

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## Notes on Squash Breeding

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Interspecific hybridization (11). The cross C. pepo x C. moschata is important from both theoretical and practical points of view. The natural reproductive isolation between these species is not as strong as that between other species of Cucurbita. Therefore, it might be possible to identify some of the major genetic barriers which keep these species apart. Furthermore, this cross can result in gene exchanges of economic value. Of special significance is the fact that, unlike the extreme susceptibility of C. pepo to cucurbit pests, C. moschata is an excellent source of resistance. Unfortunately, my notes on this cross are fragmentary.

In 1947 I obtained hybrid seed from crossing a bush line of 'Table Queen' (C. pepo) and 'Butternut' (C. moschata), using the former as seed parent. The cross was made at Fordhook Farms (W. Atlee Burpee Co.), Doylestown, PA, early in the morning (5-6 AM) of a cloudy fall day. The 20 cross-pollinated flowers of 'Table Queen' developed into mature fruits and all of them contained viable hybrid seed, ranging from 3 to 25. Parenthetically, an earlier report (2) suggested that 'Table Queen' may not be compatible with C. moschata. My results support the conclusion of some other breeders that favorable environmental conditions are essential for successful hybridization. The F<sub>1</sub> plants were large, semi-bush, and vigorous. The average fruit weight of the parents was 700g (Table Queen) and 1000g (Butternut) and that of the F<sub>1</sub>, 5000g, a remarkable heterosis for fruit size in squash. The F<sub>1</sub> fruit had a large central cavity and the "flesh" was of poor quality. The F<sub>2</sub> consisted of many male-female sterile segregates which exhibited serious growth abnormalities.

Some of the consequences of a similar cross were observed during 1979-1983. This cross was between 'Jersey Golden Acorn', BB, bearing golden, acorn-shaped fruits (C. pepo), and 'Burpee Butterbush', B<sup>+</sup>B<sup>+</sup>, bearing tan, bottle-shaped fruits with short thick "neck" (C. moschata). The fruit of the F<sub>1</sub>, BB<sup>+</sup>, was bicolor and intermediate in shape. The average fruit of the parents was 680g and 700g respectively, and that of the F<sub>1</sub>, 1041g. The F<sub>2</sub> (n = 283) was highly variable in fruit characteristics, in degree of fertility, and perhaps in proneness for parthenocarpy. With respect to fruit weight, the F<sub>2</sub> ranged from 146g to 2080g. But the most conspicuous features of the F<sub>2</sub> were severe growth abnormalities. Three other comments are of interest. First, the BC<sub>1</sub>, F<sub>1</sub>, BB<sup>+</sup>, x 'Jersey Golden Acorn', BB, consisted of a few BB plants which produced deeply scalloped disk-shaped fruits (similar to those of 'White Bush Scallop' of C. pepo) of intense golden color. Second, some F<sub>2</sub> segregates produced fruits of exceptionally thick flesh and very small central cavity. Third, some segregates were male-female sterile; others were male sterile early in plant development and male fertile later on; and still others were self-incompatible, but cross-compatible with 'Jersey Golden Acorn' as seed parent.

There is little doubt that the cross C. pepo x C. moschata deserves critical analysis as well as use in breeding. But it is evident that the ease by which F<sub>1</sub>, F<sub>2</sub>, and backcross seed can be obtained does not reflect the degree of "disharmony" between the two genomes.

Intraspecific hybridization. Each of the economically important species consists of several distinct groups of cultivars. Among other things, these groups differ from one another in fruit characteristics and duration of their artificial or geographical isolation. The short-term concerns in the squash seed industry are to perpetuate the existing groups of cultivars and to develop F<sub>1</sub> hybrids from intra-group crosses. In the long-term, it would be advisable to utilize the advantages of inter-group crosses. First the F<sub>1</sub> hybrid of inter-group crosses often exhibit greater heterosis than F<sub>1</sub> hybrids of intra-group crosses. However, the development of such hybrids will require additional breeding efforts. Second, some inter-group crosses generate tremendous genetic diversity without sterility and growth abnormalities. These crosses will provide the breeder an access to a larger portion of the gene pool including untapped potentially useful genetic elements. It is expected that most of this diversity will be inferior. But some genotypes will exhibit new useful traits; others will enhance the economic value of known desirable traits; and still others will reduce the expression of undesirable characteristics. This thought received its impetus from studies of genes B and M as well as from studies of growth habit, flowering, and sex expression.

Effects of B. This gene is widespread in C. maxima. A gene of similar behavior was transferred from the bicolor ornamental gourds to some of the major edible cultivars of C. pepo (7). In 1980, the B genes of C. maxima and C. pepo were transferred to C. moschata (9). And more recently, Paris and his colleagues in Israel reported an additional B transfer, from C. pepo and C. moschata.

Gene B conditions precocious chlorophyll depletion in fruit and this leads to precocious yellow pigmentation. Furthermore, B can bring about precocious depletion of chlorophylls in all other normally photosynthetic organs. In a broader sense, B can exhibit many "secondary effects" some of which are deleterious and others, beneficial. There is growing evidence indicating that the secondary effects result from interactions between B and other elements in the gene pool, and that the detrimental and beneficial effects are separable in breeding operations. Some of these elements are responsible for distinct phenotypes of their own, but most of them are invisible phenotypically except through their interactions with B. These "invisibles" are difficult to identify. Indeed, very few valuable invisibles (modifiers or regulators) have been identified (8, 10). Therefore, in cases in which the invisibles are not identified, the beneficial effects of B are surmised from comparison between isogenic or near-isogenic lines, BB and B<sup>+</sup>B<sup>+</sup>, of different backgrounds. But incisive tests are often lacking.

