Cucurbit Genetics Cooperative

No. 9 June, 1986

CONTENTS



U. S. Department of Agriculture
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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 Curcurbits).
>
> 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.

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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
Euphytica
HortScience
Journal of Economic Entomology
Journal of Heredity
Phytopath News

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

## REPORT OF NINTH ANNUAL MEETING

The ninth annual meeting of the Cucurbit Genetics Cooperative was held in conjunction with the American Society for Horticultural Science on 31 July 1985 at Virginia Polytechnic Institute and State University, Blacksburg. There were 24 members in attendance. The meeting was called to order by J. D. McCreight, Chairman.

CGC No. 8 was published at the University of Wisconsin. Estimated cost for 250 copies was $750.00 ($3.00 per copy) plus postage. Estimates to commercially print and bind ranged from $6.00 to $7.00. Report No. 7 would be reprinted in the coming months; there were only two copies remaining.

The Coordinating Committee raised membership dues for U.S., Canada and Mexico from $3.50 to $6.00 per biennium, and for Libraries and other Foreign countries from $6.00 to $10.00 per biennium. The membership continued to grow and had reached 206.

Gary Elmstrom was appointed to replace J. D. McCreight on the Coordinating Committee for Muskmelon. Michel Pitrat was selected to replace J. D. McCreight on the Gene List Committee for Muskmelon.

Gene Curators were appointed. The Curators (and their responsiblities) are Todd Wehner (Cucumber), Ed Cox (Muskmelon), Billy Rhodes (Watermelon), and Dick Robinson (Cucurbita sp.). One additional Curator is needed for Other Genera. Gene Curators are responsible for obtaining, maintaining and distributing stocks of the reported cucurbit genes. (Editor's Note: Members are encouraged to send samples of gene stocks along with information about their origin to the respective curators.)

The status of the Cucumis collection at the Institute for Horticultural Plant Breeding (IVT), Wageningen was discussed in light of Ton den Nijs' departure. Dick Visser, the specialist under den Nijs will continue to work with Cucumis, but CGC was encouraged to maintain contact with him. The IVT collection is broad and includes melo, anguria and metuliferous. It was suggested that Dick Robinson arrange for samples of the IVT collection be sent to Geneva, New York and to the National Seed Storage Lab, Fort. Collins.

The Vine Crops Crop Advisory Committee (CAC) held its first meeting at the Vegetable Crops Dept., University of California, Davis in September 1984. The initial task of the group is to establish descriptors for each cucurbit as part of the Germplasm Resources Network (GRIN), a database for users of plant germplasm. The second meeting was scheduled for November 1985 at the U.S. Vegatble Lab., Charleston, South Carolina. There was considerable discussion about the method of maintaining accessions at the Regional Plant Introduction Stations. Many accessions are maintained via open-pollination while others are maintained by hand pollination or by use of bees in screened cages. The Chairman agreed to write letter on behalf of the CGC to express its concerns.

## COMMENTS FROM THE COORDINATING COMMITTEE

The call for papers for the 1986 report will go out in October, 1986. Papers should be submitted to the respective Coordinating Committee member by December 31, 1986. The report will be published by July, 1987.

When CGC 8 was mailed its was realized that the prevailing dues structure (U.S. Individual, Foreign Individual, and Library), did not accurately reflect costs asociated with the U.S. and Foreign Individual memberships. Thus, we proposed that the U.S. Individual and Foreign Individual memberships be replaced by a single Individual membership. Membership invoices sent this spring refelected this change. Any difference between the proposed membership structure and dues and that approved at the Tenth Annual meeting will be credited or debited accordingly.

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

It is a pleasure to acknowledge the assistance of five people who did much for CGC this past year: Dayna Lamar and Lu Torres for typing correspondence and portions of this report, and assisting in the day-to-day business of CGC. Janet Foreman for assistance in updating the membership list and records. And, R. L. Lower and Madelyn Alt in the printing and binding of CGC No. 9 at the University of Wisconsin.

Coordinating Committee:

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

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## MEETINGS

The Tenth Annual Meeting of the CGC will be held in conjunction with the XXII International Horticultural Congress at the University of California, Davis, 10-18 August 1986. CGC will meet at 7:00 PM on Monday 11 August in Room 25 of Wellman Hall.

There will be three meetings at the Department of Horticulture, Texas A&M University, College Station, Texas during the week of October 27, 1986:

The Pickling Cucumber Improvement Committee (PCIC), October 27, 28, 29.
Contact Leonard Pike at the Department of Horticulture.

Vine Crops Crop Advisory Committee (VCAC), October 30.
Contact J. D. McCreight.

The National Muskmelon Research Group (NMRG), October 30, 31.
Contact Edward C. Cox at the Department of Horticulture.

## RESEARCH NOTES

I. Cucumber

# Callus Initiation from Cucumber (Cucumis sativus L.) Fruits

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A tissue culture system may be useful in rescuing potentially-resistant fruits from tests using Rhizoctonia solani and Pythium aphanidermatum. A screening program with subsequent callus initiation and plantlet regeneration from the selected tissues has been proposed. Callus initiation from fruit tissues of some plants has been reported in the literature (1,2,3,4, 5,6), but not from cucumber fruits.

Prior to experimentation with fruit tissue, cucumber shoot cultures were established using shoot meristems of non-embryonic origin. Callus was initiated from interstem pieces taken from shoot cultures and subsequent plantlet regeneration was attempted. Shoots were easily cultured, but exuberant callus growth was difficult to achieve. Root regeneration was easily obtained, but subsequent shoot regeneration was difficult. Greenish nodules from this callus were postulated as potential sites for plantlet regeneration. Following this preliminary work, callus initiation from fruit tissues was explored.

Fruit tissue may be more recalcitrant than stem tissue, thus a strong auxin such as 2,4-dichlorophenoxy acetic acid (2,4-D) may be needed. Slices of cucumber fruits of 4 different ages (3,5,7, and 14 days after pollination) were placed onto solidified Murashige and Skoog medium supplemented with 0.1 uM benzyladenine (BA), 1 uM naphthalene acetic acid (NAA), and one of the following concentrations of 2,4-D: 0, 0.01, 0.1 or 1 uM. Two slices were placed in each culture jar and each treatment was replicated 5 times. Cultures were grown under continuous fluorescent light at 27°C - 30°C. The ability of each explant to form callus was determined at the end of the fourth week of growth.

Less vigorous callus initiation was observed on fruit tissue as compared to that of stem internode tissue. The youngest tissue (3 days after pollination) produced the greatest growth (Table 1). Whitish-green gel-like callus occasionally formed at the peripheral layer and at cut surfaces. This callus was subcultured onto MS medium supplemented with 0.1 uM BA and 1 uM NAA and greenish nodule formation was observed. No callus was initiated in 7 and 14 day old tissues. On these explants, fleshy tissues turned dark brown, and browning of the epidermal layer was observed. Very low or no 2,4-D resulted in more callus growth than higher levels of 2,4-D.

Our results showed that the best callus growth was initiated from younger tissues. Sommer et al. (6) showed that peach mesocarp cells retained the ability of cell division after ripening. The ability of mature fruit tissue to form callus has also been reported by others in apple (1), avocado (5), citron (3) and peach (2). Whether the same is true for an herbaceous annual, such as cucumber, is not fully known. However, our results indicate that mature fruits cannot be induced to form callus and, subsequently, shoots.

Table 1. Effects of fruit age and 2,4-D concentration on callus initiation from cucumber fruit tissues after 4 weeks in culture[z].

| 2,4-D Conc (uM) | Fruit Age After Pollination (Days) | | | |
|---|---|---|---|---|
| | 3 | 5 | 7 | 14 |
| 0 | 1.0 ± 0.71 | 0.8 ± 0.84 | 0 | 0 |
| 0.01 | 1.4 ± 0.89 | 0.6 ± 0.55 | 0 | 0 |
| 0.1 | 0.8 ± 0.84 | 0.6 ± 0.55 | 0 | 0 |
| 1 | 0.6 ± 0.55 | 0.2 ± 0.45 | 0 | 0 |

[z]Results are the mean number of explants per jar with callus formation ± S.E. for 5 replicates. F-test at 5% level shows no significant differences among concentrations used.

Besides the physiological stage of growth of the explants, the growing medium also plays an important part in callus initiation. This includes the nutrients as well as the hormones added to the medium. According to Schroeder (4), fruit tissue can be established *in vitro* if proper nutritional conditions are provided.

Results from our experiment showed that callus initiation from fruit tissue is possible, but age is a major obstacle in mature fruits. More detailed research should be conducted to determine if viable tissue which is capable of mitosis exists. Pedicel and epidermal layers are 2 good candidates. Greenish nodules may be the site of plantlet regeneration and further work on the developmental biology of such nodules is warranted.

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# Evaluation of Resistance of Cucumber Lines to Damping-Off Caused by *Rhizoctonia solani*

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This research was supported by a grant from Vlasic Foods, Inc.

The soil-borne pathogen *Rhizoctonia solani* can cause damping-off in cucumber seedlings. The rot originates at or near the surface of the ground and weakens the stem so that the plant topples over or damps off. Subsequently, the whole plant may decay, either from the primary cause or from secondary rot organisms (4). *R. solani* can be divided into 5 anastomosis groups (AG). Isolates of AG 4 exist at or near the soil surface and are generally responsible for damping-off as well as other plant symptoms such as fruit rots (2,3).

The purpose of this study was to develop a screening test for resistance to damping-off in cucumber seedlings and to determine whether the results were comparable to those of fruit rot tests of the plants at the fruiting stage.

*Inoculum preparation*. The isolates of *R. solani* AG 4 (R8C, R8D) used in this study were originally collected from cucumber fields in Arkansas. Inoculum was increased by transferring the fungus from stock cultures stored in paraffin oil in test tubes to potato dextrose agar. The final increase of inoculum took place on sterile oat grains as follows. Pieces of about 1 $cm^2$ of the colonized agar were transferred onto sterile oat grains in autoclavable bags, which were obtained by autoclaving 300 $cm^3$ of oat grains and 250 ml water 2 times for 1 hr. with at least 10 hr. between to ensure that all microorganisms were killed. After about 7 days at 20 to 25°C (shaking the bags every 2 days), when the oats were completely colonized by the fungus, they were dried and then stored at 4°C until needed.

Reaction to damping-off by *R. solani* was tested by planting seeds in flats of inoculated soil. The soil was inoculated by mixing it with a predetermined number of colonized oat grains per volume of soil. Soil was inoculated 7 days before planting the seeds to enable the fungus to colonize it completely.

*Damping-off screening tests*. The first test was conducted in a germination chamber held at 18°C as well as in a greenhouse (held at 25 to 30°C). Inoculum concentrations were 0, 40, 80, 160, 320, 640 and 1280 oat grains per 1000 $cm^3$ of one isolate (R-8C). Sixteen seeds of 2 cultivars (Marketmore 76 and Sumter) were planted and rated each day for 18 days. This experiment, replicated once, was conducted to determine the optimum temperature and inoculum concentration for future evaluation of damping-off resistance. A second test was run using 6 concentration (0, 10, 20, 40, 80, 160 oats per liter) of 2 isolates (R-8C, R-8D), and 7 cultivars (Addis, Clinton, Earlipik 14, Marketmore 76, Pacer, Sumter and Supergreen) which differed in resistance

for fruit rot. The seeded flats were placed in a chamber at 20°C and 14 hr. daylength using fluorescent lights. Twenty seeds of each line per isolate per concentration were planted in 3 replications. The objective was to refine further the screening tests. The experimental design was a randomized complete block with 3 replications.

In the third test, seedlings of 35 lines were evaluated for their resistance to damping-off using one concentration (40 oat grains/1000 $cm^3$) of one isolate (R-8C). Treatments were arranged in a randomized complete block design with 4 replications. Twenty seeds of each line were planted for each treatment combination. Seedlings were grown in uninoculated flats (in the same experimental design, but with 3 replications) as a check on germination and emergence under non-stress conditions. All flats were kept at 25-30°C on greenhouse benches with a 12 hr photoperiod.

Data analysis. Days to emergence as well as the percentage of emergence of the seedlings was calculated by counting the number emerged each day until the seedlings were scored for final disease severity. After 18 days for the first test, and 20 for the second and third tests, the emerged seedlings were rated for damping-off. Counts were used to calculate percentage of infected seedlings. Also, a disease rating was given to the seedlings, using a scale ranging from 0 to 9, in which 0 = no disease, 1-3 = slight damage, 4-6 = moderate damage, 7-9 = plants dying. A corrected disease rating was calculated by correcting the disease rating for the percentage of emergence measured in the control treatments as follows:

corrected rating = [(rating) - (9(total - emerged))]/total
where: rating = disease rating for the seedlings at end of test,
total = total number of emerged seedlings in the control,
emerged = number of emerged seedlings in the inoculated flats.

The number of non-emerged seedlings (total - emerged) was multiplied by 9 because it was assumed that those seedlings were dead.

The results were compared to data for fruit rot resistance of these lines (data not shown), which were obtained from a field test at the Horticultural Crops Research Station, Clinton, N.C. and in a laboratory test run in a mist chamber at Raleigh, N.C. in July 1985. Correlation analysis was run for the damping-off scores and the percentage of the fruits covered with lesions in the fruit rot tests using means for each line (over replications and harvest dates).

Results. As a result of the first test, a concentration range from 0 to 160 oat grains/1000 $cm^3$ was chosen for further testing to find an optimum concentration. An incubation temperature of 20°C was chosen, because at a lower temperature, the percentage of germination of some lines was low and infections seem to be most severe at temperatures which were relatively less favorable for the pathogen than for the host (1). The second test indicated that an inoculum concentration of 40 oat grains/1000 $cm^3$ using isolate R-8C was optimum. A wide range among lines for percentage of diseased seedlings and for corrected disease rating was used as the criterion for the optimum inoculum concentration.

In the third test, the number of days before first emergence and the mean number of days for emergence were not effective variables for measurement of damping-off. That is shown by the nonsignificant F value for treated vs. control seedlings (Table 1). There was a large treatment effect and also a large line effect for percentage of emergence. In addition, those variables were not correlated with the disease ratings (Table 2). Correlation with the disease ratings was not high. Percentage of emergence was only moderately correlated with the corrected disease rating. There were significant treatment effects and line effects for mean disease rating and for corrected disease rating, and those were highly correlated with each other (Tables 1 and 2).

We believe the corrected disease rating to be the best variable to use because it includes a correction for the vigor of the seeds, which was measured in the control treatment. The mean of 3.6 for the corrected disease rating was not high, however, indicating some damping-off resistance in the lines tested. Also, the range among lines for corrected disease rating was not large, indicating a similarity in resistance to this disease.

Fruit rot correlations. Correlations between damping-off rating and percentage of the fruit damaged in the fruit rot tests was low and not statistically significant (Table 2). The damping-off test was faster and easier to run than the fruit rot test, but does not appear to be a useful substitute due to the low correlation. All tests were run using the same isolates and similar soil inoculation techniques, so the lack of correlation indicates different mechanisms of resistance are acting, or that the isolates of R. solani used differ in virulence as fruit rot or seedling pathogens. That result is not surprising since damping-off is mainly a root and hypocotyl response, whereas belly rot response relates to the fruit surface. Further studies are needed to evaluate the cucumber germplasm collections for resistance to damping-off, and to determine the effect of isolates from the different AG's on damping-off resistance.

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Table 1. Percentage of emergence (PE), number of days to first emergence (DFE), mean days to emergence (DME), mean disease score[z] (MS) and corrected disease rating (CR) for seeds of 35 lines tested in uninoculated and inoculated soil (lines are ordered by CR).

| | 0 oat grains per/1000 cm³ | | | 40 oat grains per/1000 cm³ | | | | |
|---|---|---|---|---|---|---|---|---|
| Line | PE | DFE | DME | PE | DFE | DME | MS[y] | CR |
| PI 163216 | - | - | - | 38 | 7 | 7.5 | 0.9 | - |
| National Pickling | 97 | 7 | 7.8 | 96 | 7 | 8.1 | 1.3 | 1.5 |
| Earlipik 14 | 55 | 9 | 11.7 | 44 | 8 | 11.4 | 1.1 | 2.7 |
| DEXP 130 | 90 | 7 | 8.5 | 83 | 6 | 9.2 | 2.0 | 2.8 |
| Raider | 98 | 7 | 7.4 | 90 | 6 | 7.6 | 2.3 | 2.8 |
| GY 14A | 98 | 7 | 8.1 | 91 | 7 | 7.4 | 2.5 | 2.8 |
| Sprint 440 | 70 | 7 | 8.6 | 75 | 7 | 8.5 | 2.8 | 3.0 |
| Straight 8 | 93 | 7 | 8.1 | 93 | 7 | 8.0 | 2.7 | 3.0 |
| Ashley | 83 | 7 | 9.0 | 71 | 7 | 9.8 | 2.3 | 3.0 |
| Sumter | 98 | 7 | 7.7 | 85 | 7 | 8.2 | 2.4 | 3.1 |
| Supergreen | 72 | 7 | 8.3 | 60 | 7 | 8.4 | 2.7 | 3.2 |
| Little Leaf | 93 | 7 | 7.2 | 93 | 6 | 7.5 | 3.0 | 3.2 |
| M 16 | 90 | 6 | 7.7 | 93 | 7 | 7.6 | 3.2 | 3.2 |
| Carolina | 80 | 7 | 9.1 | 88 | 7 | 9.0 | 3.1 | 3.3 |
| Commander | 48 | 8 | 9.4 | 49 | 8 | 10.5 | 2.3 | 3.3 |
| Castlepik | 82 | 7 | 7.7 | 93 | 7 | 7.6 | 3.0 | 3.3 |
| Score | 93 | 7 | 7.7 | 83 | 6 | 7.5 | 3.1 | 3.4 |
| Pikmaster | 100 | 6 | 6.7 | 96 | 7 | 7.1 | 3.3 | 3.7 |
| Dasher II | 90 | 6 | 7.3 | 83 | 6 | 7.5 | 2.9 | 3.5 |
| M 23 | 95 | 7 | 7.1 | 89 | 7 | 8.6 | 3.0 | 3.5 |
| Verino | 92 | 7 | 10.1 | 76 | 7 | 8.9 | 2.5 | 3.6 |
| Pioneer | 92 | 7 | 6.7 | 81 | 6 | 7.5 | 2.8 | 3.6 |
| M 15 | 87 | 7 | 8.5 | 84 | 7 | 8.1 | 3.4 | 3.6 |
| Guardian | 100 | 8 | 8.3 | 93 | 6 | 7.1 | 3.5 | 3.6 |
| Pacer | 98 | 7 | 8.7 | 93 | 6 | 7.5 | 3.5 | 3.7 |
| Poinsett 76 | 100 | 7 | 9.3 | 80 | 7 | 8.5 | 2.8 | 3.7 |
| Clinton | 72 | 7 | 8.1 | 63 | 7 | 8.6 | 3.1 | 3.9 |
| Calypso | 85 | 7 | 10.6 | 74 | 7 | 8.8 | 3.2 | 3.9 |
| SMR 18 | 88 | 7 | 7.9 | 83 | 7 | 7.7 | 3.7 | 4.0 |
| Tamor | 75 | 7 | 8.4 | 60 | 7 | 8.5 | 2.9 | 4.2 |
| Castlemaster | 88 | 7 | 9.3 | 68 | 9 | 10.3 | 3.1 | 4.3 |
| M 21 | 75 | 7 | 9.3 | 56 | 8 | 9.9 | 3.2 | 4.4 |
| Marketmore 76 | 73 | 7 | 8.5 | 50 | 8 | 9.9 | 2.8 | 5.4 |
| GY 3 | 37 | 12 | 14.0 | 19 | 14 | 16.0 | 2.5 | 5.7 |
| Addis | 30 | 9 | 10.8 | 24 | 8 | 10.7 | 4.6 | 5.9 |
| $\bar{x}$ | 83 | 7 | 8.6 | 75 | 7 | 8.8 | 2.9 | 3.6 |
| LSD (5%) | 22 | 2 | 2.0 | 17 | 2 | 1.5 | 1.4 | 1.8 |
| F (O vs 1) | - | - | - | 18 | 1 | 0 | 777 | 728 |

[z]Data are means of 3 replications in the uninoculated treatments, and of 4 replications of each 20 seedlings tested with 40 infested oat grains/1000 cm³ of soil.

[y]The MS and CR for the control were 0 for all lines, so they were not listed in the table.

Table 2. Correlation among 5 variables for damping-off resistance to *Rhizoctonia solani* in cucumber[z].

| Variable | Days to first emergence | Mean days to emergence | Mean rating | Corrected rating | Percent fruit damage | |
|---|---|---|---|---|---|---|
| | | | | | field | Lab |
| Percentage of emegence | -0.58 | -0.63 | -0.16 | -0.37 | -0.09 | -0.25 |
| Days to first emergence | | 0.83 | -0.11 | 0.11 | 0.16 | -0.06 |
| Mean days to emergence | | | -0.33 | 0.16 | 0.12 | -0.10 |
| Mean rating | | | | 0.89 | 0.00 | -0.24 |
| Corrected rating | | | | | -0.10 | -0.19 |
| % damage-Field | | | | | | 0.58** |

[z]Correlations were calculated among 35 lines and 2 inoculum levels (0 and 40 oat grains/1000 $cm^3$) with 3 and 4 replications, respectively.
**,*Correlation significant at the 1 and 5% levels, respectively.

# Germplasm Resources of Cucumis sativus L. from Spain

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In 1984, our group started a project for collecting many vegetable crop species in Spain, which was partially sponsored by I.B.P.G.R./F.A.O. In addition to many other crops, 50 accessions of Cucumis sativus L. were collected. Samples of all of them have been sent to the National seed Storage Laboratory in Fort Collins, U.S.A.

The first character in the identification label of the samples shows the area where the sample was collected: V=Valencia, AN-Andalucia, A=Aragon, C=Cataluna, CM=Castilla-La Mancha, E=Extremadura. Table 1 shows the items collected.

Acknowledgements: We are extremely grateful to the Diputacion Provincial de Valencia, Servicio de Extension Agraria and to all those who have collected vegetable crop germplasm: G. Palomares, M.L. Gomez-Guillamon, P. Corella, G. Anastasio, M.S. Catala, F. Benayas, A. Alonso-Allende, M.J. Diez, C. Ferrando, C. Cortes and J.M. Oliveras.