There exists a wide range of variation in the levels of fruit carotenoids among standard, B<sup>+</sup>B<sup>+</sup>, cultivars of Cucurbita. Previous observations (1956-1962) of the B effect on fruit color in C. pepo suggested that this gene can increase the carotenoid content, but that increases of large

magnitudes can be achieved through interactions between B and genes for dark green fruit color such as L, and provided gene inhibitors are not present (7). Subsequent chemical analyses by S. A. Garrison (in reference 6) and others (Table 1; see also reference 5) essentially confirmed this suggestion. Similar interactions exist in C. maxima and C. moschata.

The mechanism governing the synthesis of different fruit carotenoids is not understood. The specific role of environmental factors is not known. And most of the genes (including enhancers and inhibitors) which control pigmentation have not been identified. Weak or strong inhibitors appear to act a few days after B. They affect either the external portions of the fruit or both. They study of Schaffer et al (4) and other observations pose a number of intriguing questions concerning the role of B on plastid transformation in different tissues. It seems that a product of B, perhaps a diffusible substance (7), is the signal that regulates plastid transformation.

The first commercially available carotenoid-rich cultivar of summer squash was Burpee Golden Zucchini. It was developed by T. C. Torrey and introduced in 1973. This cultivar is similar to our PFZ (Precocious Fordhook Zucchini) breeding line developed in 1963. Both lines originated from the same germplasm. The use of cross IL-B x NJ-B (9) in breeding could lead to the development of nutrient-rich BB cultivars adapted to mechanized harvesting. Except for their green leaf blades, all other plant parts of these cultivars would be precociously golden. Such cultivars might be valuable to the food processing industry for various purposes, including dehydrated meal; stems for feed supplement, and fruit and seeds for human consumption.

In summer squash, the fruits of several BB lines have firmer flesh textures and exhibit new pleasing flavors to the extent not found in any of the known standard B<sup>+</sup>B<sup>+</sup> cultivars. Thus, gene B could be valuable for the development of new cultivars that are better adapted for use in freezing. 'Blondie' is the first BB<sup>+</sup> hybrid designed specifically for this purpose (David Groff, personal communication). The fruits of some "precocious" winter squash, such as 'Jersey Golden Acorn', BB<sup>+</sup> have a flavor reminiscent of yellow sweet corn. This is a new incipient trait which might be greatly reinforced through incorporation of genes for higher sugar content and absence of remnant of bitterness. I see the possibility for the development of dual-purpose B cultivars of high quality in C. pepo and C. maxima. These cultivars will serve both as "summer" and "winter" squash. And among them there will be cultivars used in food as water chestnut.

In 1966, I suggested that gene B can enhance female expression of the background of one of the summer squash cultivars. Since then this effect of B has been confirmed in repeated comparative tests of isogenic lines, BB and B<sup>+</sup>B<sup>+</sup>, of Early Prolific straightneck background. Results of one of these tests are presented in Table 2. The BB line involved is known as PEP. It was distributed among many breeders and it would be helpful if some of them publish their own findings on this subject. PEP is one of the parents of "multipik", a hybrid known for its strong female expression. As far as I know, the most strongly female line among the monocious cultivars of Cucurbita is inbred NJ260, BB. Yet, the effect of B on female expression

was not reported or has not been clearly evident in other backgrounds.

The recently synthesized closely related gynoecious lines (U.S. Patent pending), BB and B<sup>+</sup>B<sup>+</sup>, are late-flowering and 100% female in some environments, but they differentiate varying proportions of male flowers in other environments. Although we have not identified all the non-genetic factors which may promote late-flowering and male expression, it is clear that high temperatures favor both. The fact that B was not essential for the synthesis of these female lines does not necessarily exclude the possible role of B as an accelerator of pistillate flower differentiation in some backgrounds.

One of the advantages of the new gynoecious lines is that they can be used in strongly female monoecious cultivars which branch and differentiates their pistillate flowers more or less simultaneously, a desirable trait for both manual and mechanized harvesting.

Although gene B is a highly stable element in some backgrounds, it originated through the consequences of nuclear instability and is prone to instability in some other backgrounds. Its behavior recalls the behavior of a mobile element. This gene can manifest at least 2 kinds of variegation: one kind appears as a developmentally fleeting, imprecise, and unpredictable pattern seemingly due to phenotypic plasticity; and the other kind appears as a precise and predictable pattern due to mutation of B. Fruits, leaves and other parts of the plant may be affected. As a result, B is a potential source of variations some of which may be valuable in breeding of both edible and ornamental cultivars.

Finally, according to my observations all the known deleterious effects of gene B can be suppressed without interference on the expression of its beneficial effect. These observations support the following hypothesis. A potentially deleterious gene can become beneficial if two requirements are fulfilled. First, the gene must have one or more beneficial effects. Second, the gene pool must carry elements which suppress the deleterious effect independently of the beneficial one.

Virus infection. Some late-maturing pumpkins of C. pepo possess mild field tolerance to virus infection. In addition, the extremely intense dark green foliage of some breeding lines is more tolerant to virus infection than their fruit, particularly golden fruit.

The implication of gene B in fruit tolerance to the adverse effects of virus infection is difficult to assess. The best documented case is that of 'Multipik', introduced in 1981. According to T. H. Superak (personal communication), 'Multipik', BB is near-isogenic to 'Golden Girl', B<sup>+</sup>B<sup>+</sup>, but the former is less adversely affected by CMV-induced fruit symptoms than the latter. In addition, Adlerz et al (1) reported that the fruits of 'Multipik' are partially tolerant to WMV-2. And Paris et al (3) noted fewer virus-induced fruit symptoms (presumably CMV and WMV-2) in 'Goldy', BB<sup>+</sup>, than in 'Gold Rush', BB<sup>+</sup>. Obviously, if B is a contributing factor it alone cannot account for the difference in response between these hybrids.



Another issue worthy of further exploration is the possible escape mechanism of the silvery-leaf fruit trait against aphids (see communications on this subject in previous issues of CGC Rpt.). It is known that silvery leaves reflect more light than green leaves. And the inference is that greater light reflection tends to repel aphids. Our first silvery line was NJ260, BB. This line, however, proved to be highly susceptible to wilting under mild water stress conditions in the field. We transferred the genotype for the silvery trait to 'Precocious Caserta', BB, and found that the resulting silvery line, BB, is not more susceptible to wilting than standard B<sup>+</sup>B<sup>+</sup>, green cultivars.

The full genotype of the silvery trait has not been identified. The hypothesis is that it consists of gene M, for leaf mottling, and several modifiers. The silvery phenotype greatly fluctuates, in response to non-genetic variations, from different degrees of mottling (silvery spots) to uniformly silvery leaves. Attempts should be made to stabilize this trait through crosses between silvery lines which exhibit developmentally different modes of expressions.

Future direction. The long range goal in squash breeding is to restructure the Cucurbita genome through interspecific and intraspecific crosses for the purpose of transforming squash into a staple food crop of worldwide distribution.\*

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\*Some of the above notes, including tables 1 and 2, were taken from a six year old manuscript originally written for a cucurbit book whose publication has been delayed.

Table 1. Levels of carotenes and xanthophylls in ripe fruits of  $B^+B^+$  and  $BB$  inbreds of four cultivar backgrounds of *Cucurbita*. Each determination was based on a composite fresh sample obtained from the mesocarp of ten fruits. All inbreds were grown in a replicated test. From field studies, New Brunswick, New Jersey, 1978.

| Cultivar back-grounds <sup>x</sup> | Genotype | Carotenes <sup>y</sup><br>( $\mu\text{g/g}$ ) | $BB/B^+B^+$<br>(carotenes) | Xanthophylls <sup>y</sup><br>( $\mu\text{g/g}$ ) | $BB/B^+B^+$<br>(xanthophylls) | Total carotenoids<br>( $\mu\text{g/g}$ ) | $BB/B^+B^+$<br>(carotenoids) | X Carotenes |
|------------------------------------|----------|-----------------------------------------------|----------------------------|--------------------------------------------------|-------------------------------|------------------------------------------|------------------------------|-------------|
| EP                                 | $B^+B^+$ | 0.77                                          |                            | 1.74                                             |                               | 2.51                                     |                              | 30.7        |
|                                    | BB       | 1.94                                          | 2.5                        | 2.38                                             | 1.4                           | 4.32                                     | 1.7                          | 44.9        |
| FZ                                 | $B^+B^+$ | 0.35                                          |                            | 0.73                                             |                               | 1.08                                     |                              | 32.4        |
|                                    | BB       | 3.48                                          | 9.9                        | 22.13                                            | 30.3                          | 25.61                                    | 23.7                         | 13.6        |
| TK                                 | $B^+B^+$ | 2.40                                          |                            | 2.54                                             |                               | 4.94                                     |                              | 48.6        |
|                                    | BB       | 8.17                                          | 3.4                        | 12.38                                            | 4.9                           | 20.55                                    | 4.2                          | 39.8        |
| GD                                 | $B^+B^+$ | 10.6                                          |                            | 78.5                                             |                               | 89.1                                     |                              | 11.9        |
|                                    | BB       | 26.5                                          | 2.5                        | 123.3                                            | 1.6                           | 149.8                                    | 1.7                          | 21.5        |

<sup>x</sup>Key to cultivar backgrounds: EP = 'Early Prolific Straightneck', FZ = 'Fordhook Zucchini', TK = 'Table King', all three of *Cucurbita pepo*; GD = 'Green Delicious' (Munger's strain) of *Cucurbita maxima*.  $B^+B^+$  and  $BB$  inbreds of GD may not be entirely isogenic.

<sup>y</sup>Determinations were made by the New Jersey Feed Laboratory, 910 Pennsylvania Avenue, Trenton, NJ 08603, based on AOAC procedure.

Table 2. Female expression in  $B^+B^+$  and  $BB$  inbreds of 'Early Prolific Straightneck' background. The data are based on a replicated test of 10 plants per inbred. From field studies, New Brunswick, New Jersey, 1976.

| Genotype          | Cumulative number of pistillate flowers per plant<br>(Mean $\pm$ SD) <sup>a</sup> |                 |                 |                 |                 |                 |
|-------------------|-----------------------------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                   | Sequence of weeks from the time of anthesis of the first pistillate flower        |                 |                 |                 |                 |                 |
|                   | 1                                                                                 | 2               | 3               | 4               | 5               | 6               |
| $B^+B^+$          | 3.7 $\pm$ 1.89                                                                    | 6.8 $\pm$ 2.86  | 13.4 $\pm$ 4.99 | 16.4 $\pm$ 5.42 | 27.2 $\pm$ 9.03 | 30.8 $\pm$ 9.22 |
| BB                | 6.6 $\pm$ 2.46                                                                    | 11.3 $\pm$ 3.39 | 19.9 $\pm$ 5.00 | 26.8 $\pm$ 7.33 | 38.0 $\pm$ 7.66 | 45.1 $\pm$ 9.85 |
| $BB/B^+B^+$ ratio | 1.8                                                                               | 1.7             | 1.5             | 1.6             | 1.4             | 1.5             |

<sup>a</sup> The difference in cumulative number of pistillate flowers between  $BB$  and  $B^+B^+$  plants is statistically significant at each of the six weeks ( $P < 0.01$ ).