Table 1. Accessions of Cucumis sativus L. collected in Spain.

| Label | Locality | Observations |
|---|---|---|
| V-C-1 | Concentaina | Called "Tendral" |
| V-C-3 | La Punta | - |
| V-C-10 | Venta del Moro | Called "Conqueno" |
| V-C-16 | Puebla de Benifasar | - |
| V-C-27 | Benisa | - |
| V-C-49 | Alcoleja | Dry farming, late crop |
| V-C-58 | Ademuz | - |
| V-C-59 | Ademuz | - |
| V-C-60 | Ademuz | - |
| V-C-61 | Ademuz | - |
| V-C-65 | Casas Altas | Grown at 900 m |
| V-C-69 | Chulilla | - |
| V-C-85 | Jativa | late crop |
| V-C-88 | Figueroles | For pickling |
| V-C-89 | Villahermosa del Rio | - |
| V-C-91 | Giraba | - |
| V-C-93 | Fanzara | Very long cycle |
| V-C-94 | Argelita | - |
| V-C-99 | Sinarcas | - |
| V-C-104 | Torrebaja | - |
| AN-C-17 | Portugos | Late crop, grown at 1300 m |
| AN-C-18 | Ugijar | Late crop, grown at 900 m |
| AN-C-19 | Ugijar | Late crop, grown at 900 m |
| AN-C-21 | Purchil | - |
| AN-C-23 | Jinesa de Libar | - |
| AN-C-26 | Benaojan | - |
| AN-C-27 | Ronda | Grown at 700 m |
| AN-C-51 | Competa | - |
| AN-C-54 | Los Barrios | Called "Enano" |
| AN-C-61 | Benaocaz | Grown at 800 m |
| AN-C-62 | Benaocaz | Grown at 800 m |
| AN-C-65 | Grazalema | Grown at 800 m |
| AN-C-67 | Tarifa | Late crop |
| A-C-1 | Sarrion | Short cycle |
| A-C-2 | Albarracin | Grown at 1200 m |
| A-C-3 | Toores de Albarracin | Grown at 1237 m |
| A-C-5 | Gea de Albarracin | Grown at 1031 m |
| A-C-6 | Gea de Albarracin | Grown at 1031 m |
| A-C-7 | Tramacastilla | Grown at 1260 m |
| A-C-8 | Gea de Albarracin | Grown at 1031 m |
| A-C-10 | Quicena | Big size. Called "Gordo" |
| A-C-12 | Rueda de Jalon | Called "Antiguo" |
| A-C-15 | Lumpiaque | - |
| C-C-1 | Miravet | - |
| C-C-4 | Torello | Short cycle |
| C-C-9 | Gratallops | Dry farming |
| CM-C-10 | Mondejar | Short cycle. Called "Tronquero" |
| CM-C-11 | Bolarque | Long fruit |
| E-C-26 | Azabal | - |
| E-C-31 | Hoyos | Short cycle |

# Rooting Cucumber Cuttings when Water Quality is Poor

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In many regions of the world, horticulturalists have to cope with poor water quality, particularly high salt levels which are damaging to many species. Affected by this is the cucumber breeder who is concerned with taking field cuttings from gynoecious selections and rooting them for greenhouse seed production. Fortunately, Cucumis sativus L. has the ability to root fairly easily, but when subjected to saline water, the cuttings may be damaged.

Probably the most common rooting technique is the use of a misting system, either continuous or intermittent. However, where salt levels are a problem, survival and rooting of the cuttings may be impaired. Since large volumes of water are required, use of distilled water is impractical.

Another method employed the initiation of root formation in aerated water (1). Here distilled water is a reliable water source, but quite often this is a slower technique and associated with higher mortality rates. Its primary limitation is in getting rooted cuttings to survive the initial water to soil transition. For these reasons, a very simple and inexpensive technique was developed for rooting cuttings. It provides a rapid field to greenhouse cycling of selected plants because cuttings are rooted directly in a soil medium, and because it requires a small amount of distilled water.

Several 3 x 8 x 1.5 foot (w x l x h) chambers were constructed for rooting cuttings. These were made with 2 x 2 inch yellow pine and covered with 6 mil greenhouse plastic (fig. 1). Each chamber rested on a stainless steel table with a 0.75 inch drain hole in the center. The table top sloped toward the drain. A 10-bulb VHO fluorescent light bank operated by a 24 hr timer was suspended 6 inches above each chamber to give a 12 hr photoperiod. The external chamber temperature was maintained at 19 $\pm$ 1 $^{o}$C while the internal temperature fluctuated between 23 $^{o}$C while the lights were on and 19 $^{o}$C when they were off. This provided an ideal diurnal regime associated with beneficial rooting of many species.

Side port cool mist humidifiers were located at each end of the chambers with their ports penetrating the chamber walls. These humidifiers operated on a second 24 hr time clock, and utilized distilled water to create a high relative humidity without salt buildup in the humidifier. The table top drain allowed excess condensed water to escape, while a double plastic flap system was used in front to keep excess humidity from escaping (Fig. 2). Soapy water was sprayed between these two plastic layers in order to maintain a good seal.

The humidifiers were run continuously until 96 $\pm$ 1% relative humidity was reached. Then the timers were set to allow the humidifiers to cycle on and off occasionally for maintenance of this level. The total operating time varied with different brands. It was best to have a matched set if a single chamber was to maintain a uniform humidity pattern. Adjustments were made to temporarily decrease humidity levels if disease became apparent.

Selections were sprayed in the field with SADH (2) one week prior to cutting harvest. At harvest, cuttings five or six nodes long were taken from the most stocky terminals and dipped into a mixture of Banrot[z] (1.1gm/gal) and Agri-mycin[y] (.4gm/gal) for 5 min. The cuttings were then trimmed to 3 or 4 nodes with all leaves left intact. They were inserted into 3 inch square peat pots containing artificial media saturated with distilled water. These pots were then placed in the chambers on top of inverted flats with a 1 inch spacing between each pot. This maximized air circulation and decreased the rate of disease spread if it occurred. The inverted flats allowed the pots to be off the cool, damp table surface.

Once roots began to emerge through the pots, they were moved to a light bank at 23 °C and normal room humidity for 3 to 5 days for a hardening period. A single fertilizer application was made at that time. After this, they were transplanted to larger pots in a greenhouse. A full fertilizer feeding program then brought them to normal growth in one to two weeks.

This system had several advantages. There was a relatively rapid turn-around period involved. It minimized the amount of distilled water required. Disease could be controlled fairly easily. Rooting actually took place within a soil medium, so transplant shock was minimized and an 85% survival rate was achieved. This technique may also be useful for propagation of other cucurbit species.

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[z]Supplied as "Banrot", a 40% wettable powder; product of Mallindckrodt Inc.

[y]Supplied as "Agri-mycin 17" with 21.2% streptomycin sulfate; product of Pfizer Inc.

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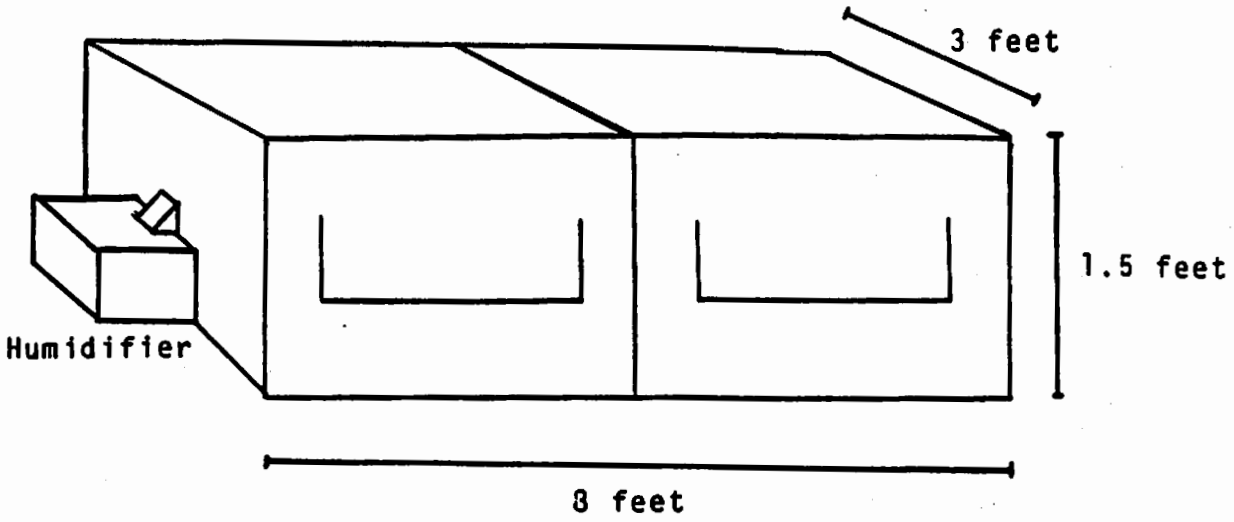


Figure 1. Chamber for rooting cucumber cuttings.

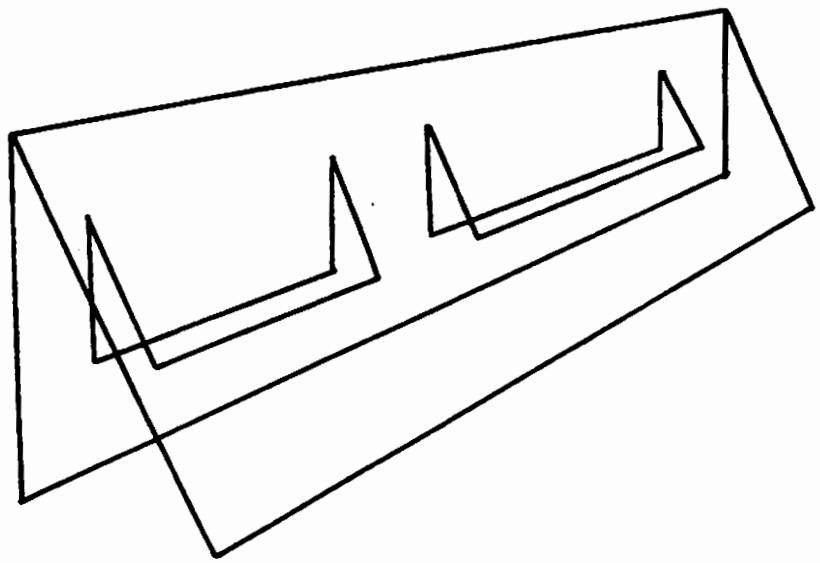

Figure 2. Double plastic flap on front of chamber.

# Leaf Area Prediction Model For Field Grown Cucumbers

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Several recent studies have suggested that fruit yield in cucumber might be limited by photoassimilate supply (1, 2). Changes in potential for photoassimilate production are related to photosynthetic rate per unit area of leaf, leaf area and number of leaves. It would be desirable to provide a nondestructive and rapid method for estimation of leaf area as related to the size of the photosynthetic canopy of cucumber genotypes. The purpose of this study was to develop a simple regression model for prediction of cucumber leaf area.

Methods. The cucumbers selected for use in this study included 4 pickling and 4 fresh-market cultivars. The fresh-market cucumbers used were A&C 1810 (Abbott & Cobb), Verino (Sluis & Groot), Supersett (PetoSeed), and Maximore 100 (Abbott & Cobb). Pickling cultivars included Calypso (Harris-Moran Seed), Volley (PetoSeed), Fancipak (Asgrow Seed), and Blitz (PetoSeed). Plots were planted at the Horticultural Crops Station at Clinton, N.C., on 18 July, 1985. Plots consisted of 3 rows 9 m long and 1.5 m apart. Plots were overplanted and thinned to 120 plants/row, for a plant density of 86,000 plants/ha. Plants were grown using standard cultural practices.

Leaves from each cultivar were collected 8 weeks after planting, placed in plastic bags, and stored on ice until measured. Leaves from each cultivar were removed for measurement beginning with the oldest leaves at the base of the plant and progressing to the apex. Length and width were measured along the leaf midrib and from tip to tip of the widest lobe, respectively (Fig. 1). These leaf positions were selected because of the ease in identifying the same position for repeated measurements. Values for length and width were recorded to the nearest 0.1 cm. In addition, the actual area (A) for each leaf was measured using a Li-cor 3000 leaf area meter (Lambda Instrument Corporation, Lincoln, Neb.) which had been calibrated to 0.01 $cm^2$.

Regression analysis was performed using length, width and actual leaf areas of 69 leaves from 'Calypso'. A search for the best model was conducted using the independent variables of length (L), length squared ($L^2$), width (W), width squared ($W^2$), length times width (LW) and the dependent variable of actual area in various combinations of the independent variables. The coefficient of determination ($R^2$), F ratio and error mean square (MSE) were calculated for each regression. Based on evaluation of these calculations a model was selected (3). The slopes of regressions for the individual cultivars were tested for equality in comparison to the initial regression derived from the 'Calypso' observations (4).

Figure 1. Diagram of a cucumber leaf showing positions of length and width measurements.

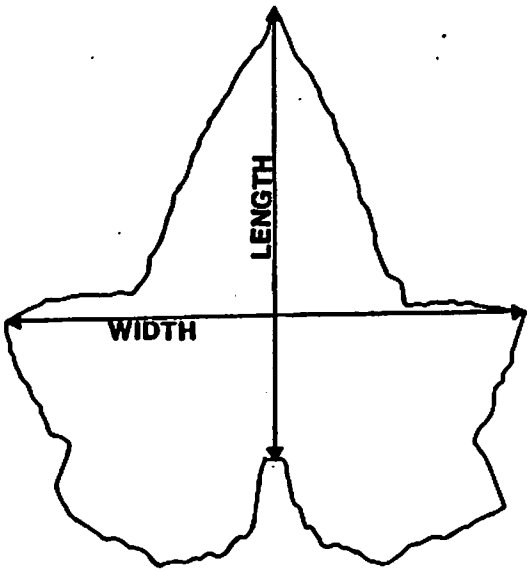


Table 1. Total[z] leaf area for leaves from 8 cultivars as measured and calculated.

| Cultivar | Measured leaf area ($cm^2$) | Calculated leaf area ($cm^2$)[y] |
|---|---|---|
| Fresh-Market Cultivars | | |
| Verino | 794.80 | 794.82 |
| Supersett | 853.10 | 786.78 |
| Maximore 100 | 732.50 | 749.80 |
| A&C 1810 | 896.40 | 902.26 |
| Pickling Cultivars | | |
| Volley | 718.40 | 719.46 |
| Calypso | 761.00 | 779.48 |
| Blitz | 628.30 | 628.46 |
| Fancipak | 912.50 | 913.97 |

[z]Sum of 8 leaves from each cultivar.

[y]Calculated as Area = $-11.306 + 0.109(L) + 1.1381(L^2)$

Results. Regression analysis using the independent variables L, $L^2$, W, $W^2$, and LW indicated that several combinations yielded equations that would be adequate for use in predicting cucumber leaf area. Major criteria used to select a common regression equation for predicting the leaf area of field grown cucumbers from these variables included simplicity of the model and the measurements it required. Analysis indicated that the equation:

$$A = 11.306 + 0.109(L) + 1.1381(L^2),$$

using a single measurement of length produced a regression equation with high predictive ability ($R^2 = 0.9604$). No significant differences were found between the regression relations for the 'Calypso' data used to develop the model and the individual cultivars used to test the model. The residuals for each regression were plotted, and no significant pattern was observed. The data presented in Table 1 show comparison between the measured and predicted area for the cultivars used in this study.

Conclusion. This study indicates that a simple regression model can accurately predict the leaf area for the 8 field-grown cucumber cultivars studied. Applicability of this model to other cultivars should be tested. The single leaf measurement of length affords the user of this model ease in recording observations as well as high predictive ability.

Acknowledgments. The authors wish to thank T. C. Wehner and K. D. Robbins for their assistance towards the completion of this study.

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# Malate Dehydrogenase Variation in African Cucumis species: A Testable Genetic Hypothesis.

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Electrophoretic, cucurbitacin and flavonoid variation; chromosome homology; and plant morphology have been studied to determine taxonomic relationships among cross-compatible species of Cucumis (1,2,4,7,8,10,11,12,15). These studies have furnished estimates of interspecific divergence which has led to increased precision in the taxonomic classification of this genus. In a study in which the production, vigour, and fertility of $F_1$ hybrids of 9 African species was used as a measure of intraspecific relationships, two species appeared to be closely related: C. prophetarum L. and C. anguria var. longipes A. Meeuse (personal communication, 10). Dane (2), was able to distinguish C. anguria var. anguria Meeuse and var. longipes supporting previous studies (3,9) which proposed that these are varieties of a single species. Although, during an electrophoretic survey of 6 enzymes, Esquinas-Alcazar (4) failed to detect appreciable differences between these botanical varieties, we observed varietal differences while staining for peptidase with phenyl-alanyl-proline (PEP-PAP) and phosphoglucomutase (PGM)(unpublished data).

In an earlier report (13), we provided evidence that C. africanus Lindley F., C. anguria, C. ficifolius A. Rich, C. myriocarpus Naud. and C. zeyheri Sond. were monomorphic for fructose diphosphatase, glutathione reductase, isocitrate dehydrogenase, and phosphogluconate dehydrogenase. Using a limited number of accessions, we gave a preliminary description of malate dehydrogenase (MDH) banding patterns in several African species. It was thought desirable to acquire more information regarding the range of electrophoretic variation in African species for MDH. Results from such a study could be used in designing future experiments which would test the genetic basis of the banding patterns observed, and to examine evolutionary relationships. This communication describes the polymorphisms observed in 7 Cucumis species for MDH and proposes a genetic model for testing this variation using cross-compatible species.

Cotyledons of 3 individuals of 7 C. africanus, 11 C. anguria var. anguria, 4 C. anguria var. longipes, 4 C. dipsaceus Ehrenb. ex. Spach, 3 C. ficifolius, 2 C. metuliferus E. Mey ex. Schrad., 3 C. myriocarpus, and 1 C. prophetarum L. collections were surveyed using horizontal starch gel electrophoresis. In order to standardize the relative mobilities of the observed electromorphs, extracts of the C. sativus L.inbred processing cucumber line GY-14A (X=7) were loaded on each gel, and band mobilities were recorded in relation to it. The least mobile (most cathodal) electromorph of GY-14A was designated 100 and all other bands were assigned a value based on their mobility relative to this electromorph.

A "Centroid" cluster analysis (5) was performed using the electromorphs observed to provide information on potential interspecific relationships not directly discernable from frequency data. Using this method, groups are depicted to lie in Euclidean space, and are replaced on formation by the co-ordinates of their centroid. The distance between groups is defined as the distance between the group centroids.

The frequencies of MDH electromorphs for each collection are given in Table 1. Each numeral indicates the number of times that a particular band was observed in 3 individuals of an accession. Although similarities among accessions are suggested by the presence of common electromorphs, their absence provides equivocal information since sample numbers were small. Bands which occur in C. anguria var. anguria are absent in var. longipes, possibly reflecting their varietal nature. Cucumis prophetarum possesses bands which are common to C. anguria var. longipes, but not to var. anguria. Several of the electromorphs recorded in C. anguria var. longipes were also observed in C. dipsaceus, C. ficifolius, and C. myriocarpus. Moreover, C. metuliferus, C. ficifolius, and C. myriocarpus shared some common electromorphs. Cluster analysis (Figure 1) may provide groupings which are meaningful if they parallel known biological facts. The classification of C. anguria var. anguria accessions from Iran and those from other sources into two groupings is in agreement with a previous study (14). In contrast, the similarities observed in shikimic dehydrogenase, PEP-PAP, and PGM zymograms of C. metuliferus and C. myriocarpus were not recorded for MDH. Likewise, the lack of common electromorphs among C. myriocarpus and C. anguria would not have been predicted based on their ability to produce good to moderately self fertile hybrids (personal communication, A. P. M. den Nijs, 10).

It would be desirable to determine the genetic basis of MDH variation and thereby use allelic frequencies (allozymes) to identify relationships among species. The fertility relationships among C. myriocarpus, C. prophetarum, and C. anguria var. anguria and var. longipes offer an avenue in which a genetic hypothesis of this dimeric enzyme (6) could be tested. Since these species have several electromorphs in common (Figure 2), isozyme patterns of individuals of each species could be identified, vegetative cuttings made and appropriate self and cross pollinations could be made which would allow testing of a proposed hypothesis. From the data provided by the present study, it would be reasonable to hypothesize a simple one locus model with 5 alleles. This hypothesis is based on the fact that all electromorphs became visible at approximately the same time and the presence of electromorphs located approximately midway between other electromorphs. Studies are being designed which test this hypothesis.

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Table 1. The frequency of malate dehydrogenase electromorphs observed in 8 species of Cucumis.

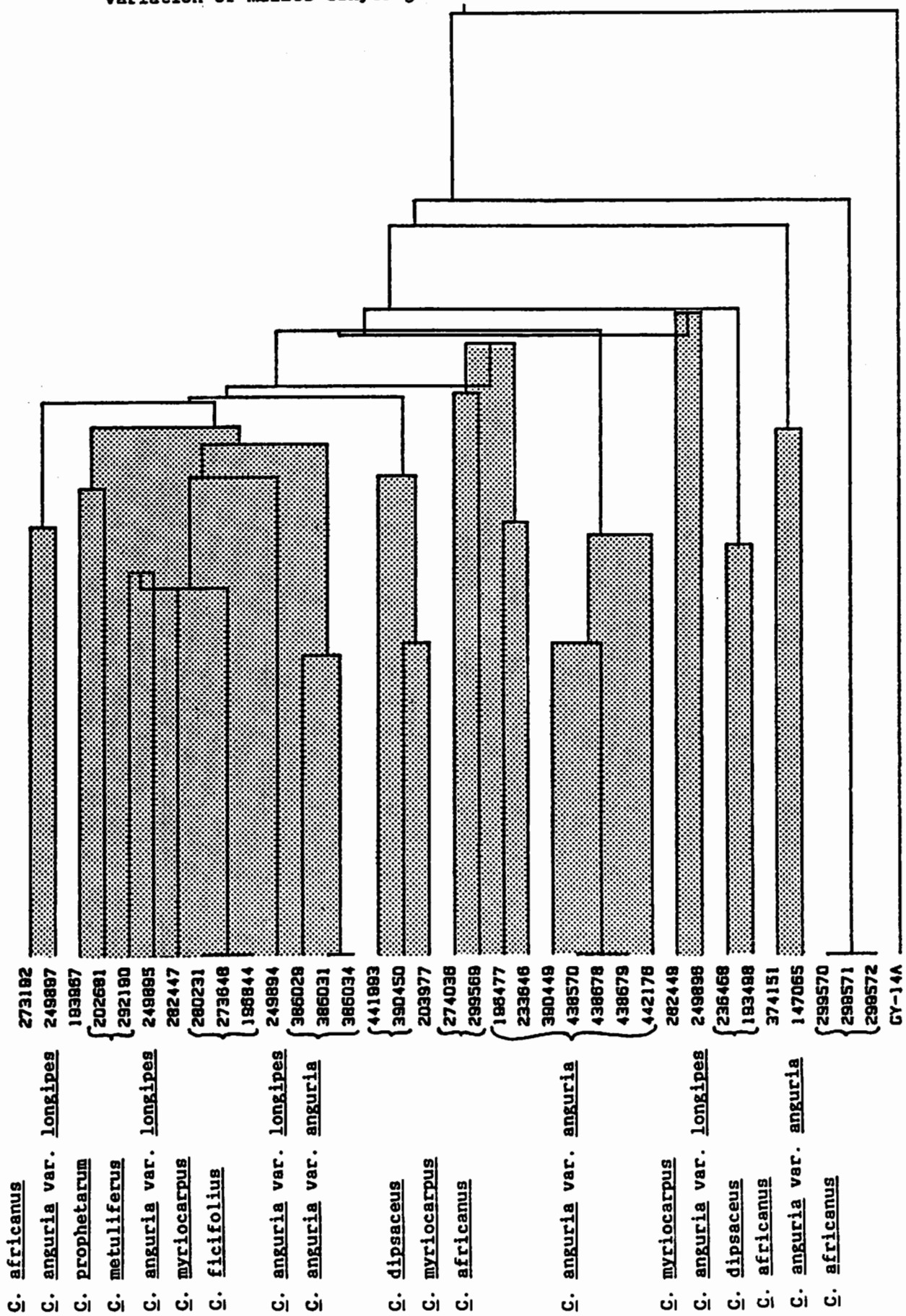
| | | Relative Electrophoretic Mobility of Malate Dehydrogenase Electromorphs* | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Source | 95 | 97 | 98 | 99 | 100 | 104 | 106 | 107 | 109 | 110 | 113 | 115 | 118 | 125 | 127 | 131 | 132 | 133 | 135 | 138 |
| C. africanus | | | | | | | | | | | | | | | | | | | | | |
| 273192 | S. Africa | | | | | 3 | | | 3 | | | | | | | | | 3 | | 3 | |
| 274036 | " | | | 2 | | 1 | | | 1 | 2 | | | | | | | 2 | | | 2 | 2 |
| 299569 | " | | | 3 | | | | | 3 | | | | | | | | 3 | | | | 3 |
| 299570 | Natal | | | 3 | | | | | 3 | | | | 3 | | | | | 3 | | | 3 |
| 299571 | S. Africa | | | 3 | | | | | 2 | | | | 2 | | | | | 2 | | | 2 |
| 299572 | " | | | 3 | | | | | 3 | | | | 3 | | | | | 3 | | | 3 |
| 374151 | USA | | | 3 | | | | | 1 | | 1 | | | | | | | | 3 | | |
| C. anguria var. anguria | | | | | | | | | | | | | | | | | | | | | |
| 147065 | Brazil | | | 3 | | | | | | 2 | 1 | | | | | | | 1 | 2 | | |
| 196477 | " | | | 3 | | | | | 2 | 1 | 2 | | | | | | 1 | 2 | | | |
| 233646 | Ethiopia | | | 3 | | | | | 3 | 1 | | | | | | | | 3 | | | |
| 386029 | Iran | | | | 3 | | | | | | 2 | | | | | | | | | | |
| 386031 | " | | | | 3 | | | | | | | | | | | | | | | | |
| 386034 | " | | | | 3 | | | | | | | | | | | | | | | | |
| 390449 | Ecuador | | | | 3 | | | | 3 | | 2 | | | | 1 | | | | | | |
| 438570 | Guatemala | | | | 3 | | | | 3 | | 3 | | | | 1 | | | | | 3 | |
| 438678 | Mexico | | | | 3 | | | | 3 | | 3 | | | | 3 | | | | | 3 | |
| 438679 | " | | | | 3 | | | | 3 | | 2 | | | | 1 | | | | | 3 | |
| 442176 | " | | | | 3 | | | | 2 | | 2 | | | | | | | | | 3 | |
| C. anguria var. longipes | | | | | | | | | | | | | | | | | | | | | |
| 249894 | S. Rhodesia | | 3 | | | | 2 | | | 1 | | | | | | | | | | 1 | |
| 249895 | " | | 3 | | | | | | | | | | | | | | | | | | |
| 249896 | " | 1 | 2 | | | | | | | | | | | | | | | | | | |
| 249897 | " | | | | | 3 | 3 | | 1 | | | | | | | | | | | 3 | |
| C. dipsaceus | | | | | | | | | | | | | | | | | | | | | |
| 193498 | Ethiopia | | 3 | | | | | 1 | 2 | 1 | | | | | | | | | | | |
| 236468 | " | | 3 | | | | | 1 | | | | | | | | | | | | | |
| 390450 | Ecuador | | 3 | | | | 2 | | 1 | | | | | 2 | | | | | | | |
| 441993 | Netherlands | | 3 | | | | 2 | | 1 | 1 | | | | 1 | | 2 | | | | | |
| C. myriocarpus | | | | | | | | | | | | | | | | | | | | | |
| 203977 | S. Africa | | 3 | | | | 2 | | 2 | | | | | 3 | | | 3 | | | | |
| 282447 | " | | 3 | | | | 2 | | | | | | | | | | 3 | | | | |
| 282449 | " | 3 | | | | | 1 | | 1 | 1 | | | | | | | 2 | | | | |
| C. sativus | | | | | | | | | | | | | | | | | | | | | |
| GY-14A | USA | | | | | 3 | | | | | | 3 | | | | 3 | | | | | |
| C. ficifolius | | | | | | | | | | | | | | | | | | | | | |
| 196844 | Ethiopia | | 3 | | | | 2 | | | | | | | | | 3 | | | | | |
| 273648 | " | | 3 | | | | 2 | | | | | | | | | 2 | | | | | |
| 280231 | " | | 3 | | | | 3 | | | | | | | | | 3 | | | | | |
| C. metuliferus | | | | | | | | | | | | | | | | | | | | | |
| 202681 | S. Africa | | | | | 3 | 1 | | 3 | | | | | | | 2 | 1 | | | | |
| 292190 | Transvaal | | 3 | | | | 3 | | 3 | | | | | | | | | | | | |
| C. prophetarum | | | | | | | | | | | | | | | | | | | | | |
| 193967 | Ethiopia | | 1 | | | 2 | | | | | | | | | | 1 | 1 | | | | |

* Electromorph with the least mobility in C. sativus (GY-14A) was designated as 100. All other electromorphs classified relative to 100.

ʸ Frequency of electromorph observed within an accession.

* R. L. Lower University of Wisconsin.

Figure 1. Cluster analysis of 8 Cucumis species based on electrophoretic variation of malate dehydrogenase.



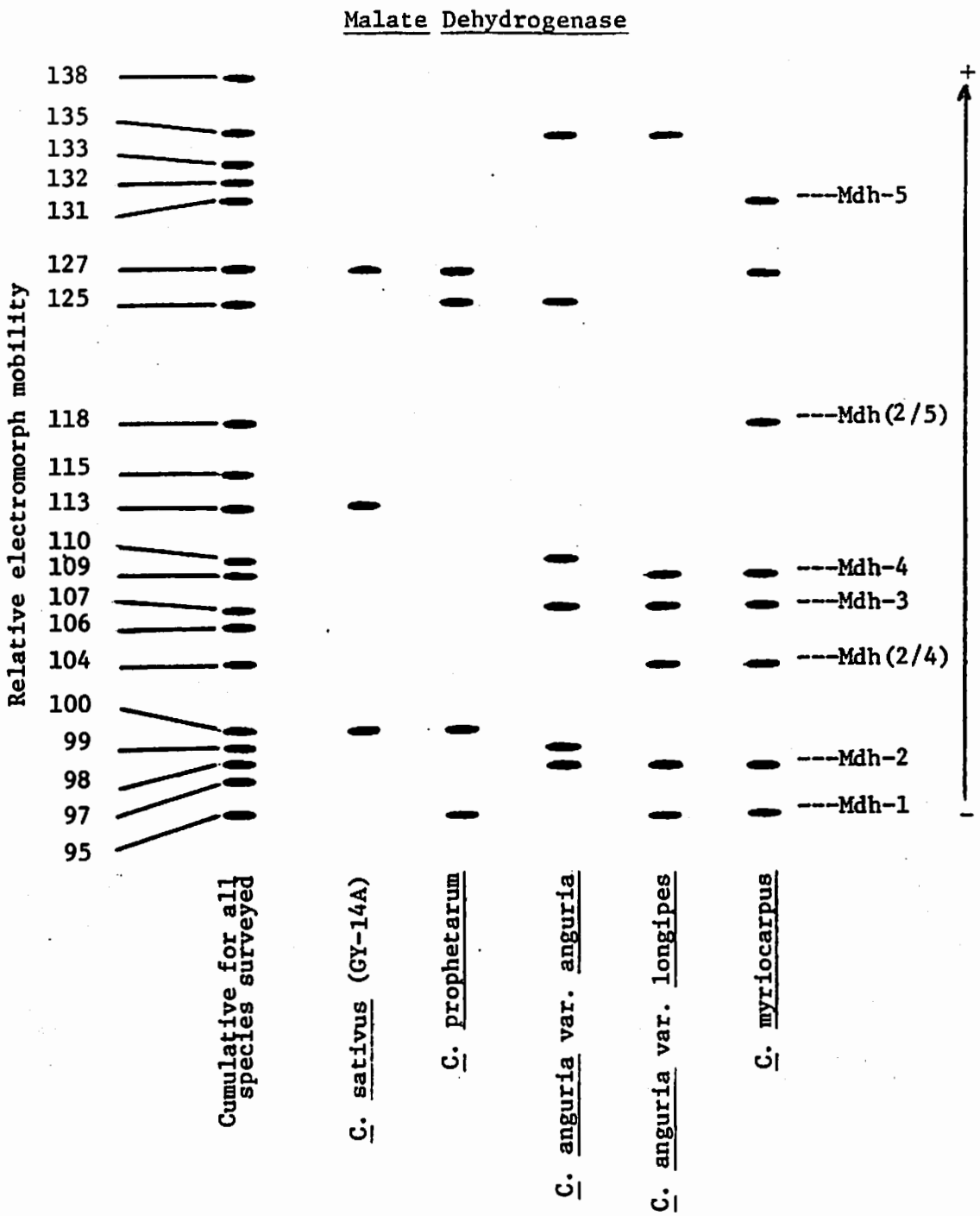



Figure 2. A diagramatic representation of electrophoretic variants of malate dehydrogenase observed in a preliminary survey. The possible position of homo- and heterodimers of a hypothetical model for the explanation of observed isozyme variation is also presented.

# Differences in Net Apparent Photosynthetic Rates and Chlorophyll Content Among and Between Cucumis anguria var. anguria and var. longipes Meeuse.

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The gherkin, Cucumis anguria var. anguria Meeuse, produces an abundance of fruit which can be prepared in several ways to provide a source of nourishment. In the West Indies and Brazil young fruits are consumed directly, pickled or prepared as a popular soup "maxixada" (1,5). Although originally thought to be native to the Americas, sexual compatibility and electrophoretic studies indicate that the gherkin is descended from a non-bitter mutant of the African C. anguria var. longipes Meeuse (2,4). It was introduced into South America (Brazil) in the 1600's, and became semi-wild. Ecotypes with a wide range of fruit characteristics now exist (1).

Previously we have reported that electrophoretic polymorphisms exist among and between plant introductions of C. anguria var. anguria and var. longipes (8, and Staub, this issue of CGC). These studies and those of Dane (3) support their classification as conspecific botanical varieties (4,6,7). Moreover, it appears that C. anguria var. longipes and C. prophetarum L. are closely related (personal communication, A. P. M. den Nijs, 1985).

Since both biochemical and morphological differences exist among these closely related cross-compatible Cucumis species, a survey was designed to assess differences in apparent photosynthetic activity. If consistent differences exist, then there would be an opportunity to investigate the genetic and physiological nature of this variation.

Thirty-day-old seedlings of 3 individuals of 10 C. anguria var. anguria and 3 var. longipes accessions were transplanted (3 x 3 m spacing) on 6/1/1984 to a field nursery at Arlington, Wisconsin. Of the 10 C. anguria var. anguria collections, 5 were of Iranian origin and 5 came from African or South American sources. Plants were grown to maturity under standard cultural practices used for cucumber (C. sativus L.). The apparent photosynthetic activity of 2 fully expanded mature leaves from one plant of each series was measured eight times on at least 2 clear days beginning 8/14/1984 with a portable photosynthesis apparatus (LI 6000). The LI 6000 (Li-Cor, Inc., Lincoln, Nebraska) consists of a battery powered non-dispersive infra-red gas analyzer, a porometer, a communications device, and a dedicated datalogger with a maximum 64K bytes of continuous memory. When a photosynthetically active leaf is enclosed in the instrument's leaf monitoring chamber, $CO_2$ concentration will decrease as photosynthesis occurs, and water vapor concentration will increase due to transpiration. Knowing the volume of the chamber and the area of the leaf, photosynthetic rate and stomatal resistance can be calculated from changes in $CO_2$ and air flow needed to maintain constant humidity, and other environmental conditions measured by the instrument. The LI-6000 makes 10 individual measurements over a pre-programed time period (40 seconds in our studies) and uses these values to calculate a mean stomatal resistance and photosynthetic value for each leaf. On the first day of measurement, 3 leaves of each plant were collected from the fourth node from the terminal whorl of a lateral branch for chlorophyll analysis.

Chlorophyll from each leaf was extracted separately in ethanol and measurements of chlorophyll a and b were made on a spectrophotometer at 649 and 665 nM,respectively. For informational purposes, field notes were taken on vegetative characteristics (foliage and fruit) during September.

There was a wide range in mean net apparent photosynthetic rate (0.45 to 0.88 mg $CO_2$ $m^{-1}$ $s^{-1}$) and chlorophyll content (a = 4.46 to 7.8 and b = 1.72 to 4.46 mg/g dry weight) among C. anguria var. anguria collections (Table 1). The mean net apparent photosynthetic rate of var. anguria collections from Iran was higher than those of other var. anguria collections taken collectively. While the chlorophyll content was, on the average, lower in Iranian collections, chlorophyll b was higher when compared with other var. anguria accessions. The total chlorophyll content of all var. anguria accessions was similar except for one collection (PI 386029) which was comparatively lower due to low chlorophyll b values. The apparent photosynthetic rate of this plant introduction was not remarkable. Compared to var. anguria collections, the mean net apparent photosynthetic rate was lower and total chlorophyll content higher in leaves of var. longipes. The observed higher chlorophyll content was due to higher values of both chlorophyll a and b.

The data of this preliminary survey suggests that there are gross differences in apparent photosynthetic activity and chlorophyll content among and between anguria var. anguria and var. longipes. This study is being repeated under more stringent conditions in the field and under controlled environment conditions. If these initial results can be verified, then the nature of net apparent photosynthetic activity could be characterized.

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Table 1. Net apparent photosynthetic rate, chlorophyll content and field notes of Cucumis anguria var. anguria and var. longipes Heeuse.

| | | Net Apparent Photosynthesis Rate[u] ($mg\ CO_2 m^{-1} s^{-1}$) | | | | | | Chlorophyll[v] (mg/g dry wt.) | | | Field Notes[z] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Foliage[w] | Vegetative[x] | | Fruit[y] |
| PI | Source | N[t] | $\bar{X}$ | SD | Min. Value | Max. Value | $SD_{\bar{X}}$ | A | B | A+B | Color | Matte | Branching | Spines |
| C. anguria var. anguria | | | | | | | | | | | | | | |
| 147065 | Brazil | 32 | 0.79 | 0.14 | 0.55 | 1.13 | 0.02 | 6.49 | 2.54 | 9.03 | 3-4 | 1,5 | 1,4 | 3,4,8 |
| 196477 | Ethiopia | 32 | 0.68 | 0.35 | 0.20 | 1.43 | 0.06 | 6.29 | 1.72 | 8.01 | 3-4 | 3,5 | 3,6 | 3,4,9 |
| 233646 | " | 32 | 0.45 | 0.18 | 0.21 | 0.78 | 0.05 | -- | -- | -- | 3-4 | 3,5 | 3,7 | 3,4,9 |
| 282442 | S. Africa | 32 | 0.47 | 0.16 | 0.20 | 0.82 | 0.03 | 7.80 | 3.78 | 11.58 | 1-4 | 1,5 | 1,4 | 3,4,9 |
| 320052 | Ethiopia | 32 | 0.50 | 0.20 | 0.15 | 0.83 | 0.04 | 5.38 | 2.30 | 7.68 | 3-4 | 1,5 | 1,6 | 3,4,9 |
| $\bar{X}$ | | | 0.58 | | 0.26 | 1.00 | | 6.49 | 2.59 | 9.08 | | | | |
| SD | | | 0.15 | | 0.16 | 0.28 | | 0.99 | 0.86 | 1.77 | | | | |
| C. anguria var. anguria | | | | | | | | | | | | | | |
| 386029 | Iran | 48 | 0.65 | 0.14 | 0.37 | 0.91 | 0.02 | 5.07 | 1.18 | 6.25 | 4 | 3,5 | 3.8 | 3,4,8 |
| 386031 | " | 48 | 0.63 | 0.33 | 0.05 | 1.50 | 0.04 | 5.70 | 3.73 | 9.43 | 2 | 3.5 | 3.6 | 3,4,9 |
| 386035 | " | 48 | 0.62 | 0.15 | 0.33 | 0.96 | 0.02 | 4.46 | 4.97 | 9.43 | 2-4 | 3,5 | 3,6 | 3,5,8 |
| 386036 | " | 48 | 0.76 | 0.24 | 0.35 | 1.40 | 0.04 | 5.79 | 5.44 | 11.23 | 3-4 | 3,7 | 4,7 | 3,4,8 |
| 386037 | " | 48 | 0.88 | 0.35 | 0.33 | 1.62 | 0.05 | 5.80 | 3.63 | 9.43 | 4 | 3,5 | 3,6 | 3,4,8 |
| $\bar{X}$ | | | 0.71 | | 0.29 | 1.28 | | 5.36 | 3.79 | 9.15 | | | | |
| SD | | | 0.11 | | 0.13 | 0.32 | | 0.59 | 1.65 | 1.80 | | | | |
| C. anguria var. longipes | | | | | | | | | | | | | | |
| 249894 | Africa | 94 | 0.41 | 0.16 | 0.11 | 0.82 | 0.02 | 6.72 | 2.93 | 9.65 | 4 | 1,7 | 4,7 | 3,4,9 |
| 249896 | " | 86 | 0.37 | 0.15 | 0.13 | 0.78 | 0.02 | 6.56 | 2.90 | 9.46 | 4 | 1,7 | 1,6 | 3,4,7 |
| 249897 | " | 80 | 0.53 | 0.26 | 0.10 | 1.14 | 0.03 | 6.95 | 2.60 | 9.55 | 4 | 1,5 | 3,6 | 3,4,7 |
| $\bar{X}$ | | | 0.44 | | 0.11 | 0.91 | | 6.74 | 2.81 | 9.55 | | | | |
| SD | | | 0.08 | | 0.01 | 0.20 | | 0.20 | 0.18 | 0.10 | | | | |

[z] Taken 12 weeks after transplanting.
[y] 1 = sharp, 2 = medium, 3 = soft/dull, 4 = many, 5 = few, 6 = none, 7 = short, 8 = long, 9 = medium.
[x] Vegetative matte: 1 = dense, 2 = sparse, 3 =few, small leaves but very branchy, 4 = many large leaves but few branches, 5 = average size, 6 = above average size; vegetative branching: 1 = many, 2 = few, 3 = average, 4 = thick stems, 5 = average stem thickness.
[w] 1 = dark green, 2 = yellow green, 3 = cabbage green, 4 = green.
[v] Average of 3 samples.
[u] $\bar{X}$ = mean, SD = standard deviation; $SD_{\bar{X}}$ = standard error of $\bar{X}$.
[t] Observations with 10 sampling times (subsamples) per observation measured over at least 2 days (i.e., 2 days x 2 leaves x 8 measurements = 32). Each observation represents data obtained from one mature fully expanded leaf.

CGC 9:26 (1986)

# Discrimination Among Seven Cucumis hardwickii (R) Alef. Accessions Based on Principal Component Analysis.

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Fruit yield in cucumber (Cucumis sativus L.) is suppressed due to the physiological nature of its fruiting habit. Fruit developing from the first pollinated flower inhibits the development of subsequent fruits. It is unclear whether fruit set inhibition is due to a substance translocated from the fruit, a substrate limited source-sink relationship, or some other physiological mechanism (3,5,7,8,14).

The incorporation of quantitatively inherited characters into commercially adapted cultivars using exotic germplasm can be an effective way to obtain greater genetic variability and response to selection (1,4). Cucumis sativus var. hardwickii (R.) Alef., a multiple fruiting phenotype, has been suggested as a potential source for increasing the genetic variability for yield in cucumber since it lacks fruit set inhibition (6,11).

One thrust of the USDA cucumber breeding program involves the utilization of hardwickii germplasm in cucumber improvement. Potentially useful multiple fruiting populations derived from initial hardwickii x sativus matings are being developed which possess multiple disease resistance along with high levels of gynoecy and the non-bitter character (12,13). Data indicate that there are morphological and anatomical differences among several of the hardwickii plant introductions used in development of these populations (10). We have used two hardwickii collections in our breeding program, PI 215589 and LJ 90430. It would be useful to determine if these and other hardwickii collections could be grouped, thus providing more efficient use of this germplasm. Therefore an experiment was designed to characterize differences among 7 hardwickii accessions currently available for use in plant improvement.

Three diverse sativus gynoecious inbred lines (WI 1379, WI 1909, and GY-14) were selected for crossing with 7 lines of hardwickii (LJ 90430, LJ 91176, PI 183967, PI 215589, PI 273648, PI 462369, PI 486336). A Design II mating scheme (2) was initiated by crossing each sativus line with each hardwickii line to produce 21 $F_1$ families (3x7). This mating scheme was used to negate the potential photoperiodic responses associated with hardwickii (13) and to provide information on the combining ability of the hardwickii collections used. The $F_1$ progenies were planted in field nurseries at the University of Wisconsin experimental stations at Hancock and Arlington, Wisconsin. The soil type at Hancock is Planefield loamy sand (Typic Udipsamment; sandy, mixed, mesic) while the Arlington soil type is Plano silt loam (Typic Argiudoll; fine-silty, mixed, mesic). Three and 6 replications were arranged in a randomized complete block design at Arlington and Hancock, respectively. In each block, 9 individuals of each cross were spaced 1.52 m apart within a row, and parallel rows were designated as plot borders. Data were collected from the 7 innermost plants of a row. Border rows of similar lineage were also planted on the outside of each block. Supplemental irrigation was used along with standard cultural practices.