Precocious Fruit Pigmentation in  $B^w B^+$  Plants of Cucurbita pepo L.

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A  $B^w B^w$  line of 'Table King',  $B^+ B^+$ , background was developed in 1979. Gene  $B^w$  is a weak allele of  $B$  (1). The fruits of the  $B^w B^w$  line are precociously pigmented and similar to the fruits of 'Jersey Golden Acorn' except that their distal and proximal ends tend to be green. The fruits of the heterozygote,  $B^w B^+$ , of 'Table King' background are highly variable in color, ranging from completely green to different grades of bicolor. Some of these fruits exhibit one or more precociously pigmented golden spots. Sectioning fruits through 200 spots of different size showed that each spot is made up of 2 contiguous golden areas, in the "skin" (rind, exocarp) and in the "flesh" (mesocarp). No golden spot or area in the "flesh" was unassociated with a contiguous counterpart in the "skin". Furthermore, casual observations suggested that the sizes of the contiguous areas of each spot are positively correlated (Shifriss, unpublished; see Figure 1). The present statistical data confirm this suggestion.

Heterozygous  $B^w B^+$  plants were grown together and 33 of their fruits, each exhibiting at least 2 spots of different size, were selected for measurement. The fruits were sectioned through the widest external ("skin") diameter of these spots and 2 measurements were taken of each spot: the length of the borderline between the contiguous golden areas and the depth of the internal golden area (Figure 1). We assumed that these parameters reflect the sizes of the two areas of each spot.

As Table 1 shows, the coefficient of correlation ( $r$ ) of the 2 parameters is highly significant statistically ( $P < 0.001$ ), indicating that the sizes of the contiguous areas vary in the same direction.

The consistent contiguity of the external and internal golden areas of the fruit and the statistically significant correlation between the measured parameters lead us to the following conclusion: Precocious pigmentation in "skin" and "flesh" has a common ontogenetic origin with respect to site and time at which gene  $B$  is expressed.

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Figure 1. Diagrammatic representation of a spotted fruit, illustrating external (left) and internal phenotypes.

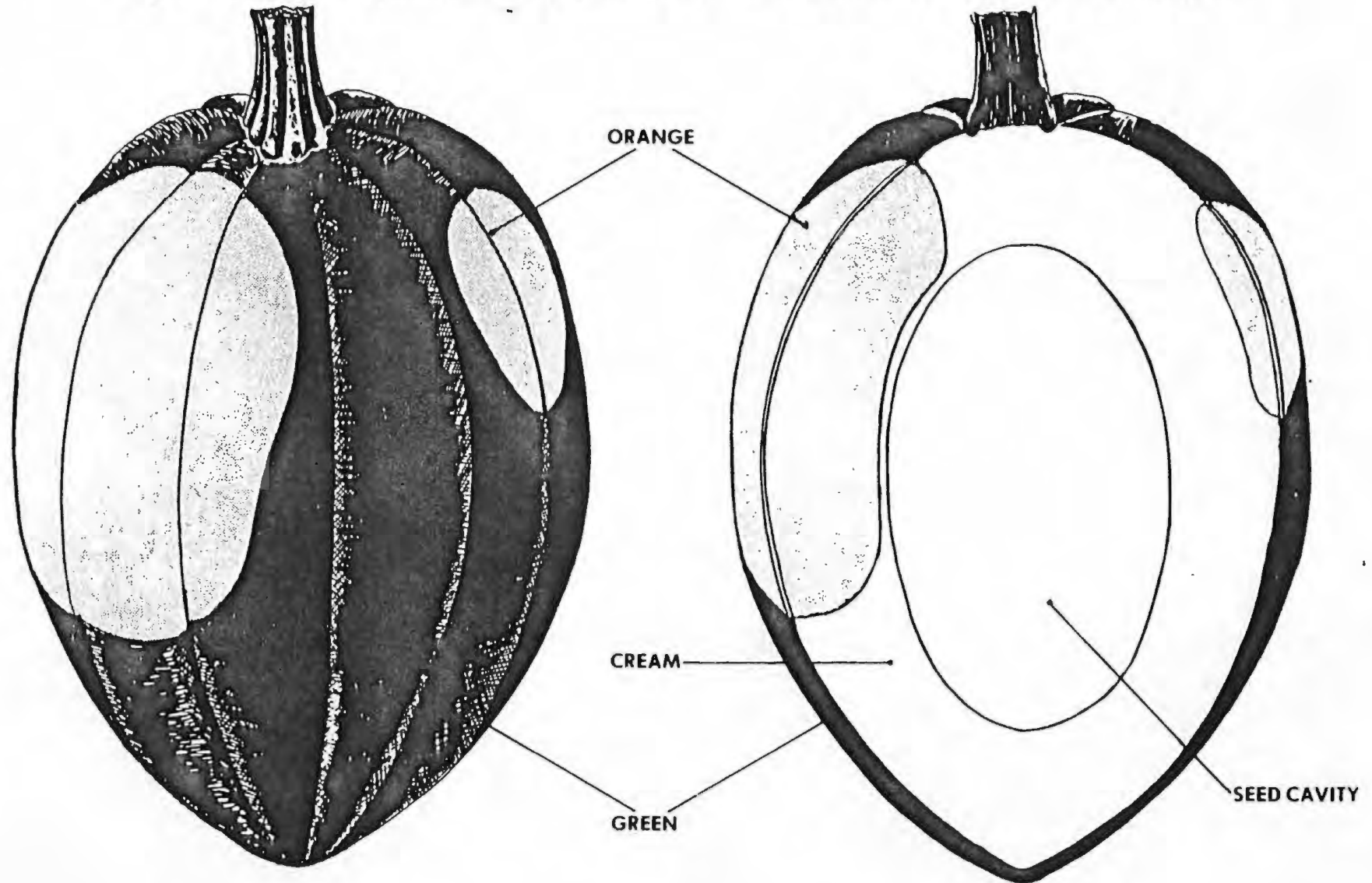


Table 1. Precocious pigmentation in spotted fruits of  $B^{WB+}$  plants of 'Table King' background. Correlation coefficient ( $r$ ) for the exterior diameter of a spot and its interior depth. From field studies, Rochester, NY, 1986.