Data from each plant within a plot were collected on the number of days to anthesis, number of female nodes, fruit number, length and diameter, number of lateral branches, and plant dry weight. Fruit length/diameter ratios (L/D) were also calculated. For each parameter, measurements of the 7 plants within a plot were averaged and these means were the experimental units used for analysis. Cumulative means (over replications) for each series of $F_1$ progeny are presented in Tables 1 and 2.

An ordination of variables by principal component analysis (PCA) was performed in order to aid in the interpretation of the multivariate data (9). The ordination obtained by PCA allows for the grouping of hardwickii collections into subpopulations based on their relative differences. Although in PCA one works from the data towards a hypothetical model (grouping into subpopulations), PCA should be considered an initial step in which complex data sets are simplified to make them more amenable to interpretation. Principal component analysis is an analytical procedure for transforming one set of variates into another set of component variates which are linear, orthogonal functions (eigenvectors) of the original variates. The total variation is equal to the total variation of the original variates such that the variance associated with each component decreases in order (i.e., the first variate will account for the largest possible proportion of the total variation, the second will account for the largest portion of the remainder and so forth). An assumption basic to PCA is that the observed variation is caused by the effects that the underlying (causal) factors have on each of the original variates. The consistency of these underlying factors on character expression and therefore on ordination (the ordering of units within a multidimensional space) can be examined by comparison of data from different locations.

The intravarietal relationships among hardwickii plant introductions was examined by casting the eigenvectors and associated components derived from $F_1$ data into a multidimensional hyperspace (9; Figure 1). Location data are presented separately such that the X-axis corresponds to the first eigenvector and the Y-axis to the second eigenvector for each location. The distance among these points is proportional to the degree of dissimilarity in terms of the set of variates (parameters) used. If discrete subpopulations with some degree of biological integrity can be defined, then hardwickii accessions can be classified.

Analysis indicates that PI 183967 and LJ 90430 could be grouped into a distinct subpopulation, while PI 273648 and LJ 91176 could form another. Although PCA of Arlington data indicate that PI 215589 and PI 486336 show similarities, analysis of Hancock data provides no such representation. The accession, PI 486336, appears to be most similar to PI 273648 and therefore bears some resemblance to LJ 91176. Principle component analysis of both locations revealed that PI 462369 could be classified as a unique subpopulation.

The grouping of PI 183967 and LJ 90430 is consistent with the fact that LJ 90430 is a derived line from the intermating PI 183967 (personal communication, J. D. McCreight). The PI 462369 was obtained from the V. I. Vavilov Institute of Plant Breeding, USSR and is itself closer to sativus than any other accession examined with regard to fruit characteristics. These fruit characteristics appear to be transferable (apparent in $F_1$ progeny)

and undoubtedly contribute to the ordination of this accession. The PI 215589 was collected near Dehra Dun, India (H. S. Gentry, 1954) while PI 486336 was acquired on Mount Abu, India (personal communication, B. Dutt). These collection sites are, ecologically speaking, very dissimilar. It might be reasonable to expect that the performance of $F_1$ progeny derived from these accessions would be dissimilar in different locations.

These data indicate that there are differences among $F_1$ progeny derived from hardwickii x sativus matings. The 8 parameters monitored at 2 locations allowed for the discrimination of 7 hardwickii parental accessions into 3 distinct subpopulations by PCA. Although any hypothesis obtained from such an analysis must be considered subjective until confirmed by additional studies, it is likely from these and other data (12) that the hardwickii accessions used in this study are not similar and that their utilization in a plant improvement program will be dictated by project objectives.

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Table 1. Means by cross of morphological traits of $F_1$ hybrids of Cucumis sativus L. and C. sativus var. hardwickii (R.) Alef. at Hancock, WI.

| Cross | | Fruit no. | Lateral branch no. | Plant dry wt. (g) | Fruit | | | No. of female nodes[z] | No. of days to anthesis |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Length (cm) | Diameter (cm) | Length/ diameter ratio | | |
| GY14 x | LJ 90430 | 62.84 | 11.86 | 253.63 | 8.97 | 5.30 | 1.69 | 0.260 | 40.42 |
| | LJ 91176 | 55.35 | 11.17 | 292.61 | 9.26 | 5.79 | 1.60 | 0.318 | 41.64 |
| | PI 183967 | 72.17 | 11.90 | 285.82 | 9.18 | 5.32 | 1.72 | 0.284 | 40.49 |
| | PI 215589 | 34.79 | 10.67 | 206.79 | 9.60 | 5.85 | 1.64 | 0.366 | 42.61 |
| | PI 273648 | 44.82 | 11.31 | 280.79 | 10.02 | 6.14 | 1.63 | 0.259 | 41.04 |
| | PI 462369 | 25.92 | 9.21 | 201.46 | 12.61 | 6.27 | 2.01 | 0.264 | 38.84 |
| | PI 486336 | 57.59 | 11.69 | 282.87 | 9.58 | 5.97 | 1.60 | 0.330 | 39.10 |
| NI 1379 x | LJ 90430 | 62.84 | 10.73 | 248.49 | 9.04 | 5.44 | 1.66 | 0.281 | 39.57 |
| | LJ 91176 | 52.48 | 10.25 | 257.07 | 9.28 | 5.71 | 1.63 | 0.275 | 40.21 |
| | PI 183967 | 62.93 | 11.48 | 239.37 | 9.01 | 5.34 | 1.69 | 0.274 | 39.86 |
| | PI 215589 | 44.02 | 10.74 | 229.95 | 9.46 | 5.60 | 1.69 | 0.353 | 37.22 |
| | PI 273648 | 44.90 | 10.41 | 240.40 | 9.70 | 5.93 | 1.63 | 0.265 | 39.69 |
| | PI 462369 | 23.09 | 8.46 | 176.52 | 12.78 | 6.32 | 2.02 | 0.307 | 38.12 |
| | PI 486336 | 52.24 | 10.55 | 246.85 | 9.40 | 5.84 | 1.61 | 0.348 | 37.92 |
| NI 1909 x | LJ 90430 | 63.64 | 11.09 | 233.28 | 10.26 | 5.20 | 1.97 | 0.336 | 40.33 |
| | LJ 91176 | 52.71 | 10.40 | 259.52 | 10.62 | 5.48 | 1.94 | 0.316 | 39.83 |
| | PI 183967 | 56.95 | 10.85 | 243.06 | 10.56 | 5.16 | 2.05 | 0.338 | 40.52 |
| | PI 215589 | 35.80 | 9.88 | 185.49 | 11.02 | 5.68 | 1.94 | 0.375 | 41.43 |
| | PI 273648 | 43.36 | 10.55 | 244.42 | 10.90 | 5.80 | 1.88 | 0.325 | 40.02 |
| | PI 462369 | 24.25 | 8.37 | 192.18 | 14.18 | 6.10 | 2.32 | 0.292 | 39.19 |
| | PI 486336 | 48.53 | 10.60 | 227.28 | 10.21 | 5.68 | 1.80 | 0.338 | 39.55 |
| Grand Mean | | 48.64 | 10.58 | 239.42 | 10.27 | 5.71 | 1.80 | 0.310 | 37.87 |

[z]Arcsine squareroot transformation of the data.

Table 2. Means by cross of morphological traits of $F_1$ hybrids of Cucumis sativus L. and C. sativus var. hardwickii (R.) Alef. at Arlington, WI.

| Cross | | Fruit no. | Lateral branch no. | Plant dry wt. (g) | Fruit | | | No. of female nodes[z] | No. of days to anthesis |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Length (cm) | Diameter (cm) | Length/ diameter ratio | | |
| GY14 x | LJ 90430 | 114.83 | 11.62 | 436.97 | 8.80 | 5.13 | 1.71 | 0.256 | 49.70 |
| | LJ 91176 | 64.76 | 8.71 | 274.48 | 8.77 | 5.71 | 1.53 | 0.295 | 51.57 |
| | PI 183967 | 78.09 | 11.14 | 363.29 | 8.79 | 5.04 | 1.74 | 0.223 | 53.29 |
| | PI 215589 | 46.61 | 9.05 | 186.62 | 9.05 | 5.64 | 1.60 | 0.355 | 48.39 |
| | PI 273648 | 53.66 | 10.02 | 287.54 | 9.99 | 6.20 | 1.61 | 0.244 | 50.12 |
| | PI 462369 | 22.06 | 7.31 | 105.80 | 12.27 | 6.30 | 1.95 | 0.253 | 49.71 |
| | PI 486336 | 46.63 | 9.35 | 142.58 | 8.75 | 5.62 | 1.56 | 0.319 | 50.22 |
| NI 1379 x | LJ 90430 | 81.52 | 10.95 | 260.59 | 8.39 | 5.11 | 1.64 | 0.286 | 48.95 |
| | LJ 91176 | 48.99 | 7.95 | 215.38 | 8.87 | 5.64 | 1.57 | 0.262 | 51.09 |
| | PI 183967 | 81.12 | 9.56 | 254.05 | 8.69 | 5.07 | 1.71 | 0.294 | 48.43 |
| | PI 215589 | 42.28 | 8.44 | 148.60 | 9.19 | 5.66 | 1.62 | 0.371 | 47.81 |
| | PI 273648 | 46.33 | 8.38 | 195.47 | 9.28 | 5.85 | 1.58 | 0.300 | 51.05 |
| | PI 462369 | 17.67 | 6.11 | 96.20 | 12.20 | 6.28 | 1.94 | 0.308 | 47.33 |
| | PI 486336 | 54.28 | 8.70 | 186.47 | 9.03 | 5.77 | 1.56 | 0.350 | 47.29 |
| NI 1909 x | LJ 90430 | 73.77 | 10.54 | 307.43 | 9.74 | 5.04 | 1.93 | 0.330 | 49.96 |
| | LJ 91176 | 41.86 | 8.54 | 209.18 | 10.24 | 5.43 | 1.89 | 0.274 | 54.19 |
| | PI 183967 | 79.51 | 11.27 | 355.69 | 10.12 | 4.93 | 2.05 | 0.341 | 50.84 |
| | PI 215589 | 37.78 | 9.04 | 126.84 | 9.97 | 5.53 | 1.80 | 0.376 | 51.83 |
| | PI 273648 | 52.12 | 10.86 | 264.53 | 10.75 | 5.81 | 1.85 | 0.349 | 52.00 |
| | PI 462369 | 19.79 | 6.97 | 144.76 | 13.40 | 5.87 | 2.28 | 0.327 | 50.62 |
| | PI 486336 | 42.64 | 8.66 | 159.20 | 10.03 | 5.49 | 1.82 | 0.361 | 53.16 |
| Grand Mean | | 54.57 | 9.20 | 224.81 | 9.82 | 5.58 | 1.76 | 0.308 | 50.36 |

[z]Arcsine squareroot transformation of the data.

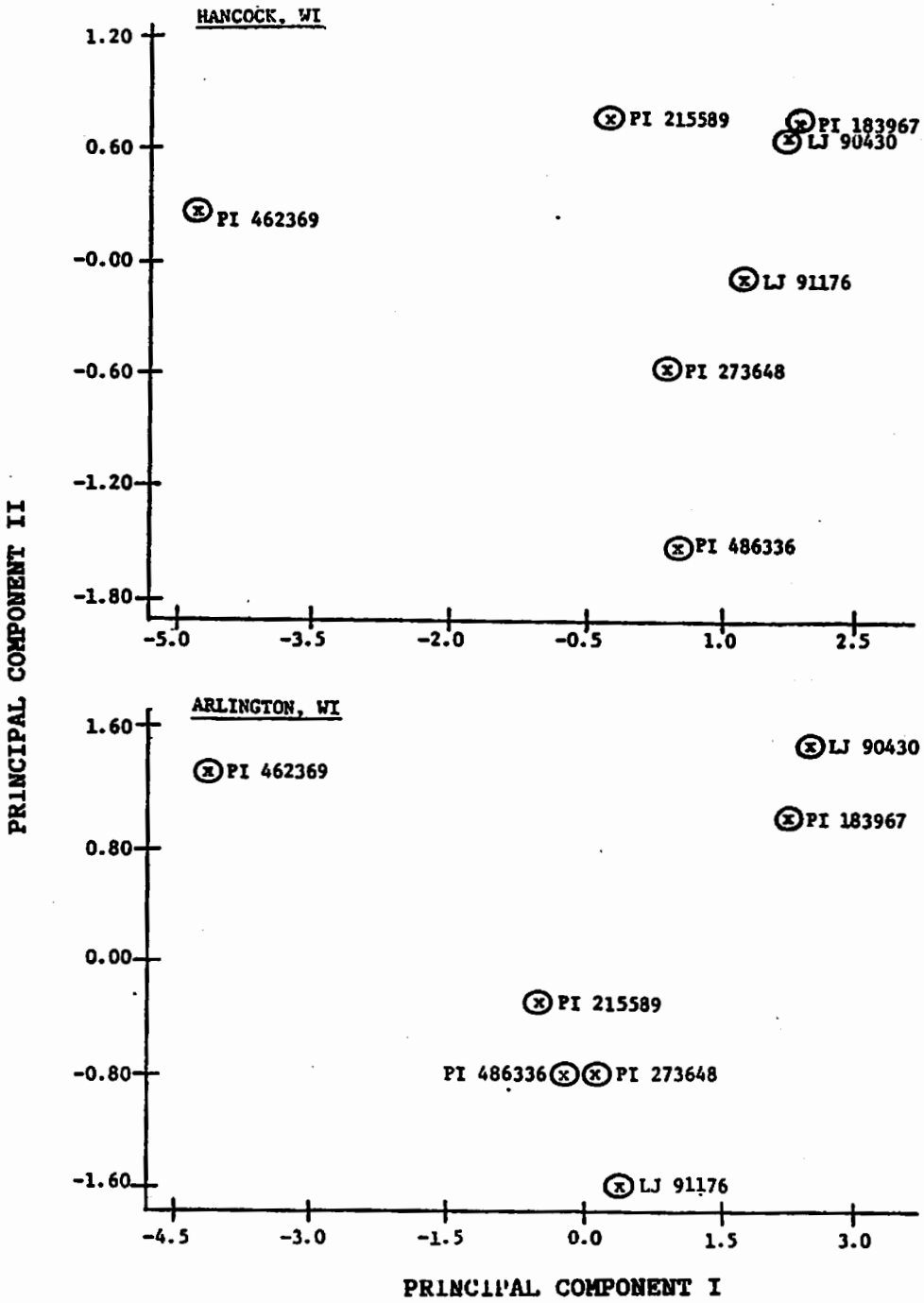


**Figure 1.** Plot of individual C. sativus var. hardwickii (R) Alef. accessions based on value scores of $F_1$ progeny (C. sativus var. sativus x var. hardwickii) on principal component axes I and II at 2 locations. Each point represents the measurement of 8 parameters in each of 6 replications per location.

# A Rapid and Non-Destructive Technique for Measuring the Area of Cucumber Leaves.

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Leaf area measurements are an essential part of growth analyses. For most measurements, plants need to be defoliated so that leaf area can be measured with a photo-electrical area meter. Growth analyses have shown that in cucumber a large leaf area is desired for quick growth (3). For selection of fast-growing lines and for experiments with repeated measurements of the same plants, a non-destructive measuring technique is required. Also, when many leaves have to be measured, a rapid and efficient method is necessary.

As early as 1921, leaf area calculation was used for area measurements in one cucumber cultivar, based on three linear and two corner measurements (1). Differences from the actual area occurred and a correction factor was necessary. The error with single leaf measurements was $\pm$ 5%, whereas it was only $\pm$ 1.6% for total leaf area. In poplar a rapid method was developed using only length and width to calculate leaf area (4). The accuracy of this method was limited for individual leaves, but it was sufficient for total leaf area. A correction factor was necessary to calculate the actual leaf area. The correction factor was unique for each poplar clone in the study. Liebig (2) used this method in his experiments on productivity of two cucumber cultivars for which he used one correction factor.

Methods. At IVT, an experiment was run to determine whether the method of Liebig can be used in plant breeding, where different genotypes are involved. In the autumn of 1983 5 plants of 4 slicing cucumbers ($F_1$ hybrids Farbio and Birgit and 2 genetically uniform inbred lines K79341 and K79351) and 2 pickling cucumber cultivars (Hokus and Levo) were randomly grown in pots under normal glasshouse conditions. When most plants had 9 or more leaves, length and width of all leaves of each plant were measured 3 times and recorded to the nearest cm, according to a previously established random scheme. Width was measured on the narrowest leaf part between the first and the second main vein, and length between the base of the leaf and the point where the leaf is 1 cm wide (Fig. 1). This was done to avoid errors caused by differences in the shape of the leaf tips of different genotypes. Leaves smaller than 3 x 3 cm were not measured. Leaf area (LAC) was calculated as length x width. The leaves of each plant were also measured individually with a Li-cor 3100 area meter (LAM) to measure the actual leaf area.

Results: When measuring length and width of leaves ranging from 3 to 20 cm on a 1 cm scale, rounding off to the nearest cm causes errors. Therefore three separate measurements were made. The results of the LAC measurements on single leaves gave a variation of 5 to 30%, whereas for total leaf area it was about 2%. The use of a smaller measuring scale will reduce the difference between these measurements. However, this will increase the time necessary for collecting the data. For total leaf area, LAC can normally be calculated on the basis of one measurement.

The shape of the leaf depends on its position on the plant. Therefore the difference between LAC and LAM of individual leaves vary. In slicing cucumbers, LAC was nearly equal to LAM for young leaves, whereas for old leaves it was much greater than the LAM. With the pickling cultivars, however, the LAC of young leaves deviated more from LAM than the LAC of old leaves, as shown in Fig. 2. Differences between LAC and LAM of the middle leaves was intermediate to the difference of LAC and LAM of old and of young leaves.

A better agreement of LAC with LAM was found with measurements of total leaf area because the middle leaves, which were stable in shape, represented most of the total area and the influence of measuring errors was limited. The average total area per plant shows identical significant differences both for LAC and LAM between the six genotypes (Table 1). LAC gives a systematic overestimation of the total leaf area. This difference between LAC and LAM (LAC/LAM ratio) is caused by the different shape of the leaves of each genotype. The pickling cucumbers, Levo and Hokus, have a somewhat higher LAC/LAM ratio than the slicing cucumbers. Within the group of slicing cucumbers, K79341 and Farbio have a lower LAC/LAM ratio than K79351 and Birgit, indicating that for an accurate conversion of LAC into LAM a correction factor is necessary, which may be different for each genotype. The overall correlation between the average of total leaf area per plant of the 3 LAC measurements and the LAM is 0.99 ($y = 0.9005 x - 54.5$).

Conclusions. This experiment shows that for breeding and selection, total leaf area can easily be approximated by measuring length and width on a 1 cm scale, because the breeder is most interested in comparing inbred lines. For growth analyses, where the real leaf area is necessary, a more accurate method is needed.

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Table 1. Number of leaves, and total leaf area as measured (LAM) and as calculated (LAC) for 3 separate measurements.[z]

| Genotype | Average number of leaves | LAM ($cm^2$) | LAC ($cm^2$) | LAC as % of LAM |
|---|---|---|---|---|
| K79341 | 9.2 | 1679 b | 1876 b | (112) |
| Farbio | 9.2 | 1459 b | 1656 b | (114) |
| K79351 | 11.4 | 1966 a | 2279 a | (116) |
| Birgit | 9.2 | 1609 b | 1893 b | (118) |
| Hokus | 8.8 | 646 c | 781 c | (121) |
| Levo | 9.0 | 710 c | 867 c | (122) |

[z]Data are means over 5 plants and 3 measurements per genotype.

Fig.1: Diagrams of leaves with measuring points for length (L) and width (W).