| Fruit # | Spot <sup>z</sup> | Ex. <sup>y</sup> (mm) | In. <sup>x</sup> (mm) | Fruit # | Spot <sup>z</sup> | Ex. <sup>y</sup> (mm) | In. <sup>y</sup> (mm) | Fruit # | Spot <sup>z</sup> | Ex. <sup>y</sup> (mm) | In. <sup>x</sup> (mm) |
|---------|-------------------|-----------------------|-----------------------|---------|-------------------|-----------------------|-----------------------|---------|-------------------|-----------------------|-----------------------|
| 1       | a                 | 85                    | 12                    | 12      | a                 | 95                    | 18                    | 23      | a                 | 63                    | 6                     |
|         | b                 | 43                    | 5                     |         | b                 | 69                    | 14                    |         | b                 | 50                    | 1                     |
| 2       | a                 | 32                    | 4                     | 13      | a                 | 73                    | 15                    | 24      | a                 | 42                    | 8                     |
|         | b                 | 10                    | 1                     |         | b                 | 70                    | 9                     |         | b                 | 9                     | 1                     |
| 3       | a                 | 70                    | 9                     | 14      | a                 | 90                    | 18                    | 25      | a                 | 55                    | 7                     |
|         | b                 | 45                    | 4                     |         | b                 | 64                    | 9                     |         | b                 | 64                    | 8                     |
| 4       | a                 | 60                    | 10                    | 15      | a                 | 65                    | 9                     | 26      | a                 | 66                    | 6                     |
|         | b                 | 42                    | 13                    |         | b                 | 43                    | 6                     |         | b                 | 20                    | 3                     |
| 5       | a                 | 32                    | 5                     | 16      | a                 | 85                    | 10                    | 27      | a                 | 44                    | 5                     |
|         | b                 | 17                    | 1                     |         | b                 | 69                    | 10                    |         | b                 | 39                    | 6                     |
| 6       | a                 | 58                    | 15                    | 17      | a                 | 95                    | 14                    | 28      | a                 | 102                   | 10                    |
|         | b                 | 40                    | 13                    |         | b                 | 78                    | 4                     |         | b                 | 55                    | 6                     |
| 7       | a                 | 87                    | 5                     | 18      | a                 | 65                    | 16                    | 29      | a                 | 115                   | 9                     |
|         | b                 | 80                    | 4                     |         | b                 | 34                    | 10                    |         | b                 | 84                    | 9                     |
| 8       | a                 | 45                    | 8                     | 19      | a                 | 94                    | 8                     | 30      | a                 | 74                    | 5                     |
|         | b                 | 10                    | 4                     |         | b                 | 70                    | 4                     |         | b                 | 50                    | 6                     |
| 9       | a                 | 50                    | 5                     | 20      | a                 | 80                    | 6                     | 31      | a                 | 74                    | 7                     |
|         | b                 | 42                    | 3                     |         | b                 | 46                    | 1                     |         | b                 | 40                    | 3                     |
| 10      | a                 | 85                    | 15                    | 21      | a                 | 100                   | 7                     | 32      | a                 | 52                    | 6                     |
|         | b                 | 64                    | 11                    |         | b                 | 70                    | 1                     |         | b                 | 41                    | 5                     |
| 11      | a                 | 100                   | 6                     | 22      | a                 | 95                    | 7                     | 33      | a                 | 35                    | 4                     |
|         | b                 | 81                    | 5                     |         | b                 | 80                    | 6                     |         | b                 | 25                    | 3                     |

$r = 0.48$ ,  $df = 65$ ,  $P < 0.001$

<sup>z</sup> Two spots of each fruit were measured, one spot being larger (a) than the other (b)

<sup>y</sup> Refers to the length of the borderline common to the contiguous golden areas of each spot. See text and Figure 1.

<sup>x</sup> Refers to the depth of the internal golden area of each spot. See text and Figure 1.

## A Green Corolla Mutant in Cucurbita pepo

Theodore H. Superak  
Harris Moran Seed Company, 3760 Buffalo Road, Rochester, NY 14624

During the summer of 1986, an F<sub>2</sub> population of Cucurbita pepo was observed to segregate for a new flower mutant. The mutant had a green, leaf-like corolla in both male and female flowers. Early male flowers were sterile with brown anthers; later male flowers had normal appearing yellow anthers, but were still sterile. Corollas of both male and female flowers were partially open at an early stage and remained that way, giving no indication of a distinct time of opening. Female flowers appeared to be fertile, but repeated sib pollinations failed and there were no fruit from open pollination. In addition, the mutant flowers were large, although this may be related to the genetic backgrounds of the parents.

The segregation ratio of 19 normal to 7 mutant plants suggests a single recessive gene. It is proposed that the mutant be called green corolla and the gene symbol gc be adopted. Among the Cucurbits this mutant most closely resembles the co gene discovered in Cucumis sativus by Hutchins (1). A green corolla gene in Cucumis melo, described by Mockaitis and Kivilaan (2), is similar, but affected plants have only non-functional female-like flowers. Zink (3) recently described a greenish-yellow corolla mutant of Cucumis melo, but this is fully fertile.