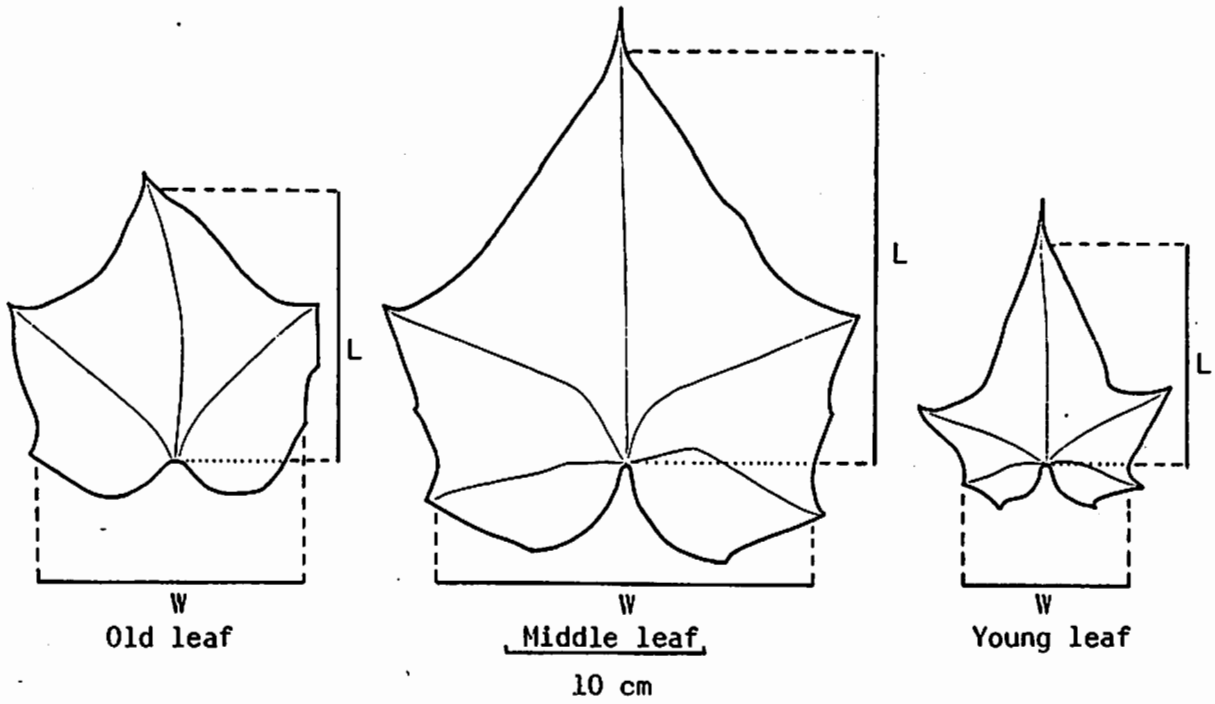
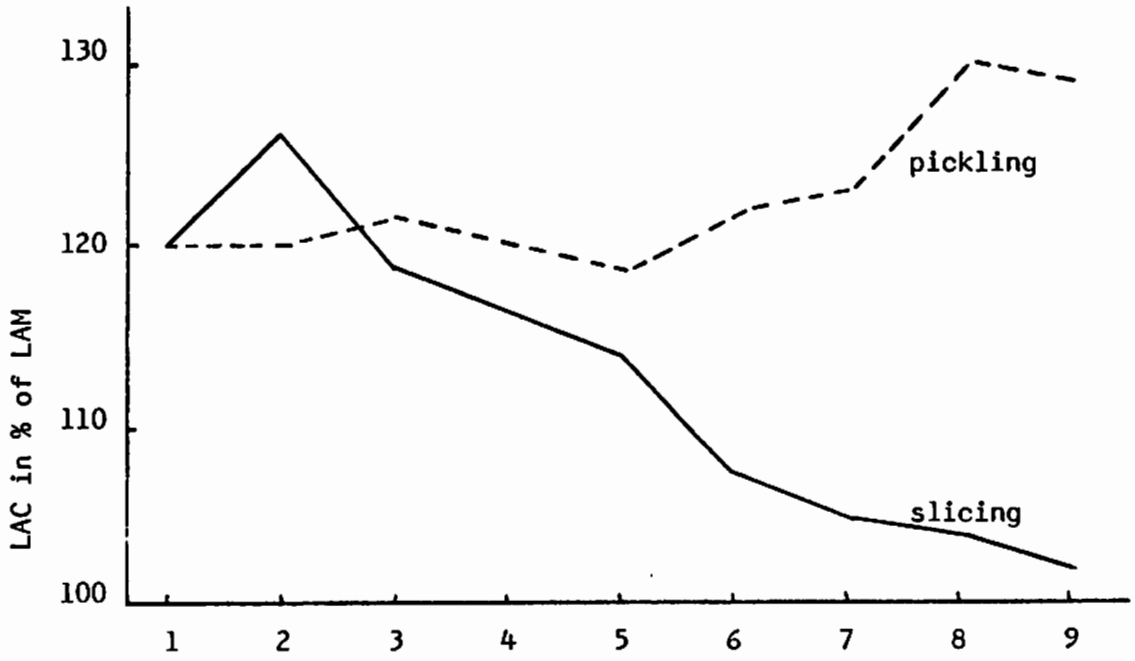



Fig.2: LAC in % of LAM according to leaf position for four slicing cucumbers and two pickling cucumbers.



# An Electronic Clipboard for Field Data Collection

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Horticultural researchers are often in the position of having to collect a lot of data for their experiments, and then get it analyzed and summarized in a short period of time, and all that within the constraints of a limited budget. I have converted to a new data collection system recently which offers a number of advantages over the old system of writing the data on sheets of paper, entering the data from the data sheets into the computer for analysis, and then writing the article using a "rough" printout from the computer (Fig. 1). This is a good time to convert to electronic data collection because the necesary equipment is easy to use and within reach of many research budgets. Computer programming skills are not required to set up and use the system, since all of the programs used are available commercially, and are easy to learn.

The general procedure is to collect data on a portable microcomputer using a word processing program, then transmit the dataset to a desktop microcomputer for printing, and storage on disks. Subsequently, the data may be analyzed using either a statistical program on the microcomputer, or by transmitting it to a mainframe computer for analysis using programs such as the Statistical Analysis System (SAS Institute, Cary, North Carolina).

Equipment. The data collection system that I use consists of a Tandy 200 portable microcomputer and an Apple Macintosh Plus desktop microcomputer. The Model 100 portable microcomputer is slightly smaller and lighter, and also less expensive, but the Tandy 200 has a larger screen and better keys for cursor control (used frequently during data collection). Accessories useful to have are an external, rechargeable battery for the Tandy 200 (to increase the time that one can go without replacing or recharging the internal AA size batteries), and 2 extra banks of random access memory for a total of 72 kilobytes. The Macintosh system includes a second disk drive, an Imagewriter II printer, MacWrite version 2.2 (the new 4.5 version is not as easy to use for documents that are handled as text only) word processing program, and Red Ryder 7.0 communications program.

The procedure for data collection is similar to the old method, but is faster and easier since the data is not transcribed from paper to computer, saving additional time spent previously in proofreading.

Procedure. The system we used until 1984 involved paper data sheets which were held in binders until needed (Fig. 1). Data sheets were transferred to a clipboard for data collection, then photocopied and given to data entry services to type into a file on the mainframe computer used for data analysis. The data was then printed for checking against the original and mistakes corrected. Finally, the data was printed, analyzed using a SAS program, and the output used to write a report.

The new system involving electronic data collection was tested in 1983 and 1984 with 2 data collectors (a Datamyte 1000 and a Tandy 100) to evaluate the performance under field conditions which included exposure to rain, dust

and physical abuse. In 1985, the present system was used exclusively with no backup data sheets since we felt that the problems with the system had been mostly solved.

The new system involves writing blank data sheets on the desktop computer and transferring the data sheets to the portable computer the day before needed. After collecting the data, it was transferred back to the desktop computer, and stored and printed. When all of the data was collected for an experiment, the data was combined with a SAS program and sent to the mainframe computer for analysis. The results were then transferred from mainframe to desktop computer in a form that was nearly ready for publication.

Example session. The portable microcomputer used for data collection (the Tandy 200) has a screen that is 40 columns wide. Therefore, the data sheet is limited to 39 columns of text (leaving the last column in each line for the carriage return). That is usually sufficient for the data we collect during one session.

An experiment might involve collecting yield data for 6 harvests, quality data for 3 samples, and disease data at the end of the season. For such an experiment, we would use 8 data sheets: 6 for yield, and 1 each for quality and disease. The yield and quality data are collected simultaneously during 3 of the harvests using 2 microcomputers (previously, we used 2 clipboards). Thus, even for an experiment involving much data, the 40 column screen is adequate. The data is collected at different times or in different places, and is, therefore, easily divided into sets. An example of the way one of our data sheets looks is shown in Fig. 2.

Problems. The problems we have noticed with this system are not serious, and are easily solved. Battery power is a limitation in the Tandy 200, since the internal AA alkaline cells last about 14 hr. For that resaon, we generally use an external rechargeable battery as mentioned above. The electrical contact of the "down arrow" cursor key of the Model 100 became worn after one season of data collection (since it was used to go down to the next line of data, each line corresponding to a plot number). For that reason, we started using a Tandy 200, which has better cursor keys. Dust in the computer was not a problem, but rain was. Both of those problems can be solved by enclosing the computer in a clear plastic bag and typing on the keys through the bag.

With the old system, it was easy to train new workers to enter data on paper. The new system required additional time to teach the operation of the Tandy 200. However, that was generally not a problem for us since we use the same 2 or 3 people to collect data all year. Another problem we noticed in one experiment is that it is difficult to collect data on a portable computer that is not in order. When data is being called out by many people for one person who is writing in several different places on the data sheet, it is easier to use pencil and paper. The experiment can usually be planned to avoid such confusion, however.

Finally, the communication program in the Tandy 200 does not add a line feed to the carriage return at the end of each line when transmitting to another computer. Therefore, when unloading data from the portable, it is necessary to have a communications program in the desktop computer that will add a line feed. The program, Red Ryder, for the Macintosh will do that.

Advantages. A number of advantages of the electronic data collection system were apparent after 2 seasons of field use. It is easier to use when working under windy or rainy conditions (which formerly made it difficult to handle paper data sheets). Once the data has been collected, a report can be generated within hours with little additional effort. That helps to cut labor costs, and makes it possible to base field selection in a breeding program on complete data (for example, summarized over replications and locations).

Figure 1. Procedures for data collection using paper data sheets, or electronic datasets on a portable microcomputer.

| Paper | Electronic |
|---|---|
| Write out data sheets on paper | Make up datasets on desktop computer and print on paper |
| Store data sheets for each experiment in binder | Store datasets on disk and put printout in a binder |
| Move data sheets for present experiment to a clipboard | Transfer datasets for present experiment to portable computer |
| Fill in the data sheet | Fill in the dataset |
| Photocopy the data sheets when finished | Transfer dataset to desktop computer |
| Give photocopy to data entry service | Store on disk and print on paper |
| Print data set when entered on computer | |
| Proof photocopy against computer printout | |
| Correct errors on computer dataset and print | |
| Write program for data analysis | Write program for data analysis |
| Analyze the data and print | Transfer data with program to mainframe computer for analysis |
| Write report | Transfer analysis summary to desktop computer and write report |

Figure 2. Example of a 40-column data sheet as it would appear on the Tandy 200 screen (as well as on the computer it was transferred to)[z].

```
Genotype x Environment Study
Summer, 1985
----------------------------------------
DATES
  Planted-
    Clayton:      7/12
    Clinton:      7/18
    Stress:       7/16
    Castle Hayne:7/17
  Harvested-
    Clayton:      8/28 and 9/11
    Clinton:      8/29 and 9/5
    Stress:       8/29 and 9/5
    Castle Hayne:8/30 and 9/5
----------------------------------------
  Identification                  Quality
------------------      Fruit No -------
      S C Plot R Ln St -------- S C S O
Yr Lc n r  No. p No Ct To Cl OS h o C P
-- -- - - ---- - -- -- -- -- -- - - - -
85 CA M 1 0353 1 19 30 36 03 05 5 5 3 6
85 CA M 1 0354 1 06 30 29 05 05 2 5 3 6
85 CA M 1 0355 1 01 30 30 03 05 3 8 6 8
85 CA M 1 0356 1 16 30 22 03 05 3 7 4 7

 etc

85 CH F 2 1056 2 01 30 08 04 00 8 8 6 7
----------------------------------------
```

[z]The identification columns were filled out when the experiment was planned. The dates and the remaining data columns were filled out during the data collection operation. Column headings are abbreviated to help the person collecting the data to remember what was needed. The headings are: Year, Location, Season, Crop, Plot Number, Replication, Line Number, Stand Count, Total, Cull and Oversized Fruit Numbers, and Shape, Color, Seedcell and Overall Performance Quality Score.

# Field and Detached-Fruit Tests 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. Research on resistance has shown quantitative differences among cucumber lines tested, with a narrow-sense heritability of 0.24 (3). Lines with netted fruit skin seem to be most resistant. Resistance in one study was controlled by a single dominant gene (1). It is not known whether the gene for resistance is the same as the single dominant gene, H, which controls netted fruit skin (2). The objective of this study was to evaluate the most resistant and susceptible lines found in previous studies using inoculated-field and detached-fruit tests. Also, we evaluated the effect of fruit skin removal and fruit firmness on resistance.

Methods. Two studies were run in 1985, a field test and a detached-fruit test. The field test was run at Clinton, N.C. The field was used for screening the previous 3 years, and no fungicides ever used. The soil is inoculated each year when the crop is at vine tip-over stage. A mixture of Rhizoctonia solani isolates growing on oat grains is used for inoculation. Oat grains (6400 grains/$m^2$) were spread over the soil to ensure a high disease incidence, and the crop was watered with overhead irrigation 3 times/week (30mm/week).

The isolates of R. solani (R8C and R8D, anastomosis group 4) used in this study were originally collected from cucumber fields in Arkansas. Inoculum was increased by transferring the fungus from stock cultures stored in paraffin oil in test tubes to potato dextrose agar. The final increase of inoculum took place on sterile oat grains as follows. Pieces of about 1 $cm^2$ of the colonized agar were transferred onto sterile oat grains in autoclavable bags, which were obtained by autoclaving 300 $cm^3$ of oat grains and 250 ml water 2 times for 1 hr with at least 10 hr between to ensure that all microorganisms were killed. After about 7 days at 20 to 25°C (shaking the bags every 2 days), when the oats were completely colonized by the fungus, they were dried and then stored at 4°C until needed.

The detached-fruit test was run using sterilized soil placed in flats in a mist chamber. The soil was inoculated (as described for the field test) on the day before fruits were harvested and placed on the soil. The fruits and soil were watered every few days as needed to keep the soil moist.

The field test was planted 29 April, and scored for disease on 5 and 11

Table 1. Resistance of 18 lines (out of 85 tested) to Rhizoctonia fruit rot in the field and detached-fruit (lab) tests run in 1985.[z]

| Cultivar or line | Seed source | Mean damage | | Max. damage | | Firm-ness |
|---|---|---|---|---|---|---|
| | | Field | Lab | Field | Lab | |
| Marketmore 76 | Asgrow Seed | 1 | 1 | 5 | 2 | 20 |
| Guardian | Northrup King | 1 | 2 | 6 | 4 | 20 |
| M 21 | NC State Univ | 2 | 1 | 7 | 2 | 20 |
| M 16 | NC State Univ | 1 | 3 | 6 | 5 | 24 |
| PI 63216-B2 | India | 2 | 5 | 8 | 7 | 22 |
| Wautoma | Wis.-USDA | 2 | 2 | 7 | 3 | 24 |
| Little Leaf | Univ. Arkansas | 2 | 5 | 6 | 10 | 16 |
| Calypso | Asgrow Seed | 2 | 4 | 8 | 7 | 20 |
| Castlemaster | Castle Seed | 2 | 2 | 7 | 4 | 19 |
| Poinsett 76 | PetoSeed | 2 | 2 | 9 | 5 | 20 |
| Carolina | Northrup King | 3 | 5 | 8 | 6 | 23 |
| Sumter | Asgrow Seed | 3 | 4 | 8 | 7 | 23 |
| SMR 18 | PetoSeed | 4 | 5 | 10 | 9 | 23 |
| Score | Asgrow Seed | 4 | 3 | 12 | 5 | 23 |
| WI 1701 | Wis.-USDA | 4 | 6 | 15 | 8 | 21 |
| WI 2757 | Wis.-USDA | 6 | 23 | 17 | 34 | 23 |
| PI 419108-5A | P. Rep. China | 11 | 30 | 24 | 50 | 14 |
| Supergreen | Harris-Moran | 10 | 34 | 26 | 35 | 20 |
| $\bar{x}$ | | 3 | 7 | 9 | 11 | 21 |
| LSD (5%) | | 3 | 11 | 9 | 16 | 4 |
| CV (%) | | 54 | 77 | 58 | 74 | 11 |

[z]Firnmess data were obtained by punching 2 fruits of size grade 3 in each replication and harvest in the field and lab tests. Fruits were chosen that were not touching the soil, and that were free of disease. A Magness-Taylor punch tester with a 5/16" diameter tip was used.

Table 2. Correlations among 85 lines for Rhizoctonia fruit rot in the field and detached-fruit (lab) tests run in 1985.

| Trait | % fruit damage | | Firm-ness |
|---|---|---|---|
| | Field | Lab | |
| % Fruit damage - Peeled[z] | 0.17* | 0.12 | -0.11 |
| - Field | - | 0.58** | -0.21** |
| - Lab | - | - | -0.33** |

[z]Two fruits in each plot were peeled and placed in contact with the soil 1 week before measuring damage.

July. The detached-fruit test was planted on 9 May and harvested on 12 and 19 July. On 1 July, the skin was peeled off 2 fruits in each plot where they touched the soil. One fruit in each treatment of the detached-fruit test was peeled at harvest before placing the fruits on the soil in flats.

The field and detached-fruit tests were run with 85 lines, consisting of the most resistant and most susceptible lines from previous tests, along with several cultivars of interest for production in the southern U.S.A. The field test used 3 replications, 2 harvests, and 6 fruits per line. The detached-fruit test used 1 replication, 2 harvests, and 3 fruits per line. Fruits were tested when they reached 50 mm diameter.

Results. The most resistance pickling cucumbers were NCSU M 16 and NCSU M 21 (Table 1). The most resistant slicing cucumbers were 'Marketmore 76' and 'Guardian'. PI 163216 selection B2 showed a useful level of resistance. However, this year, we were careful to allow for its later maturity, and it had more fruit damage as a result. Part of its resistance appears to be due to escape (delayed fruit setting). 'Sumter' and 'Supergreen' are useful as check lines for intermediate and susceptible levels, respectively.

The correlation between field and detached-fruit tests was 0.58, about the same as in 1984 (Table 2). The correlation was highly significant, but not high enough to substitute for the field test. The most useful application for the detached-fruit test is for testing lines that may have low yield, late maturity or low resistance to other prevalent diseases that make field tests difficult to run. In North Carolina, however, the spring field crop can be grown with few disease problems, and insects and nematodes can be controlled with standard cultural practices. Maturity problems have been reduced by using different planting dates.

Firmness was correlated with resistance (Table 2), but not to a very high degree ($r = 0.33$). Also, peeled fruits responded somewhat similarly to unpeeled fruits, but not to a high degree ($r = 0.17$). Thus, some progress will be made by selecting for firm fruits with a resistant skin. However, there are obviously lines that do not fit that pattern since the correlations are low in both cases.

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# Optimum Allocation of Plots into Years, Seasons, Locations and Replications for Once-Over Harvest Trials of Cucumber

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Many cucumber breeders test large numbers of lines before discarding them or releasing them as cultivars. Lines that perform well over a wide range of environmental conditions are useful to growers and seed companies, because such lines have a high probability of being useful in future (untested) years, and in many production areas. Since, it is possible to test lines in trials over years, seasons, locations, and replications, the question arises as to the most efficient way to distribute resources in the planning of trials. The question of optimum sample size has been recently reviewed (5).

Optimum sample size and distribution has been examined for nested designs (2), and for crossed designs with tobacco (1), cotton (3) and maize (4). Generally, it was concluded that additional locations and years were more efficient in providing cultivar performance data than additional replications within locations or years.

Data analysis. Cucumbers were grown in 2 years (1984, 1985), 3 seasons (spring, summer, fall), 4 North Carolina locations (Clayton, Clinton, Castle Hayne, and stress conditions of low fertilizer, irrigation and pest control), and 2 replications. Twenty-two genotypes in each of 2 crop types (pickle, slicer) were grown. The genotypes represented a diverse sample of available cultivars and lines (gynoecious vs. monoecious, dwarf vs. tall, indeterminate vs. determinate, disease resistant vs. susceptible, hybrid vs. inbred, adapted to southern vs. northern U.S.A., newly released vs. outdated).

Cucumbers were harvested once-over when the check plots ('Calypso' and 'Poinsett 76') had 10% oversized fruits. Data were collected on total fruit number, and average fruit quality (scored 1 to 9, where 1=poor, 5=average, 9=excellent).

The variance for a genotype mean can be expressed as in equation (A).

$$(A)\quad V_{\bar{x}} = \frac{\sigma^2_{GY}}{y} + \frac{\sigma^2_{GS}}{s} + \frac{\sigma^2_{GL}}{l} + \frac{\sigma^2_{GYS}}{ys} + \frac{\sigma^2_{GYL}}{yl} + \frac{\sigma^2_{GSL}}{sl} + \frac{\sigma^2_{GYSL}}{ysl} + \frac{\sigma^2_{e}}{rysl}$$

for data collected over y years, s seasons, l locations, r replications and g genotypes, with all factors viewed as random.

For each crop type, the variance components for total yield and average quality score were estimated (Table 1) using Type I estimates from the VARCOMP procedure of the Statistical Analysis System (SAS Institute, Cary, North Carolina).

Table 1. Estimates of variance components for effects of genotype and environment.

| Crop and Variable | Variance component | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $\sigma^2_{GY}$ | $\sigma^2_{GS}$ | $\sigma^2_{GL}$ | $\sigma^2_{GYS}$ | $\sigma^2_{GYL}$ | $\sigma^2_{GSL}$ | $\sigma^2_{GYSL}$ | $\sigma^2_{e}$ |
| Pickle | | | | | | | | |
| Yield | 17.13 | 0.25 | -2.60 | 11.92 | 3.07 | 8.20 | 15.58 | 67.64 |
| Quality | 0.020 | 0.035 | -0.015 | -0.013 | -0.045 | 0.061 | 0.033 | 0.821 |
| Slicer | | | | | | | | |
| Yield | 11.65 | 7.79 | 2.83 | 6.67 | 3.71 | 6.89 | 6.58 | 64.20 |
| Quality | 0.009 | 0.099 | -0.017 | -0.007 | -0.011 | 0.013 | 0.141 | 0.741 |

Estimated variances of genotype means were calculated for each of 5 designs (combinations of y, s, l and r), using equation (A) with the variance components replaced by their estimated values. The basic design took y=s=l=r=2. The other designs increased, in turn, the number of levels of one factor to 3, while holding the others at 2. We then compared the estimated variances of a genotype mean across designs in order to provide insight on the relative benefit (variance reduction) of increased allocation of resources in one direction or another.

Results. Comparing estimated variances of genotype means across the designs (Table 2) showed that the response was similar for pickling and slicing cucumber lines. The following conclusions can be made. If total yield is of principal interest, allocating resources in favor of more years will be most beneficial in reducing the variance of a genotype mean. Increasing the number of replications will be least effective. If increasing the number of years is impractical, increasing the number of seasons is second best, followed by locations. If average quality is considered instead, increasing number of seasons of data collection will produce the greatest benefits.

Considering yield and quality together for either pickling or slicing cucumbers, the best allocation of resources is to use tests over years and/or seasons, rather than locations or replications. For initial testing in a breeding program, it would be easiest to use a spring and summer (or fall) test to sample the effect of seasons. Tests over years would be better mainly for advanced lines, since additional years of testing in early stages will slow the progress in a breeding program.

Table 2. Estimated variances of genotype means for total yield and average quality score of pickles and slicers using 5 designs for performance trials[z].

| Model | | | | | Total yield | | Average quality | |
|---|---|---|---|---|---|---|---|---|
| Increased | r | l | s | y | Pickle | Slicer | Pickle | Slicer |
| Base | 2 | 2 | 2 | 2 | 19.4 | 20.3 | 0.076 | 0.108 |
| Reps | 3 | 2 | 2 | 2 | 18.0 | 19.0 | 0.059 | 0.093 |
| Locations | 2 | 3 | 2 | 2 | 16.8 | 17.3 | 0.059 | 0.090 |
| Seasons | 2 | 2 | 3 | 2 | 15.6 | 16.2 | 0.048 | 0.070 |
| Years | 2 | 2 | 2 | 3 | 13.2 | 15.9 | 0.059 | 0.087 |

[z]Variances estimated for trials run with r replications, l locations, s seasons and y years (using 2 of each for the base model).