Seed of F<sub>2</sub> has been given to the curator of the genus Cucurbita, Dr. R. W. Robinson, who kindly supplied the literature citations, and any requests for seed should be directed to him.

### Literature Cited

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3. Zink, F. W. 1986. Inheritance of a greenish-yellow corolla mutant in muskmelon. Jour. Heredity 77:363.

## Cold Tolerance in the Cucurbitaceae

R. W. Robinson

Horticultural Sciences Department, New York State Agricultural Experiment Station, Geneva, NY

All cultivated cucurbits prefer warm temperature. They are subject to chilling injury and are very sensitive to frost. A very light frost, not sufficiently cold to injure other tender crops such as beans and tomatoes, may blacken the leaves of squash plants. Numerous accessions of wild species of Cucumis, Cucurbita, Citrullus and other genera of the Cucurbitaceae have been grown at Geneva, New York in recent years but none was found to have a useful degree of frost tolerance that might be transferred by conventional breeding techniques to a cultivated cucurbit. One cucurbit, however, that proved to be remarkably frost tolerant is Ecballium elaterium (L.) A. Rich. A frost 10 hours long and as low as  $-4^{\circ}\text{C}$  killed all other cucurbits in the field but did not injure the foliage of this species, although it caused the fruit to lose their capacity to forcibly eject seed. Two months later, when the ground was covered by snow in December, the foliage of E. elaterium was still green and showed no evidence of injury from the cold. The plants did not survive the winter, but volunteer plants from overwintering seed were abundant the next season.

Cucurbita ficifolia Bouche', a native of high elevations, appears to be well adapted to low temperature. Although it appeared to be equally sensitive to killing frost, it was more tolerant than other Cucurbita species to temperatures between 0 and  $10^{\circ}\text{C}$ . The onset of cold weather in the fall caused other Cucurbita species to cease floral and vegetative development, but C. ficifolia continued to grow vigorously and flower profusely. Most accessions of this species are short day plants and do not flower at Geneva, NY until shortly before frost, too late to make crosses in the field. It was possible, however, to make interspecific crosses in September by pollinating C. pepo plants in the greenhouse with pollen from field grown C. ficifolia. The C. ficifolia plants produced abundant, functional pollen despite the chilling temperatures during microsporogenesis. The fruit of C. ficifolia are tolerant of chilling temperature. When fruit of C. ficifolia and different cultivars of C. pepo, C. maxima, and C. moschata were stored at  $5^{\circ}\text{F}$ , all except C. ficifolia quickly rotted. Fruit of C. ficifolia have a very long storage life; fruit remained in sound condition for over a year when stored at room temperature, but storage life was shortened by prolonged exposure to chilling temperature.

Cyclanthera pedata (L.) Schrad. has edible fruit that may be eaten raw, like cucumber, or cooked like summer squash. It grows well at low temperature, and continued to be productive in the fall after fruit production of cucumbers and summer squash was curtailed by low temperature.



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

MEMBERS ARE REQUESTED

TO FORWARD SAMPLES OF CURRENTLY HELD GENE STOCKS

TO THE RESPECTIVE CURATOR.

Cucumber

Todd C. Wehner, Dept. of Horticultural Science, North Carolina State University, Raleigh, NC 2766695-7609

Muskmelon

Edward L. Cpx, Texas Agricultural Experiment Station, 2415 East Hwy. 83, Weslaco, TX 78596-8399

Watermelon

Billy B. Rhodes, Clemson University, Edisto Research and Education Center, Blackville, SC 29817

Cucurbita spp.

Richard W. Robinson, New York Agricultural Experiment Station, Department of Horticultural Sciences, Hedrick Hall, Geneva, NY 14456

### Gene List for Watermelon

Lists of the known genes for the Cucurbitaceae have been published previously in 3 installments (3, 4, 21) and a complete, updated list of cucumber genes as published in CGC No. 8 (5). In the interest of updating and collecting the information on watermelon in one place, following is a complete list of the known genes for Citrullus lanatus (Thunb.) Matsum & Nakai. We hope to continue this practice, and publish a complete list for watermelon every four years.

| <u>Gene symbol</u><br><u>Preferred</u> <u>Synonym</u> | <u>Character</u>                                                                                                                             | <u>Reference</u> |
|-------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| <u>a</u>                                              | <u>andromonoecious</u> . Recessive to monoecious.                                                                                            | 17, 18, 24       |
| <u>Af</u>                                             | <u>aulacophora foveicollis</u> resistance.<br>Resistance to the red pumpkin beetle.<br>Dominant to susceptibility.                           | 28               |
| <u>Ar-1</u> ( <u>B</u> , <u>Gc</u> )                  | <u>Anthracnose resistance</u> to race 1 of<br><u>Glomerella cingulata</u> var. <u>orbiculare</u> .                                           | 6, 9, 32         |
| <u>Ar-2</u>                                           | <u>Anthracnose resistance</u> to race 2 of<br><u>Colletotrichum lagenarium</u> .                                                             | 25, 26, 32       |
| <u>C</u>                                              | <u>Canary yellow flesh</u> . Dominant to pink.                                                                                               | 17               |
| <u>d</u>                                              | <u>dotted seed coat</u> . Black dotted seed when<br>dominant for <u>r</u> , <u>t</u> and <u>w</u> .                                          | 7, 17, 19        |
| <u>db</u>                                             | Resistance to gummy stem blight caused by<br><u>Didymella bryoniae</u> from PI 189225.<br>Recessive to susceptibility.                       | 16               |
| <u>dg</u>                                             | <u>delayed green</u> . Cotyledons and young leaves<br>are initially pale green but later develop<br>chlorophyll. Hypostatic to <u>I-dg</u> . | 21               |
| <u>dw-1</u>                                           | <u>dwarf-1</u> . Short internodes with fewer,<br>shorter cells than normal.                                                                  | 10, 13           |
| <u>dw-2</u>                                           | <u>dwarf-2</u> . Short internodes, due to fewer<br>cells.                                                                                    | 10, 14           |
| <u>e</u> ( <u>t</u> )                                 | <u>explosive rind</u> . Thin, tender rind,<br>bursting when cut.                                                                             | 17, 20           |
| <u>f</u>                                              | <u>furrowed fruit surface</u> . Recessive to<br>smooth.                                                                                      | 17               |
| <u>Fo-1</u>                                           | Dominant gene for resistance to race 1<br>of <u>Fusarium oxysporum</u> .                                                                     | 15               |