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# Effect of Pot Size on Growth and Flowering of Cucumbers in the Greenhouse

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Cucumbers grow rapidly compared to most crop species, so that it is possible to get 3 to 5 generations per year in a breeding program using greenhouse facilities. Four generations per year is possible if the plants flower within 6 weeks of planting so that pollinations can be made in time to allow up to 6 weeks for the seeds to mature and up to 1 week to harvest and replant the greenhouse. It is often difficult to stay on a 13-week generation time due to the extra labor requirements at planting, pollination and harvest times.

Researchers with other crops have been successful in getting more generations per year by crowding the plants. Goulden (2) proposed the idea of using growth chambers to speed the growth of small grains by crowding. Grafius (3) applied the method by planting barley and oats in greenhouses using sand culture. Water was applied as needed, but fertilizer was given only 2 to 3 times. The plants remained small and produced only 0 to 4 (rarely 0) seeds per inflorescence. Many plants could be advanced in a small space in less time under this system. The system was modified by Brim (1) for his single-seed descent breeding method. Robbins and Pharr (4) grew cucumber plants in pots ranging from 0.4 to 5.9 l in volume to restrict root growth. Water and nutrients were provided to prevent stress, and shoots were given unlimited growth space in the greenhouse. However, the shoots were proportionally smaller on plants with restricted root size (root/shoot ratio remained constant).

Peterson (personal communication) found that seeds could be harvested 4 weeks after pollination, cleaned with high pressure water, dried for 1 day, soaked in acetone for 1 day, incubated at 32°C in petri plates containing moist vermiculite, and transplanted to pots 1 day later. That method saves 2 weeks on post-pollination seed development time, and 1 week on fermentation, drying and packeting time. Thus, with gynoecious plants, it is possible to go from seed to seed in 10 weeks for 5 generations per year.

In working with genotypes that grow fairly large, and are not as rapid to flower and set seed, it would be desirable to reduce further the generation time. It is possible that if cucumber plants were crowded into a small space they would grow smaller, flower faster, and set seeds sooner than normal. The objective of this study was to determine whether growing plants in small pots could reduce the generation time of cucumbers in the greenhouse.

Methods. The experiment was a randomized complete block with 2 cultivars, a gynoecious inbred pickle (Gy 14A) and a monoecious inbred slicer (Poinsett 76). Plants were grown in one of 4 pot sizes (10, 13, 15, or 20 cm diameter), and fruits were either held on the plants without watering 2, 4, or 6 weeks after pollination (summer, 1983), or harvested 2, 4, or 6 weeks after pollination (spring, 1984).

Data were collected at fruit harvest time. The vine length, fruit weight, number of seeds per fruit, weight per seed, and percentage of germination at 22°C were measured. Also, the number of days from planting to first pistillate flower was recorded.

Results. Plants grown in large pots had longer vines and heavier fruits than those grown in small pots (Tables 1 and 2). The larger fruits had more seeds, but the weight per seed did not change. Days to flower and percentage of germination of the harvested seeds did not change with pot size, except that no seeds were produced from fruits harvested 2 weeks after pollination. Although 'Poinsett 76' flowered as soon as Gy 14A, the flowers were staminate for the first 5 to 10 days (data not shown). Thus, for monoecious lines, self-pollincations cannot be made until 41 to 49 days after planting (Tables 1 and 2). The extra 1 to 2 weeks time for pollination of monoecious lines would make it possible to get 4 generations a year, but not 5 (as is possible for gynoecious lines).

Thus, it is possible to change the size of the plant and the number of seeds per fruit by using larger pot sizes. However, plants grown in small pots do not flower or reach mature seed stage sooner than those grown in large pots. Although there were occasional problems of small seeds and poor germination of seeds from plants grown in 10 cm diameter pots, it was possible to get sufficient seed numbers from plants grown in 13 to 20 cm diameter pots. Two weeks after pollination, watering could be stopped and seeds would still develop properly (Table 1). However, fruits harvested 2 weeks after pollination did not have viable seeds (Table 2). It required 4 weeks for seeds to develop sufficiently for good germination.

Plants grown in 13 cm pots with fruits harvested 4 weeks after pollination provided 90 to 296 seeds per fruit that were plump and germinated well. That appeared to be the minimum satisfactory system of the treatment combinations tested.

## Literature Cited


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2. Goulden, C. H. 1939. Problems in plant selection. p. 132-133. In: R. C. Punnett (ed.), Proc. 7th Internat. Genet. Cong. Cambridge Univ. Press, London.

3. Grafius, J E. 1965. Short cuts in plant breeding. Crop Sci. 5: 377.

4. Robbins, N. S. and D. M. Pharr. 1985. Effect of restricted roots on shoot growth and carbohydrate metabolism of Cucumis sativus L. leaves. HortScience 20(3): 536 (abstr.).

Table 1. Effect of pot size and weeks to end of irrigation on plant and fruit growth for summer, 1983 greenhouse crop[z].

| Cultivar | Weeks of water[y] | Pot dia. (cm) | Vine length (cm) | Fruit weight (g) | Seed no. | Weight per seed (mg) | % germination | Days to 1st flower |
|---|---|---|---|---|---|---|---|---|
| Gy 14 A | 2 | 10 | 102 | 25 | 74 | 22 | 100 | 37 |
| | | 13 | 136 | 23 | 52 | 27 | 100 | 38 |
| | | 15 | 180 | 35 | 61 | 21 | 100 | 38 |
| | | 20 | 308 | 103 | 213 | 28 | 100 | 37 |
| | 4 | 10 | 132 | 37 | 82 | 16 | 98 | 38 |
| | | 13 | 135 | 28 | 90 | 21 | 98 | 40 |
| | | 15 | 206 | 65 | 60 | 27 | 100 | 38 |
| | | 20 | 290 | 50 | 67 | 26 | 100 | 41 |
| | 6 | 10 | 75 | 20 | 45 | 26 | 95 | 37 |
| | | 13 | 126 | 40 | 61 | 25 | 98 | 37 |
| | | 15 | 236 | 68 | 197 | 18 | 100 | 38 |
| | | 20 | 390 | 95 | 219 | 28 | 100 | 38 |
| Poinsett 76 | 2 | 10 | 176 | 16 | 39 | 25 | 100 | 49 |
| | | 13 | 192 | 40 | 125 | 23 | 100 | 49 |
| | | 15 | 264 | 53 | 179 | 25 | 100 | 43 |
| | | 20 | 306 | 75 | 172 | 27 | 100 | 49 |
| | 4 | 10 | 108 | 28 | 99 | 16 | 95 | 49 |
| | | 13 | 157 | 48 | 142 | 21 | 100 | 49 |
| | | 15 | 323 | 65 | 102 | 27 | 100 | 47 |
| | | 20 | 300 | 120 | 118 | 27 | 100 | 49 |
| | 6 | 10 | 204 | 45 | 87 | 23 | 100 | 48 |
| | | 13 | 220 | 40 | 149 | 25 | 100 | 43 |
| | | 15 | 347 | 117 | 202 | 28 | 100 | 43 |
| | | 20 | 451 | 118 | 210 | 24 | 100 | 49 |
| LSD (5%) | | | 86 | 31 | 109 | 10 | 4 | 3 |
| CV (%) | | | 19 | 27 | 55 | 20 | 2 | 5 |

[z]Data are means of 2 replications.

[y]Irrigation of pots stopped 2,4, or 6 weeks after pollination.

Table 2. Effect of pot size and weeks to harvest on plant and fruit growth for summer, 1984 greenhouse crop[z].

| Cultivar | Weeks to harvest | Pot dia. (cm) | Vine length (cm) | Fruit weight (g) | Seed no. | Weight per seed (mg | % germination | Days to 1st flower |
|---|---|---|---|---|---|---|---|---|
| Gy 14 A | 2 | 10 | 74 | 201 | 0 | 0 | 0 | 37 |
| | | 13 | 102 | 411 | 0 | 0 | 0 | 29 |
| | | 15 | 107 | 347 | 0 | 0 | 0 | 35 |
| | | 20 | 108 | 333 | 0 | 0 | 0 | 33 |
| | 4 | 10 | 87 | 350 | 160 | 26 | 100 | 36 |
| | | 13 | 92 | 496 | 228 | 26 | 100 | 36 |
| | | 15 | 110 | 567 | 251 | 28 | 100 | 35 |
| | | 20 | 126 | 522 | 231 | 32 | 100 | 36 |
| | 6 | 10 | 91 | 354 | 189 | 22 | 88 | 35 |
| | | 13 | 100 | 507 | 225 | 26 | 100 | 35 |
| | | 15 | 107 | 595 | 221 | 29 | 93 | 34 |
| | | 20 | 141 | 624 | 218 | 29 | 100 | 36 |
| Poinsett 76 | 2 | 10 | 158 | 170 | 87 | 12 | 50 | 46 |
| | | 13 | 144 | 291 | 0 | 0 | 0 | 45 |
| | | 15 | 172 | 418 | 0 | 0 | 0 | 43 |
| | | 20 | 218 | 435 | 0 | 0 | 0 | 45 |
| | 4 | 10 | 122 | 366 | 234 | 14 | 45 | 46 |
| | | 13 | 164 | 534 | 296 | 25 | 100 | 45 |
| | | 15 | 182 | 532 | 197 | 25 | 90 | 44 |
| | | 20 | 195 | 665 | 230 | 28 | 100 | 45 |
| | 6 | 10 | 176 | 439 | 277 | 18 | 85 | 45 |
| | | 13 | 176 | 631 | 260 | 27 | 100 | 42 |
| | | 15 | 193 | 727 | 235 | 29 | 100 | 41 |
| | | 20 | 154 | 641 | 311 | 26 | 98 | 45 |
| LSD (5%) | | | 36 | 150 | 143 | 5 | 43 | 4 |
| CV (%) | | | 13 | 16 | 45 | 10 | 34 | 6 |

[z]Data are means of 2 replications.

# Fertilizer Effects in a Seedling Test for Gynoecious Expression

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Seedling tests for gynoecious expression are useful for checking the degree of gynoecy of inbred lines being developed in a breeding program for use in the production of gynoecious hybrids. The test is usually run with a high degree of plant stress to determine how stable the gynoecious expression is. We have generally grown plants in flats in crowded conditions to stress the plants and to save space in the greenhouse. However, recently a test run where no fertilizer was added to the soil demonstrated that there is an optimum degree of stress in the test, at least under North Carolina greenhouse conditions (high temperature and light).

The objective of this experiment was to determine the effect of fertilizer on gynoecious expression of gynoecious and monoecious lines in a crowded-flat seedling test.

Methods. Two lines (Gy 14A and SMR 18) were tested in 57 x 57 mm square peat pots (in flats) containing a mix of sand, soil and peat in equal volumes. Two seeds were planted (8 November, 1985) in each peat pot and thinned to 1 seedling at cotyledon stage. The plants were given one of 4 fertilizer treatments: none, starter fertilizer mixed in with the soil, starter plus 1 liquid fertilizer treatment added at first true leaf stage, or starter plus liquid fertilizer in all waterings (approximately 4 per week).

Fertilizer-line combinations were replicated 1 time with 24 plants per treatment unit. Data were collected at full flowering stage (12 December, 1985), 34 days after planting. Number of pistillate and staminate nodes were counted in each treatment unit.

Results. The percentage of pistillate nodes on Gy 14A increased from 0 to 70 as more fertilizer was added to the soil medium (Table 1). All treatments where fertilizer was added provided a good test, but starter fertilizer was necessary to have pistillate flowers develop. The greatest difference between Gy 14A (a gynoecious inbred) and SMR 18 (a monoecious inbred) occurred at the highest amount of fertilizer added. When no fertilizer was added, the gynoecious and monoecious lines could not be distinguished. Also, many plants died before flowering in the treatments where no fertilizer was added.

Therefore, the level of stress should not be too high if the test is to work properly. Light, fertilizer and water should be provided in optimum amounts, with stress provided by crowding of plants into a small root volume. Additional benefits of crowding are the resulting saving of space in the greenhouse.

Table 1. Number (percentage) of pistillate and staminate nodes on plants of Gy 14A and SMR 18 grown in 57 x 57 mm square peat pots.

| Fertilizer[y] | Number of pistillate nodes | | Number of staminate nodes | |
|---|---|---|---|---|
| | Gy 14A | SMR 18 | Gy 14A | SMR 18 |
| None | 0 (0) | 0 (0) | 34 (100) | 8 (100) |
| Starter | 72 (65) | 0 (0) | 38 (35) | 80 (100) |
| Starter + Once | 43 (36) | 0 (0) | 76 (64) | 111 (100) |
| Starter + Every | 78 (70) | 0 (0) | 33 (30) | 58 (100) |

[z]Data were collected on 24 plants per treatment combination.

[y]Fertilizer added as complete starter fertilizer and liquid fertilizer in the irrigation water (at first leaf stage, or in every watering).

# Performance of Cultivars of Four Different Cucumber Types for Fresh-Market use in North Carolina

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Several different fruit types of cucumbers are used commercially for fresh-market consumption throughout the world. The major types used for field production are American slicers, Middle-Eastern beit alpha slicers and Japanese trellis slicers. Lately, American pickling cucumbers have been used for fresh consumption as consumers have discovered their thin skin, small seeds, and convenient size for use for today's small families.

Little has been written about the different fruit types of cucumber to define their characteristics or performance (1). The objective of this study was to evaluate the performance and determine the fruit characteristics of fresh-market cucumber types that have potential use in North Carolina.

Methods. A trial was run in 1985 to measure yield, quality and fruit characteristics of 10 cultivars of 4 different fruit types of cucumbers. The cultivars were planted in 3 m rows 1.5 m apart. The experiment design was a randomized complete block with 3 replications. The trial was planted 25 April and harvested 6 times (twice weekly) between 10 and 25 June. Standard cultural practices were used to control weeds, diseases and insects. Fruits were sorted into marketable and cull grades based on U.S. Department of Agriculture standards (but modified to allow for different fruit diameter).

Results. The major difference among the types was in fruit length, skin color and wartiness (Table 1). The beit alpha slicers were generally lightest in color, and the American slicers generally the darkest. The Japanese trellis cucumbers had large warts which tended to form ridges down the fruit surface. There were differences in yield and quality among cultivars within each of the fruit types. The beit alpha types were generally the highest yielding (Table 2).

There were also differences among the 4 cucumber fruit types for length:diameter ratio (LD). LD ratio was smallest for the pickles: 3.0; intermediate for the beit alpha and American slicers: 4.0; and longest for the Japanese trellis slicers: 6.0 (Table 2).

All types are suited for fresh-market production of cucumbers in the field, although consumers would have to be introduced to the beit alpha and Japanese trellis fruit types in the U.S. The Japanese trellis types would have fewer culls if grown on a trellis, since they tend to curve when grown on the ground.

## Literature Cited

1. Anonymous. 1984. Modern cucumber technology. Asgrow Seed Co. Kalamazoo, Michigan.

Table 1. General fruit characteristics of 4 cucumber types for fresh-market production.

| Fruit type | Shade of green | Color uniformity | Fruit skin | | Fruit length |
|---|---|---|---|---|---|
| | | | Thickness | Surface | |
| American pickle | Lt.-Med. | Speckled | Medium | Warts | Short |
| Beit alpha | Light | Uniform | Thin | Hairs | Medium |
| American slicer | Dark | Uniform | Thick | Warts | Medium |
| Japanese trellis | Medium | Uniform | Thin | Ridges | Long |

Table 2. Yield and fruit quality[z] of 10 cultivars from 4 different fruit types of cucumbers.[y]

| Cultivar or line | Seed source | Marketable yield (Mg/ha) | Shape | Color | Seed-cell | Length (cm) | Length: diameter ratio |
|---|---|---|---|---|---|---|---|
| **American Pickling** | | | | | | | |
| Sumter | Asgrow Seed | 34 | 8.0 | 5.0 | 6.7 | 14 | 2.8 |
| Regal | Harris-Moran | 74 | 6.7 | 7.3 | 7.0 | 16 | 3.1 |
| **Middle-Eastern Beit Alpha** | | | | | | | |
| Amra | Nickerson | 84 | 5.7 | 5.0 | 7.0 | 18 | 3.4 |
| Lama | Asgrow Seed | 76 | 6.0 | 6.3 | 4.0 | 18 | 3.4 |
| Celebrity | Ferry-Morse | 81 | 4.7 | 5.0 | 5.0 | 22 | 4.1 |
| **American Slicing** | | | | | | | |
| Sprint 440 | Asgrow Seed | 70 | 7.0 | 8.3 | 7.0 | 19 | 3.6 |
| Dasher II | PetoSeed | 44 | 8.0 | 8.0 | 7.3 | 20 | 4.0 |
| **Japanese Trellis** | | | | | | | |
| Tasty Time | Sakata Seed | 73 | 5.7 | 7.3 | 6.0 | 26 | 5.2 |
| Tasty Gift | Sakata Seed | 64 | 5.7 | 7.7 | 8.0 | 32 | 6.8 |
| Tasty Gem | Sakata Seed | 28 | 7.0 | 7.3 | 9.0 | 29 | 6.7 |
| LSD (5%) | | 17 | 2.0 | 1.5 | 2.1 | 4 | 1.0 |
| CV (%) | | 20 | 21 | 15 | 26 | 13 | 16 |

[z]Quality scored 1 to 9 (Shape: 1=pointed, crooked; 5=tapered, curved; 9=blocky, straight. Seedcell: 1=extra large, 5=medium, 9=extra small. Color: 1=white, 5=medium green, 9=very dark green.)

[y]Data are means over 3 replications and 6 harvests.

# Further Results of Linkage Studies in Cucumis sativus L.

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Following earlier research (1), linkage studies for 5 pairs of genes have been continued at the Institute for Horticultural Plant Breeding (IVT). Involved were the marker genes short petiole (sp), little leaf (ll), glabrous (gl), compact (cp), umbrella leaf (ul) and long hypocotyl (lh). The mutants are designated as in the list in the 1985 CGC report.

The 3 pairs sp/ll, gl/ll and sp/cp segregated independently (Table 1). Although the number of $F_2$ plants was small for the pair sp/ll the chi-square values were low for both the monogenic segregation and the digenic segregation (linkage test). The $F_2$ population from the pair gl/ll had too many individuals of the gl genotype but the $X_3^2$ in the test of independence was satisfactorily low. The $X_3^2$ in the combination sp and cp indicated a very good fit to independent segregation.

A weak linkage was found between the genes sp and ul. The recombination percentage was calculated as 36%. However, this result should be considered with caution, because during the winter season, when the test was done, it is hard to distinguish between normal and umbrella leaf (ul). There were too many plants of the ul genotype in the $F_2$ population. A strong linkage was calculated for the gene pair sp/lh. The recombination percentage was 18%.

It is remarkable that, of all the gene loci studied, only a few are in the same linkage group. The localization of the genes is to be continued.

## Literature Cited

1. Nijs, A.P.M. den and I.W. Boukema. 1983. Results of linkage studies and the need for a cooperative effort to map the cucumber genome. Cucurbit Genetics Coop. Rpt. 6: 22-23.

Table 1. Segregation and size (N) of $F_2$ populations for linkage test, recombination percentage and calculated chi-square ratio for the 9:3:3:1 ratio ($X_3^2$) for 5 pairs of genes.

| Gene pair | Segregation ++ | +- | -+ | -- | N | % | $X_3^2$ |
|---|---|---|---|---|---|---|---|
| sp/ll | 73 | 23 | 16 | 12 | 124 | 50 | 2.95* |
| gl/ll | 79 | 25 | 49 | 9 | 162 | 50 | 1.16* |
| sp/cp | 145 | 38 | 56 | 18 | 257 | 50 | 0.45* |
| sp/ul | 97 | 81 | 47 | 14 | 239 | 36 | 8.73 |
| sp/lh | 131 | 54 | 47 | 13 | 245 | 18 | 69.24 |

* Significant at P = 0.05

RESEARCH NOTES

II. Muskmelon

# Screening for Muskmelon Resistance to a Pathogenic Strain of *Myrothecium roridum* Using Detached Leaves

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Introduction: *Myrothecium roridum* Tode ex Fries can be a serious pathogen to muskmelon (*Cucumis melo* L.) (1,3). Genetic resistance to *M. roridum* can be assessed using seed germination and seedling growth tests (2); however, these tests are labor intensive and are influenced by seed quality. The purpose of the current study was to determine whether inoculation of leaves detached from field and greenhouse plants could be used as a screening procedure for determining resistance.

Materials and Methods: Twelve muskmelon genotypes (Table 1) were grown in the greenhouse and in the field. Leaves approximately 9 cm in diameter were excised at either the third or fourth node on lateral branches of mature plants and placed in 9 cm petri dishes lined with filter paper moistened with 2 ml distilled water prior to inoculation.

A spore suspension ($10^6$ spores/ml) was prepared of a pathogenic strain of *Myrothecium roridum* (ATCC#52485). Droplets (25 $\mu$l) of the spore suspension were applied to 2 interveinal areas on detached leaves and covered with moistened filter paper discs (12 mm) for 24 hr. Controls were treated with sterile distilled water. Detached leaves were incubated under normal laboratory conditions for a total of 7 days, then measured for diameter of necrosis plus associated chlorosis at each inoculated site. A lesion index was calculated by dividing lesion diameter by 12 mm. Lesion sporulation was measured by excising diseased leaf tissue, mincing it in a vial containing 2 ml sterile water, and counting spores in the resultant solution with a hemacytometer. There were 4 replications per experiment and each experiment was repeated.

Results and Discussion: In all inoculated leaves, a susceptible reaction was characterized by necrotic lesions surrounded by chlorotic areas at the point of inoculation. There were significant differences among genotypes for lesion size and sporulation for both greenhouse and field grown leaves (Table 1). Controls for each genotype did not exhibit chlorosis or necrosis (data not shown).

In general, genotype rankings from inoculated greenhouse material were similar to those from field material, indicating the consistency of the detached leaf assay. Four of the genotypes ('Amarelo', 'Hale's Best', 'Iroquois' and 'PMR 45') were also used in the seed germination and seedling growth study (2) and exhibited the same relative performance. As the detached leaf test is less labor intensive and allows for easier quantification of results, we are using it to study the inheritance of resistance to *M. roridum* in muskmelon.

## Literature Cited


1. Bruton, B.D. 1982. Myrothecium roridum, a probable devastating pathogen of muskmelon in south Texas. Phytopath. 72:355. (Abstr.)

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3. McLean, D.M. and B. Sleeth. 1961. Myrothecium rind rot of cantaloupes. Plant Dis. Rept. 45:728-729.


Table 1. Lesion size and sporulation on detached leaves of 12 muskmelon genotypes inoculated with Myrothecium roridum.

| Genotype | Lesion index[z] | | Sporulation[y] | |
|---|---|---|---|---|
| | Greenhouse | Field | Greenhouse | Field |
| Amarelo | 3.3 | 2.9 | 4.65 | 4.10 |
| Edisto 47 | 4.7 | 4.4 | 3.16 | 2.85 |
| Hale's Best | 1.9 | 2.2 | 2.82 | 2.79 |
| Imperial 4-50 | 3.9 | 3.8 | 2.93 | 2.25 |
| Iroquois | 7.8 | 6.7 | 5.09 | 4.71 |
| MD63-53 | 7.3 | 6.9 | 5.98 | 4.42 |
| MD80-2 | 6.7 | 6.0 | 4.90 | 4.95 |
| Ogen | 2.2 | 2.7 | 2.69 | 2.30 |
| Perlita | 4.0 | 3.6 | 2.57 | 2.40 |
| PMR 6 | 3.0 | 3.4 | 3.25 | 2.85 |
| PMR 45 | 2.9 | 2.3 | 2.98 | 2.72 |
| Tam Uvalde | 3.6 | 3.5 | 2.73 | 2.94 |
| lsd(.05) | 0.5 | 0.6 | 1.35 | 0.97 |

[z]Lesion index calculated by dividing lesion diameter by 12 mm.

[y]$Log_{10}$ of spores/ml extracted from diseased tissue.

# Germplasm Resources of Cucumis melo L. from Spain

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A project designed for collecting several vegetable crop species germplasm in Spain was undertaken during 1984 and 1985. The project was partially sponsored by I.B.P.G.R./F.A.O. Cucumis melo L. specie was enclosed in this project because it is one of the most important crops in Spain. In addition to many other crops, 180 accessions were collected from different sampling areas: Valencia, Andalucía, Cataluña, Extremadura, Castilla-La Mancha and Aragón. Samples of all of the accessions have been sent to the National Seed Storage Laboratory, U.S D.A. of Fort Collins, U.S.A.

We have grouped the accessions according to several fruit characteristics.

Table 1. Accessions collected assembled in four groups (A,B,C and D). The abbreviations for the observations are: D= Dry farming; S= Big size; SW=sweet flavour; E= "Escrito"(1). R= Ribbed fruits; N= Netted fruits; C= cracked fruits

Group A.- Predominant skin colour at maturity: Green or dark green. Secondary skin colour: white or yellow. Design produced by secondary skin colour: speckled or spotted. Fruit skin texture: longitudinally wrinkled, except E-C-8. Flesh colour: white, except V-C-56, AN-C-73 and A-C-4 which have green flesh colour.

| Label | Local name | Observations | Label | Local name | Observations |
|---|---|---|---|---|---|
| V-C-2 | De todo el año | E | V-C-96 | Meló blanc | D |
| V-C-4 | Tendral | - | V-C-98 | Pinyonet | D |
| V-C-6 | De piñoncillo | - | V-C-100 | Piel de Sapo | - |
| V-C-19 | Meló Groguet | D | AN-C-3 | De tajadas señaladas | D,S,R |
| V-C-26 | De todo el año | - | AN-C-15 | De secano | E |
| V-C-29 | - | S | AN-C-73 | Amarillo Blanco Piñón | D,E |
| V-C-32 | Pinyonet | E,S | C-C-7 | Piel de Sapo | - |
| V-C-33 | Piel de Sapo | - | C-C-8 | Pinyonet | - |
| V-C-56 | Piel de Sapo | - | C-C-11 | - | - |
| V-C-67 | - | SW,N | E-C-8 | Punteado amarillo | - |
| V-C-73 | Tendral | C | E-C-15 | Verdejo | D,N |
| V-V-81 | - | S | A-C-4 | - | E |
| V-C-82 | Tendral | E | A-C-9 | Piel de Sapo | E |
| V-C-92 | Pinyonet | E | A-C-17 | De común | E |
| V-C-95 | - | E | CM-C-4 | - | D,E |

(1): In Spain the word "escrito" is used to describe the set of lines or marks that generally appear on the fruit skin and which look like letters or features made by a pen or some other very thin cutting object.

Group B.- Predominant skin colour at maturity: pale green, green or dark green, with no secondary skin colour. Fruit skin texture: longitudinally wrinkled (superficial, intermediate or deep) except AN-C-2 and CM-C-3. Flesh texture: firm. Flesh colour: white, except V-C-17, AN-C-14, E-C-1 and CM-C-2,which have green flesh colour, and C-C-12, which has salmon flesh colour.

| Label | Local name | Observations | Label | Local name | Observations |
|---|---|---|---|---|---|
| V-C-11 | Tendral | - | AN-C-10 | Tendral | D |
| V-C-12 | Largo | - | AN-C-11 | Del país | D |
| V-C-17 | - | E | AN-C-14 | Lucena | D |
| V-C-22 | Malacara | D,S | AN-C-20 | De invierno | - |
| V-C-36 | Tendral | S | AN-C-25 | Escrito | C |
| V-C-37 | Tendral | - | AN-C-28 | Escrito | S,N |
| V-C-38 | Tendral | - | AN-C-48 | Gran Alejandro | D,E |
| V-C-39 | Tendral | - | AN-C-50 | Bolero | - |
| V-C-40 | Tendral | - | AN-C-58 | Rochet | D,E |
| V-C-43 | De tot l'any | S | AN-C-60 | - | - |
| V-C-44 | Cabrasado | R,N | AN-C-76 | Redondillo de la Cañada | D,E |
| V-C-46 | Tendral | - | C-C-3 | De agua | E |
| V-C-50 | - | E | C-C-6 | Tendral | - |
| V-C-52 | Casero | - | C-C-12 | - | S |
| V-C-57 | Tendral | - | C-C-13 | Mollerusa | D,E |
| V-C-66 | - | - | C-C-18 | Mollerusa | D,E |
| V-C-72 | - | - | E-C-1 | De Calamonte | E |
| V-C-76 | Tendral | S | E-C-7 | Verde de Holguera | D |
| V-C-84 | De la Torre | - | E-C-16 | Manto del Señor | D |
| V-C-90 | - | D,E | E-C-28 | Melona verdosa | D,S,SW,E,N,C |
| AN-C-2 | De olor antiguo | D,C | CM-C-2 | - | D,SW |
| AN-C-4 | De olor | D,R | CM-C-3 | - | - |
| AN-C-7 | Verde gordo | D | A-C-16 | De común | E |
| AN-C-8 | Tendral negro | | | | |

Group C.- Predominant fruit skin colour at maturity: yellow; with no secondary skin colour, except: AN-C-24, E-C-3, E-C-6, E-C-19, E-C-32 which have green spots. Fruit skin texture: longitudinally wrinkled (superficial, intermediate or deep), except for V-C-54, AN-C-6, AN-C-29, AN-C-33, AN-C-77, E-C-14, E-C-19 and E-C-29. Flesh texture: firm, except V-C-71, AN-C-6, AN-C,29 and AN-C-77. Flesh colour: white, except V-C-54, AN-C-1, AN-C-33, AN-C-34 which have green flesh colour and V-C-42, V-C-101, E-C-10 which have salmon flesh colour.

| Label | Local name | Observations | Label | Local name | Observations |
|---|---|---|---|---|---|
| V-C-23 | Tendral | D | AN-C-56 | Amarillo | D |
| V-C-30 | De exportación | E | AN-C-69 | Blanco | S,SP,N |
| V-C-34 | Amarillo | - | AN-C-70 | Blanco | - |
| V-C-41 | Alficós | - | AN-C-71 | Blanco | - |
| V-C-42 | Alficós | - | AN-C-77 | Loperano | D,E |
| V-C-54 | - | SW | E-C-3 | Hilo Carrete | D,N |
| V-C-71 | Amarillo Oro | - | E-C-4 | Amarillo Largo | D |
| V-C-80 | Blanco | E | E-C-5 | Redondo piel lisa | D,E,C |
| V-C-83 | Amarillo Oro | D,E | E-C-6 | Fondo amarillo | D |
| V-C-101 | Acordonado | E | E-C-10 | Amarillo | S |
| AN-C-1 | Blanco | D | E-C-12 | De Badajoz | E,C |
| AN-C-6 | Loperos | D,S | E-C-13 | Perita | D |
| AN-C-24* | De olor | S | E-C-14 | Amarillo Alargado | D,E,C |

| Label | Local name | Observations | Label | Local name | Observations |
|---|---|---|---|---|---|
| AN-C-29 | Ardales | D | E-C-17 | Melona amarilla | D,C |
| AN-C-32 | Ardales | D | E-C-19 | Cosío | D |
| AN-C-33 | Ardales | D,E,N | E-C-29 | Melona amarilla | D,SW,E |
| AN-C-34 | Ardales | D | E-C-32 | Fondo verdoso | D |
| AN-C-53 | Amarillo | E | | | |

(*): AN-C-24, without Yellow-virus syntoms.

Group D.- Predominant fruit skin colour at maturity: white, with no secondary skin colour except AN-C-5 and AN-C-49, which have spots. Fruit skin texture:- longitudinally wrinkled except E-C-30. Flesh colour: white.

| Label | Local name | Observations | Label | Local name | Observations |
|---|---|---|---|---|---|
| V-C-21 | Blanquet | D | AN-C-49 | Ardales | D,C |
| V-C-35 | - | S | AN-C-68 | Blanco liso | D,E,R,C |
| AN-C-5 | Castellanos | D,C | AN-C-72 | Blanco | N,R,C |
| AN-C-19 | Blanco grueso | D,E | E-C-2 | Blanco liso | D,E,R,C |
| AN-C-31 | Ardales | D | E-C-30 | Melona blanca | D,E,R |

Other accessions.- They have not been characterized yet. The abbreviations for the observations are: SH= short cycle; L= Long cycle; EC= Early crop; LC= Late crop.

| Label | Local name | Observations | Label | Local name | Observations |
|---|---|---|---|---|---|
| V-C-28 | Amarillo | - | AN-C-44 | Ardales | D |
| V-C-31 | Alficós | "flexuosus"v. | AN-C-45 | Ardales | D |
| V-C-45 | De tot l'any | - | AN-C-46 | Ardales | D |
| V-C-47 | - | sweet | AN-C-47 | Ardales | D |
| V-C-51 | Alficós | "flexuosus"v. | AN-C-55 | Escrito | D |
| V-C-53 | Alficós | ",SH | AN-C-59 | De Tarizna | LC |
| V-C-62 | - | - | AN-C-63 | Escrito | LC |
| V-C-63 | - | - | AN-C-74 | Capitán | D |
| V-C-64 | - | - | AN-C-75 | Segovia | D,EC |
| V-C-68 | - | - | AN-C-78 | Amarillo Onteniente | D |
| V-C-74 | - | - | C-C-2 | - | - |
| V-C-75 | - | - | C-C-15 | Morellusa | D,LC |
| V-C-79 | Calvet | L | C-C-16 | Morellusa | D,LC |
| V-C-87 | - | - | C-C-17 | Morellusa | D,LC |
| V-C-97 | Franceset | D | C-C-19 | Morellusa | D,LC |
| V-C-102 | Calvet | - | E-C-9 | Verde | L |
| V-C-103 | - | - | E-C-11 | Amarillo | - |
| AN-C-12 | Rider de Lucena | D | E-C-18 | Amarillo | D |
| AN-C-13 | Lucena | D | E-C-20 | Madura | D |
| AN-C-16 | - | LC | E-C-21 | Madura amarilla | D |
| AN-C-22 | Escrito | - | E-C-22 | Verdoso | D |
| AN-C-30 | Ardales | D | E-C-23 | Maduro negro | D |
| AN-C-35 | Ardales | D | E-C-24 | Culo de estrella | D |
| AN-C-36 | Ardales | D | E-C-25 | - | D |
| AN-C-37 | Ardales | D | A-C-11 | - | - |
| AN-C-38 | Ardales | D | A-C-13 | - | - |
| AN-C-39 | Ardales | D | A-C-14 | - | L |
| AN-C-41 | Ardales | D | CM-C-8 | - | - |
| AN-C-43 | Ardales | D | | | |

| Label | Local name | Observations |
|---|---|---|
| AN-C-42 | Ardales | D |

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# Attempt at Localization of male sterile-4, cut leaf and virescent Mutants in Muskmelon

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We have tried to find some linkages between male sterile-4 (ms-4) supplied by P. LOZANOV (Bulgaria) (2), cut leaf (cl) supplied by I. VELICH (Hungary) (5) or virescent (v) supplied by P.E. NUGENT (South Carolina, USA) (1) with other genes belonging to linkage groups already described (3, 4).

Male sterile-4 (ms-4) was found independent (Table 1) from genes belonging to linkage groups 1 to 7. Cut leaf (cl) was found independent (Table 2) from genes belonging to groups 1 to 7 and from ms-4. Virescent (v) has already been found independent from groups 2, 3 and 6 (4). We found it independent from genes belonging to groups 1, 4 and 7 and from ms-4 and cl (Table 3). It must be still tested with Fom-1 or Prv (synonym Wmv) from group 5.

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Table 1. Segregation data observed in F2 progenies between male sterile -4 (ms-4) and si-1, Vat, gl, r, Zym, Fom-1, yg and nsv.

| Linkage group | Genes | [Male fertile] ms-4$^+$/- | [Male sterile] ms-4/ms-4 | chi square (9:3:3:1) Value | Probability |
|---|---|---|---|---|---|
| 1 | si-1$^+$/- | 121 | 31 | | |
| | si-1/si-1 | 41 | 7 | 4.516 | 21 % |
| 2 | Vat/- | 175 | 61 | | |
| | Vat$^+$/Vat$^+$ | 55 | 12 | 3.030 | 39 % |
| 3 | gl$^+$/- | 244 | 75 | | |
| | gl/gl | 71 | 26 | 1.171 | 76 % |
| 3 | r$^+$/- | 125 | 45 | | |
| | r/r | 33 | 13 | 2.008 | 57 % |
| 4 | Zym/- | 111 | 43 | | |
| | Zym$^+$/Zym$^+$ | 27 | 18 | 6.213 | 11 % |
| 5 | Fom-1/- | 154 | 57 | | |
| | Fom-1$^+$/Fom-1$^+$ | 38 | 25 | 7.719 | 5 % |
| 6 | yg$^+$/- | 117 | 32 | | |
| | yg/yg | 40 | 11 | 1.333 | 72 % |
| 7 | nsv$^+$/- | 226 | 83 | | |
| | nsv/nsv | 77 | 14 | 5.751 | 12 % |

Table 2. Segregation data observed in F2 progenies between cut leaf (cl) mutant and si-1, Fn, Pa, Zym, Fom-1, Fom-2, ms-2, nsv and ms-4.

| Linkage group | Genes | [normal] $cl^+/-$ | [cut leaf] cl/cl | chi-square (9:3:3:1) Value | Probability |
|---|---|---|---|---|---|
| 1 | $si\text{-}1^+/-$ | 164 | 50 | | |
| | si-1/si-1 | 58 | 28 | 5.446 | 14 % |
| 2 | $Fn/-$ | 100 | 39 | | |
| | $Fn^+/Fn^+$ | 50 | 11 | 5.796 | 12 % |
| 3 | $Pa/Pa^+$ | 114 | 36 | | |
| | $Pa^+/Pa^+$ | 59 | 22 | 0.602[z] | 90 % |
| 4 | $Zym/-$ | 137 | 52 | | |
| | $Zym^+/Zym^+$ | 50 | 14 | 0.983 | 81 % |
| 5 | $Fom\text{-}1/-$ | 110 | 38 | | |
| | $Fom\text{-}1^+/Fom\text{-}1^+$ | 38 | 14 | 0.249 | 97 % |
| 6 | $Fom\text{-}2/-$ | 227 | 72 | | |
| | $Fom\text{-}2^+/Fom\text{-}2^+$ | 66 | 34 | 4.466 | 22 % |
| 6 | $ms\text{-}2^+/-$ | 107 | 37 | | |
| | ms-2/ms-2 | 39 | 12 | 0.236 | 97 % |
| 7 | $nsv^+/-$ | 165 | 52 | | |
| | nsv/nsv | 57 | 26 | 3.218 | 36 % |
| | $ms\text{-}4^+/-$ | 109 | 44 | | |
| | ms-4/ms-4 | 32 | 15 | 2.542 | 47 % |

[z] tested for 6:3:2:1 segregation because Pa/Pa is lethal

Table 3. Segregation data observed among the normal green plants in F2 progenies segregating for virescent (v) and si-1, Zym, Fom-2, nsv, ms-4 and cl.

| Linkage group | Genotypes | segregation among normal ($v^+$/-) plants | chi-square (3:1) Value | chi-square (3:1) Probability |
|---|---|---|---|---|
| 1 | $si\text{-}1^+$/- : si-1/si-1 | 121 : 34 | 0.776 | 38 % |
| 4 | Zym/- : $Zym^+/Zym^+$ | 125 : 39 | 0.130 | 72 % |
| 6 | Fom-2/-:$Fom\text{-}2^+/Fom\text{-}2^+$ | 229 : 70 | 0.402 | 53 % |
| 7 | $nsv^+$/- : nsv/nsv | 141 : 47 | 0.428 | 93 % |
| | $ms\text{-}4^+$/- : ms-4/ms-4 | 127 : 34 | 1.294 | 26 % |
| | $cl^+$/- : cl/cl | 222 : 80 | 0.358 | 55 % |

## Flava, a Chlorophyll Deficient Mutant in Muskmelon

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A spontaneous yellow mutant appeared in 'K 2005', a muskmelon line from China supplied by M. HEDDE (Caillard Seeds Company, Sarrians, France). From the original seed stock, three plants were selfed to maintain the line and in one inbred progeny appeared yellow plantlets. One of these plants was selfed and crossed with normal green 'Charentais'. The selfed progeny of the yellow plant was uniformly yellow. The F1 was green and the F2 segregates 237 green : 75 yellow close to a 3:1 expected ratio ($\chi^2$ = 0.154 ; Prob. = 69 %) for a single recessive gene. Allelism test must be made with yellow green (yg) marker (1) but the phenotypes of the two mutants are quite different. Growth rate is reduced and the yellow color is more dull and dark than yellow green.

We propose the name flava and the symbol f for this mutant.

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# Maternal Effect on Growth of Melon Seedlings

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Seed size varies considerably in melon (Cucumis melo L.). Weight of 100 seeds varies from less than 0.5 g to more than 7 g. This seed size variation influences seedling size and is very troublesome in growth studies on young plants.

We reported here observations made on 2 lines, 'Persian Small Type' (P) and 'Freeman's Cucumber' (FC), and their reciprocal F1 hybrids.

## Methods

11 January : sowing in 6x6 cm pots in heated greenhouse
11 February : measure of one cotyledon on 10 plants
27 February : measure of young seedlings on 7 plants repotting in 12x12 cm pots
12 April : measurements on 7 plants
Plants were not pinched.
Statistical comparison of means is made by Student's t test.

## Results

The dates of unfolding of successive leaves are reported in Figure 1.
Other observations are reported in Table 1.

Figure 1. Date of unfolding of successive leaves.

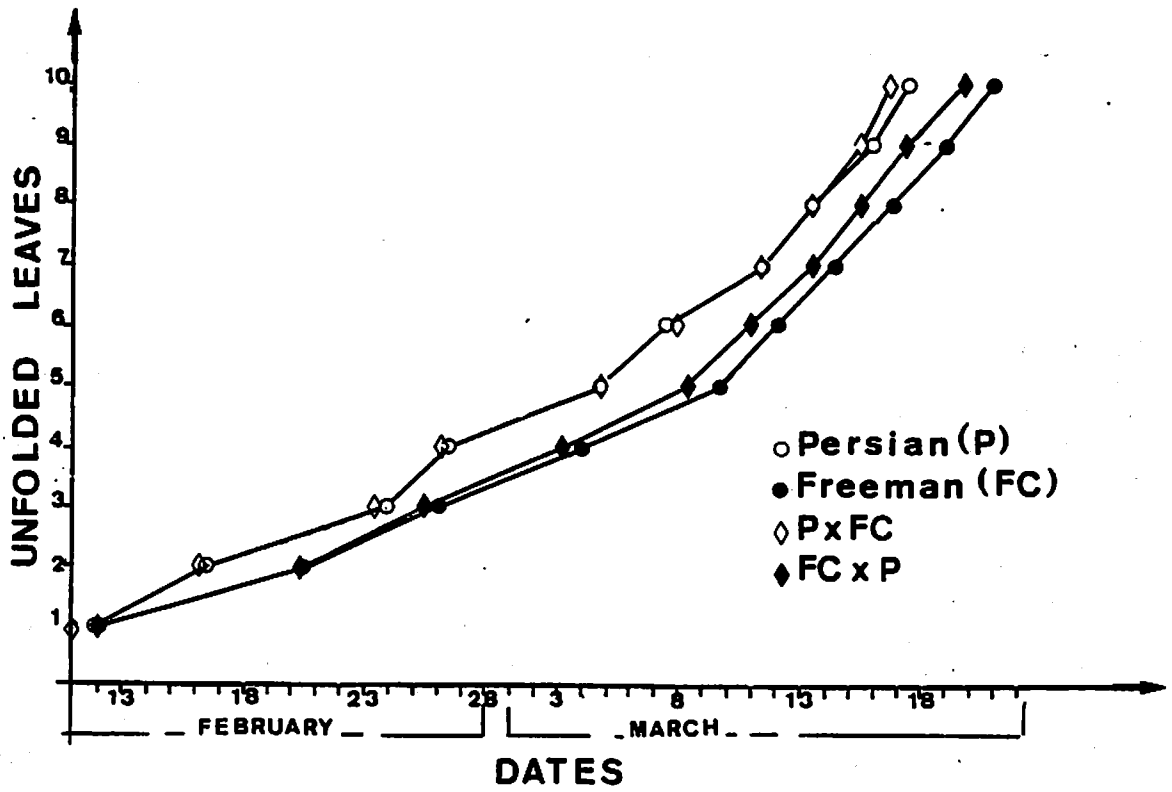

Table 1. Measures of different characters on 'Persian Small Type' (P), 'Freeman's Cucumber' (FC) and their reciprocal F1 hybrids.

| Date of measures | Observation | FC | FCxP | PxFC | P | P/F | PxPC/FCxP |
|---|---|---|---|---|---|---|---|
| | Weight of 100 seeds [y] | 2.0 | 2.0 | 5.3 | 5.0 | 2.5 | |
| 11 February | cotyledon length = L (mm) | 22 a[z] | 24 b | 38 d | 36 c | 1.6 | 1.6 |
| | cotyledon width = l (mm) | 14 a | 14 a | 23 c | 21 b | 1.5 | 1.6 |
| | cotyledon[y] Lxl ($mm^2$) | 306 | 341 | 851 | 761 | 2.5 | 2.5 |
| 27 February | hypocotyl length (mm) | 38 a | 54 b | 70 c | 84 d | 2.2 | 1.3 |
| | number of leaves [y] | 3.0 | 3.0 | 3.6 | 3.9 | 1.3 | 1.2 |
| | length of the first leaf (mm) | 59 a | 67 b | 81 d | 76 c | 1.3 | 1.2 |
| | total length of leaves | 115 a | 130 b | 192 d | 186 c | 1.6 | 1.5 |
| 12 April | fresh weight (g) | 107 a | 119 a | 139 b | 128 b | 1.2 | 1.2 |
| | number of leaves on main stem | 17.6 | 17.4 | 18.2 | 16.8 | 1.0 | 1.0 |
| | main stem length (cm) | 86 | 117 | 119 | 109 | 1.3 | 1.0 |
| | ramification number | 2.3 a | 2.9 b | 2.8 b | 3.7 c | 1.6 | 1.0 |
| | total length of ramification (cm) | 122 | 123 | 160 | 131 | 1.1 | 1.3 |
| | number of plants with female flowers [y] | 3 | 2 | 6 | 0 | | |
| | date of first ramification[y] | 14/03 | 12/03 | 10/03 | 11/03 | | |
| | date of tendril apparition [y] | 9/03 | 10/03 | 4/03 | 12/03 | | |
| | date of male flower apparition [y] | 3/04 | 3/04 | 1/04 | 3/04 | | |

z = mean separation in rows by student's t test, 5 % level

y = no statistical analysis

## Discussion

As expected, cotyledon size is very dependent on maternal seed size and a significant maternal effect is measured on one month old seedlings. At the end of the trial, when the plants were more than 2 months old, maternal effect was still significant for fresh weight but not significant for the other measured characters.