| <u>Gene symbol</u>   |                            | <u>Character</u>                                                                                                                                                       | <u>Reference</u> |
|----------------------|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| <u>Preferred</u>     | <u>Synonym</u>             |                                                                                                                                                                        |                  |
| <u>Fwr</u>           |                            | <u>Fruit fly resistance in watermelon.</u><br><u>Dominant to susceptibility to <u>Dacus cucurbitae</u>.</u>                                                            | 8                |
| <u>g</u>             | ( <u>D</u> )               | light <u>green</u> skin. Light green fruit;<br>to dark green.                                                                                                          | 17, 20, 30       |
| <u>g<sup>s</sup></u> | ( <u>d<sup>s</sup></u> )   | striped <u>green</u> skin. Recessive to dark<br>dominant to light green skin.                                                                                          | 17, 31           |
| <u>go</u>            | ( <u>c</u> )               | <u>golden</u> . Yellow color of older leaves<br>and mature fruit.                                                                                                      | 1                |
| <u>I-dg</u>          |                            | <u>Inbibitor of delayed green.</u> Epistatic to<br><u>dg: dgdg I-dgI-dg and dgdg I-dgi-dg</u><br>plants are pale green; and <u>dgdg i-dgi-dg</u><br>plants are normal. | 21               |
| <u>gms</u>           | ( <u>ms</u> )<br><u>g</u>  | <u>glabrous male sterile.</u> Foliage lacking<br>trichomes; male sterile.                                                                                              | 29, 30           |
| <u>l</u>             |                            | <u>long</u> seed. Long recessive to medium<br>length of seed; interacts with <u>s</u> .                                                                                | 18, 19           |
| <u>m</u>             |                            | <u>mottled</u> skin. Greenish white mottling<br>of fruit skin.                                                                                                         | 17, 31           |
| <u>nl</u>            |                            | <u>nonlobed</u> leaves. Leaves lack lobing;<br>dominance incomplete.                                                                                                   | 12               |
| <u>o</u>             |                            | <u>Oval</u> fruit. Incompletely dominant to<br>spherical.                                                                                                              | 18, 31           |
| <u>p</u>             |                            | <u>pencilled</u> lines on skin. Inconspicuous<br>recessive to netted fruit.                                                                                            | 17, 31           |
| <u>pm</u>            |                            | <u>powdery mildew</u> susceptibility.<br>Susceptibility to <u>Sphaerotheca</u><br><u>fuliginea</u> .                                                                   | 23               |
| <u>r</u>             |                            | <u>red</u> seed coat. Interacts with <u>w</u> and <u>t</u> .                                                                                                           | 19               |
| <u>s</u>             |                            | <u>short</u> seeds. Epistatic to <u>l</u> .                                                                                                                            | 18               |
| <u>Sp</u>            |                            | <u>Spotted</u> cotyledons, leaves and fruit.                                                                                                                           | 21               |
| <u>su</u>            | ( <u>su<sup>Bi</sup></u> ) | <u>suppressor</u> of bitterness. Nonbitter<br>fruit.                                                                                                                   | 2                |
| <u>t</u>             | ( <u>b<sup>t</sup></u> )   | <u>tan</u> seed coat. Interacts with <u>r</u> and <u>w</u> .                                                                                                           | 11               |
| <u>w</u>             |                            | <u>white</u> seed coat. Interacts with <u>r</u> and <u>t</u> .                                                                                                         | 11               |

| <u>Gene symbol</u> | <u>Preferred</u> | <u>Synonym</u> | <u>Character</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <u>Reference</u> |
|--------------------|------------------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| <u>Wf</u>          | ( <u>W</u> )     |                | <u>White flesh</u> . Dominant to yellow and red <u>Wf</u> is epistatic to a second, unnamed gene ( <u>C ?</u> ) which conditions yellow and red flesh. Thus, if the gene at the hypostatic locus is symbolized B, <u>WfWfBB</u> , <u>WfWfBb</u> , <u>WfWfbb</u> , <u>WfwfBB</u> , <u>WfwfBb</u> and <u>Wfwfbb</u> are all white fleshed; <u>wfwfBB</u> and <u>wfwfBb</u> are yellow fleshed; and <u>wfwfBB</u> and <u>wfwfBb</u> are yellow fleshed; and <u>wfwfbb</u> are red fleshed. | 25               |
| <u>y</u>           | ( <u>r</u> )     |                | <u>yellow flesh</u> . Recessive to red.                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 17, 20           |

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It is hoped that scientists will consult the above list as well as the rules of gene nomenclature for the Cucurbitaceae (3, 4) before choosing a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature were adopted in order to provide guidelines for the naming and symbolizing of genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

Gene List Committee:

|                         |                 |
|-------------------------|-----------------|
| <u>Cucumber</u> :       | T. C. Wehner    |
| <u>Muskmelon</u> :      | M. Pitrat       |
| <u>Watermelon</u> :     | W. R. Henderson |
| <u>Cucurbita spp.</u> : | T. W. Whitaker  |
| <u>Other Genera</u> :   | R. W. Robinson  |

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.

Approvals: W.P. Bemis  
W. Bemis

Joseph B. Norton  
J. B. Norton

R. W. Robinson  
R. W. Robinson

M. R. Henderson  
M. R. Henderson

M. L. Robbins  
M. L. Robbins

R. L. Lower  
R. L. Lower

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

3. Other committees may be selected by the Coordinating Committee as the need 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.

Approvals:

W.P. Bemis  
W. Bemis

Joseph D. Norton  
J. D. Norton

R. W. Robinson  
R. W. Robinson

W. R. Henderson  
W. R. Henderson

M. L. Robbins  
M. L. Robbins

R. L. Lower  
R. L. Lower



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

ARTICLE VI. Meetings

An Annual Meeting shall be held at such 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 provision of the By-Laws or any other document that might be susceptible to a contrary interpretation:

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

Approvals:

W.P. Bemis  
W. Bemis

Joseph D. Norton  
J. D. Norton

R. W. Robinson  
R. W. Robinson

W. R. Henderson  
W. R. Henderson

Ken Robbins  
M. L. Robbins

R. L. Lower  
R. L. Lower

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.

W. P. Bemis

W. Bemis  
(Cucurbits sp.)

W. R. Henderson

W. R. Henderson  
(Watermelon)

Joseph D. Norton

J. D. Norton  
(Muskmelon)

Ken Robbins

M. L. Robbins  
(Cucumber)

R. W. Robinson

R. W. Robinson  
(Other genes and species)

R. L. Lower

R. L. Lower, Chairman

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| Hassan, Mohamed Nabil                                                      | ICAR Research Complex<br>Ramachandran, C.<br>Seshadri, V. S.                                                                                                                                                                                   |
| ENGLAND                                                                    | ISRAEL                                                                                                                                                                                                                                         |
| British Library<br>Iraj Poostchi<br>National Vegetable Research<br>Station | Central Library of Agricultural<br>Sciences<br>Cohen, Yigal<br>Galun, Esra<br>Herman, Ran<br>Jehoshua, Rudich<br>Niego, Shlomo<br>Paris, Harry                                                                                                 |

ITALY

Esquinas-Alcazar, Jose T.  
Iapachino, Giovanni

JAPAN

Asian Seed Co. Ltd.  
Fujieda, K.  
Fujita, Yukio  
Fujiwara, Koju  
Hirabayashi, Tetsuo  
Igarshi, Isamu  
Kanno, Tsuguo  
Kuginuki, Yasuhisa  
Kwack, Soo-Nyeon  
Matsuo, Kazuma  
Mochizucki, Tatsuya  
Oizumi, Toshikatsu  
Shoji Kamimura  
Yukura, Yasuo

KOREA

Oh, Dae-Geun  
Om, Y. H. Om

MEXICO

Laborde, Jose Antonio

NEW ZEALAND

Mt. Albert Res. Center, DSIR

NIGERIA

Adeniji, Adeoye

NORWAY

Persson, Arnulf

PEOPLE'S REPUBLIC OF CHINA

Ming, Wang  
Wang, Yong Jian

POLAND

Mackiewicz, Henryk O.

REPUBLIC OF CHINA

Hsiang, Chi-Hsiung  
Hung, Lih

ROMANIA

Poli, Virgil

SPAIN

Biblioteca Instituto Valenciano De  
Cuartero, J.  
Escudero, V. Andres  
Gomez-Guillamom, Maria Luisa  
Leeuwen, Loes Van  
Moreno, Vicente  
Nuez, Fernando  
Roig, Luis O.  
Sanchez, Joaquin Abadia  
Servicio De Investigacion Agraria

SWEDEN

Lehmann C. Louis  
Lundin, Marianne

THAILAND

Lertrat, Kamol

THE NETHERLANDS

Arend, Wim van der  
Blokland, G. D. van  
Boorsma, P. A.  
Custers, J. B. M.P.  
Eenhuizen, P.  
Eyck, L. Van  
Groot, Steven P. C.  
Meysing, Wilbert D.  
Ruiter de, Ir. A. C.  
Verhoef, Ruud

YUGOSLAVIA

Institut Za Povrtarsrvo Palanka  
Institut Za Ratarstvo

FINANCIAL STATEMENT

31 December 1986

|                                           |               |                 |           |
|-------------------------------------------|---------------|-----------------|-----------|
| <u>Balance</u> on 31 December 1985        |               |                 | s 992.40  |
| <u>Receipts</u>                           |               |                 |           |
| Dues and Back Issues                      | 2,535.50      |                 |           |
| Interest                                  | 114.43        |                 |           |
| Correction                                | <u>.04</u>    | <u>2,649.97</u> |           |
| <u>Expenditures</u>                       |               |                 |           |
| Report #9 <sup>2</sup>                    | 1,035.51      |                 |           |
| Membership Invoices <sup>2</sup>          | 58.08         |                 |           |
| Report #10 (Call for Papers) <sup>2</sup> | <u>136.19</u> | <u>1,229.78</u> |           |
| <u>BALANCE</u> on 31 December 1986        |               |                 | s2,412.59 |

<sup>2</sup> Publishing and mailing