The F1 hybrid PxFC is earlier than the reciprocal FCxP for apparition of ramifications, tendrils, male and female flowers. We cannot exclude that other factors than seed size may contribute to the maternal effect but surely seed size is a major factor because it is found in every observed cross.

## Conclusion

The influence of seed size cannot be neglected when breeders wish to screen young plants for vigor or in adaptation studies.

# Isolation and Culture of Protoplasts from Cucumis metuliferus and Cucurbita martinezii and a Method for their Fusion with Cucumis melo Protoplasts.

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The incorporation of resistance to diverse plagues and diseases into cultivated varieties of melon is one of the most interesting targets of the genetic improvement of such a crop. Some wild species of the family Cucurbitaceae are very important sources of extraspecific variation as several of those resistances have been described in them. Thus, resistances to Sphaeroteca fugilinea SLECHT and aphids (5), pumpkin mosaic virus and watermelon mosaic virus I (13) and Meloidogyne spp. (4) have been reported in Cucumis metuliferus, while resistances to cucumber mosaic virus and Erysiphe cichoracearum sp. cucurbitae (3) have been referred in Cucurbita martinezii. In principle, protoplast fusion should help to overcome the incompatibility barriers existing for the sexual cross between melon and those wild species. In this paper we describe the isolation and culture of protoplasts from both wild species and a method to induce the fusion of these protoplasts with mesophyll protoplasts from axenic plants of Cucumis melo 'Cantaloup Charentais'.

Methods.- Seeds of C. melo 'Cantaloup Charentais', Cucumis metuliferus NAUD (gently supplied by Dr. Jacobs, University of Stellenbosch, South Africa) and Cucurbita martinezii BAILEY (gently supplied by Dr. Dumas de Vaulx, INRA, Montfavet, France) were surface sterilized and germinated on MG medium as previously described (9,10). The source of protoplasts from each plant species were as follows: i) C. melo, leaf segments precultured two days in C medium from axenic plants grown on MEL medium (9); ii) C. metuliferus, two days precultured cotyledons in C medium as well as non-precultured ones and calli from a cell line kept by subculturing every 15 days in CCM medium (C medium + 10% coconut milk) at 27±0.5°C; iii) C. martinezii, cotyledons as above and small roots (1-2 cm in length) precultured three days in C medium, obtained from axenically propagated plants by rooting the shoot apices on MB3 medium (10).

Enzyme solutions and protoplast isolation methods for each kind of source were: i) for melon mesophyll protoplasts, those previously reported (10) but at the rate of 0.15 g of tissue per ml of enzyme solution; ii) for cotyledons from both wild species, those described for C. melo cotyledons in this issue (14); iii) for calli from C. metuliferus cell line, the same method but using 2 g of calli/8 ml of RSM enzyme solution consisting of 2% cellulase Onozuka-RS + 1% Macerozyme + 0.6M-mannitol on Murashige and Skoog (12) salt solution; and iv) for roots from C. martinezii, the same method but using 1g of tissue/8 ml of the enzyme solution utilized for melon mesophyll protoplasts. Protoplasts were cultured, in all the cases, on ZEPC 0.6M-mannitol according with the procedure and conditions described earlier (9) for melon mesophyll protoplasts.

Protoplast fusion procedure: it consists in a modification of the Kao et al. (7) ones. Protoplasts from both parentals were purified by flotation over 20% sucrose and resuspended in the M solution (100 mM $CaCl_2.2H_2O$ + 300 mM glucose, at pH 6.3) at the rate of 3-4 x $10^5$ protoplasts/ml. Both suspensions were mixed at equal parts (v:v) and a volume of 0.5 ml of the mixture was settled out on a 55 mm plastic Petri dish for 10 min. Then, 0.5 ml of MPEG solution

(M solution + 60% PEG-6,000 at pH 6.3) were added to the mixed protoplasts and the mixture was kept static for another 10 min period. The resulting mililiter of aggregated protoplast suspension was eluted with 5 ml of KMml0 solution, a modification of that from Keller and Melchers (8) consisting of 100 mM $CaCl_2$ $2H_2O$ + 300 mM mannitol, buffered at pH 10 with glycine 50mM-NaOH. After 20 min the liquid medium was removed and 5 ml of ZEPC 0.6M-mannitol were added to the protoplasts which had remained attached to the bottom of the dish. This step was repeated once in order to eliminate the fusogenic solution and, finally, another 4 ml of ZEPC medium were added to culture the protoplasts and the fusion products.

Results.- Protoplast isolation and culture from the wild species. Protoplast yields were very different depending on the kind of source utilized; thus, cotyledons of both species, regardless of their preculture in C medium, yielded 0.5-3.0 x $10^6$ protoplasts/gram of tissue, similarly to those obtained with melon using the same source of material (14), while calli from the Cucumis metuliferus cell line and roots from the axenically propagated Cucurbita martinezii plants produced lower yields, around 0.8-2.0 x $10^5$ protoplasts/gram.

Morphologically, cotyledon-derived protoplasts are similar to the melon cotyledon ones, and not much different from the melon mesophyll protoplasts (close vacuolation degree and chloroplasts distribution, but being clearly superior in size). On the contrary, calli- and root-derived protoplasts are similar in size to melon mesophyll ones but more cytoplasmic and lacking mature chloroplasts.

The response to culture in ZEPC medium of the cotyledon protoplasts from both wild species was, again, absolutelv dependent on the preculture of the plant material (9,14), as non-precultured cotyledons produced protoplasts which, although regenerated their cell wall and initiated one or two cell divisions, were unable to grow further, whereas precultured cotyledons gave rise to protoplasts that maintained their mitotic activity in similar way to what melon mesophyll protoplasts did (9) and, after 20 days in culture, they could be transferred to ZEPC 0.3M-mannitol to continue their growth.

Calli and roots produced a too low protoplast yield to allow a systematic study of their behaviour in ZEPC medium. Nevertheless, their morphology, completely different from the melon mesophyll protoplasts, makes them specially useful for fusion experiments, because it facilitates the determination of fusion frequencies and, above all, they are extremely advantageous if the selection method to be used is that of visual identification and mechanical harvest of the heterokaryons.

Protoplast fusion. Several factors, such as the initial protoplast density, the type and concentration of PEG, the duration of treatment with the high $Ca^{++}$/PEG solution, the time elapsed in the high pH solution, etc., were systematically studied in preliminary experiments. As a result of these studies, the most suitable method to fuse mesophyll protoplasts of melon with calli-derived protoplasts of Cucumis metuliferus and with roots-derived protoplasts of Cucurbita martinezii is the one described under 'Methods'.

PEG-6,000 gave better results than PEG-1,500. Out of the 20%, 30% and 40% concentrations studied, the one with 30% originated an acceptable protoplast aggregation degree without affecting their viability. The aggregation period

of 10 min was the most advisable one, as shorter periods produced lower fusion frequencies while longer times resulted in reduced cell viability. The time elapsed in contact with the high pH solution was, also, critical, since periods longer than 20-25 min strongly afffected the viability of the protoplasts, although higher fusion frequencies could be achieved. Finally, the initial protoplast density was very important as concentrations higher than $3-4 \times 10^5$ protoplasts/ml led to the formation of excessive amounts of polykaryons and multiple fusion bodies, which are highly unstable, whereas lower initial densities caused too low fusion frequencies. Summing up, by using the adequate conditions and the procedure described above, fusion frequencies ranging from 5% to 12% in Cucumis melo (x) C. metuliferus and from 1% to 6% in C. melo (x) Cucurbita martinezii have been obtained in our laboratory.

We are currently applying this method to several protoplast systems, using the abovementioned sources of protoplasts according to the chosen selection procedure for the fusion products, with the following strategies: i) selective system, by visually identifying and mechanically selecting the heterokaryons and culturing them in microdroplets of conditioned culture media; in this case we are fusing mesophyll protoplasts of C. melo with calli protoplasts of C. metuliferus and with root protoplasts of Cucurbita martinezii; ii) natural semiselective system, based on the fusion of melon mesophyll protoplasts with non-precultured cotyledon protoplasts of both wild species; and iii) induced semiselective system, fusing melon mesophyll protoplasts with physical or chemically inactivated protoplasts from precultured cotyledons of both wild species. The third system has allowed, in some cases (1,2,11) the transfer of limited quantities of genetic information from the donor species (the inactivated protoplasts) to the recipient species ( the undamaged protoplasts) and, therefore, could be of great interest from the point of view of plant breeding. Moreover, the application of this semiselective system to the fusion of protoplasts between species belonging to different genera could permit the obtention of asymmetric hybrids which can be of greater interest than the symmetric ones, since, usually, the former do not present as many infertility problems, developmental disarrangements and rooting difficulties as the latter (6).

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# Plant Regeneration from Cotyledon Protoplasts of Cucumis melo L. cv. Cantaloup Charentais.

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In a previous work (1) we described suitable methods for obtaining callus from mesophyll protoplasts of axenic plants of three melon cultivars. Recently we have reported the morphogenic response of those kind of calli, both via embryogenesis and organogenesis (2). Whole plants could be regenerated from protoplast-derived calli growing on a definite sequence of media. In this paper we show the achievement of plant regeneration from protoplasts originating from another source, namely cotyledons from seedlings of Cucumis melo L. 'Cantaloup Charentais'.

Methods.- Seeds of melon were surface sterilized by immersion in 12.5% commercial bleach (equivalent to 6.25 g/l of active chlorine) for 20 min. After three rinses with sterile distilled water, they were aseptically sown on agarified MG medium (1) in 20x195 mm test tubes. Cotyledons from 11-13 days old seedlings were used for protoplast isolation, either directly or after preculturing them in C medium (1) for two days. Strips of 1-2 mm width from these explants were placed in 100 ml Erlenmeyer flasks containing 8 ml of enzyme solution at the rate of 0.15 g of tissue/ml of enzyme, following the procedure previously described (1). After removing the enzyme solution, protoplasts were purified by flotation over 20% sucrose, picking up the ring which appeared in the interphase formed between the mannitol solution containing the protoplasts and the pad of sucrose. The protoplast yield was estimated by four cell counts in a Howard mould-counting chamber. Protoplasts, at a initial density of 1.5-2 $\times 10^5$ cells/ml, were cultured on 50 mm Petri dishes containing 4 ml of ZEPC 0.6M-mannitol liquid medium (1), at 27°C under darkness, for 20 days. Cells and small cell aggregates originating from protoplasts were transferred to the same liquid medium but at 0.3M-mannitol, at the rate of 2-4 $\times 10^4$ units/ml. After 20 days more in culture, the cells and small cell clusters were subcultured in a modified CEN medium (2) consisting of Murashige and Skoog (4) salt solutions, 1 mg/l thiamine-HCl, 100 mg/l myo-inositol, 1 g/l casaminoacids, 30 g/l sucrose, 0.1 mg/l indole-3-acetic acid and 10 mg/l kinetin, at the rate of 0.5-1.0 $\times 10^4$ units/ml, in 100 ml flasks containing 20 ml of liquid medium and incubated at 25.0±0.5°C and constant illumination (400 lux) on an orbital shaker at 95 r.p.m. In order to obtain organogenesis, the resulting microcalli were successively transferred every 15 days to the sequence of solid media IK 0160 (3), IK 0060 (3) and a modified NB 00101$_s$ (2) to content 10 g/l sucrose. All seedlings and callus cultures were incubated in a growth chamber at 27±2°C during the 16 h light period (1,500 lux, cool fluorescent tubes Gro-lux, Sylvania) and at 24±1°C during the dark.

Results.- Table 1 shows the protoplast yields obtained from cotyledons under the two conditions assayed and their behaviour when they were cultivated in ZEPC 0.6M-mannitol liquid medium. Contrarily to what happened with mesophyll protoplasts (1) we do not observe here a decrease of yield due to the preculture of the source of protoplasts; in fact, the number of protoplasts per gram of tissue obtained from precultured as well as from non-precultured cotyledons was similar to the one produced by precultured leaves of axenic plants (1).

Morphologically, cotyledon protoplasts are very close to mesophyll ones, presenting a great quantity of big chloroplasts distributed in the cytoplasm and a notable vacuolization, but being clearly superior in size and containing a higher number of other intracellular organelles (mainly amyloplasts) which makes it necessary their purification by floating them over a pad of sucrose.

The behaviour of the protoplasts in the ZEPC liquid medium was absolutely dependent on the preculture of cotyledons. So, precultured cotyledons produced viable protoplasts with 75-80% of cell wall regeneration within 2-3 days of culture, and sustained divisions in the 45% of the regenerated cells after 20 days in culture. On the contrary, the non-precultured cotyledons gave rise to protoplasts which experimented cell wall regeneration at very low rates and only exceptionally initiated first divisions; sustained divisions were never observed. Previously (1) we already pointed out the advantageous effect of preculturing the source of protoplasts in the case of varieties with low response and/or when mother plants were grown under suboptimal conditions; the present results using cotyledons as source of protoplasts are a new evidence in this direction.

The percentage of cells undergoing sustained divisions in the ZEPC 0.6M as well as their further growth after being subcultured in the ZEPC 0.3M liquid medium did not differ from the data reported for mesophyll protoplasts (1), reaching in both cases a high level of mitotic activity.

When cells and small cell clusters grown on ZEPC 0.3M were transferred to the CEN agitated liquid medium, whitish or light-green microcalli were formed after 20 days in culture. These could be collected by being filtered through a 100 µm nylon mesh. Batches of around 250 mg (fresh weight) of microcalli were placed on IK 0160 solid medium and grown for 15 days, giving rise to big whitish and friable calli carrying several green zones of apparently organized growth and some peripheral true buds. The transfer of these organized tissues to auxin-free IK 0060 solid medium encouraged their growth, though the amount of unorganized friable callus formed was still high. The quantity of unorganized tissue decreased gradually after two passages of the green zones through NB $00101_S$ medium, where compact and green calli were developing greatly, giving rise to the formation of a great number of buds and shoot-buds. Clearly distinguishable shoots could be observed in some calli after their third passage through the NB $00101_S$ medium. The number of calli presenting developed shoots and the number of shoots per callus depended on two factors: the exclusive transfer of organized zones of calli in each subculture and the short interval elapsed among them (10-15 days every time). Table 2 summarizes the results obtained after each step of this procedure.

In order to obtain whole plants, the clearly developed shoots were individually transferred to a modified NB $00101_S$ medium (without 6-benzylaminopurine or casaminoacids). Most of the shoots displayed clear abnormalities due, perhaps, to changes in their chromosome number and were unable to root. However, some of them became whole plants (usually rosette-shaped plants and occasionally normal plants) which could be propagated <u>in vitro</u> by subculturing their apices and axillary buds in the abovementioned medium.

In short, we can now avail ourselves of two different protoplast systems which, under the proper cultural conditions, allow a moderate rate of plant regeneration, via organogenesis, in melon.

Nowadays, in our laboratories, we are using the protoplast fusion technique in order to obtain interspecific somatic hybrids between Cucumis melo L. and several wild species of genera Cucumis and Cucurbita, in which resistances to plagues and diseases have been described. The obtention of such a kind of hybrid plants should represent the first step in our final goal, i.e. the transfer of resistance genes from the wild species to the cultivated one. So the attainement of plant regeneration from protoplasts of melon is an important step towards the stated end.

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Table 1. Isolation and culture of protoplasts from non-precultured (NP) cotyledons and from precultured (P) ones in C medium for 20 days.

| | Isolation | Culture |
|---|---|---|
| | Protoplast yield ($\times 10^6$)/g of tissue | % of cells undergoing divisions |
| NP | 0.82 ± 0.53 | 0.0 |
| P | 1.37 ± 0.77 | 45.30 ± 9.57 |

Table 2. Morphogenic response of protoplast-derived calli after subculturing for 15 days in each of the succesive solid culture media.

| Sequence of media | No. of calli | % of calli with buds[z] | | | % of calli with shoot-buds | % of calli with shoots |
|---|---|---|---|---|---|---|
| | | ++ | + | - | | |
| 1)IK 0160 | 177 | 77.4 | 22.6 | 0 | 1.7 | 0 |
| 2)IK 0060 | 138 | 97.8 | 2.2 | 0 | 31.2 | 0 |
| 3)NB 00101$_S$ | 93 | 77.4 | 22.6 | 0 | 35.5 | 0 |
| 4)NB 00101$_S$ | 59 | 94.9 | 0 | 5.1 | 59.4 | 0 |
| 5)NB 00101$_S$ | 41 | 100.0 | 0 | 0 | 100.0 | 9.7 |
| 6)NB 00101$_S$ | 56 | 100.0 | 0 | 0 | 100.0 | 10.7 |
| 7)NB 00101$_S$ | 64 | 100.0 | 0 | 0 | 100.0 | 20.0 |

[z] ++ : calli with numerous buds peripherally distributed.
+ : calli with a few buds appeared in discrete zones.
- : calli without buds.

# Compatibility among Cucumis melo varieties inodorus, conomon, flexuosus, momordica, and utilissimus

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Naudin classified Cucumis melo into Cucumis melo var. cantaloupensis Naud.; Cucumis melo var. reticulatus Naud.; Cucumis melo var. inodorus Naud.; Cucumis melo var. flexuosus Naud.; Cucumis melo var. conomon Mak.; Cucumis melo var. chito Naud.; and Cucumis melo var. dudain Naud. based on fruit and plant characteristics (3). Pangelo reported that all the seven varieties reported by Naudin (3) hybridized readily with one another and there was apparently very little sterility even among progenies from crosses involving variant types (4). The snap melon (Cucumis melo var. momordica) and long melon (Cucumis melo var. var. utilissimus) were described by Kirtikar and Basu and were of typical Indian origin (1). The lines CS26 (Cucumis melo var. conomon) and CS52 (Cucumis melo var. momordica) collected indigenously differed from other melon varieties for their plant habit and fruit characteristics. CS26 is grown in the midlands of Kerala (India) for ripened fruits. These fruits are stored in the open for up to one year for year around use. CS52 is grown on the coasts of Kerala (India) during summer months for their ripened and cracked fruits which yield delicious flesh. The present study was carried out to determine compatibility of these two varieties with Cucumis melo var. inodorus Naud.; Cucumis melo var. flexuosus Naud.; and Cucumis melo var. utilissimus Duth and Full. (Table 1). The varieties were grown at a spacing of 1.5 m between plants and 3 m between rows with ten pits for each, having 2 plants/pit. Bagging of the male and female matured flower buds with butterpaper bags was done in the evening. Pollination was performed the next morning between 6:30-8:30 A.M., when the stigmas were receptive. The pollinated flowers were covered and labelled. along with selfs, 20 cross combinations (including reciprocals) among the five selected melons were made by hand pollination. The crossability index was then calculated (5). The genetic distances among the five botanical varieties were calculated as per Mahalanobis (2). The genetic distance was based on nodes to first female flower, fruit weight, seeds/fruit and fruits/plant.

All the five botanical varieties of Cucumis melo were found to be crossable with each other (Table 2). No significant reciprocal effect was observed indicating that the maternal parent did not have any influence on crossability index. The crossability index was the highest for oriental pickling melon x long melon (79.19%) and the lowest for muskmelon x snake melon (47.15%). It was lesser than 50% in muskmelon x snake melon, long melon x muskmelon, long melon x snap melon and snap melon x muskmelon. Crossability index was more than 70% in oriental pickling melon x long melon and snake melon x oriental pickling melon. In other crosses, crossability index varied from 50 to 70%.

Genetic divergance could also be considerd as a measure of affinity (Table 3). Muskmelon and snake melon were the most divergent ($D^2$ = 14.49) while long melon and snap melon were the closest ($D^2$ = 0.38). In the order of affinity, the five melon varieties could be arranged as oriental pickling melon, long melon, snap melon, snake melon, and muskmelon.

Table 1. Source, chromosome number, and distinguishing morphological characters of five botanical varieties of *Cucumis melo*.

| Acc. No. | Botanical varieties | 2n. | Origin | Fruit rind | Fruit shape | Flesh color | Fruit flavor | Sweetness | Cracking |
|---|---|---|---|---|---|---|---|---|---|
| CS26 | Oriental pickling melon (*C. melo* var. *conomon*) | 24 | Trichur (Kerala) | Golden yellow | Long oval | White | Poor | Less sweet | No |
| CML8 | Muskmelon (*C. melo* var. *inodorus*) | 24 | Ludhiana (Punjab) | Light green | Spher-ical | Light green | Good | Very sweet | No |
| CS4 | Long melon (*C. melo* var. *utilissimus*) | 24 | Pantnagar (U.P.) | Greenish white with stripes | Ellip-tical & elon-gated | Pale white | Poor | Less sweet | No |
| CS50 | Snake melon (*C. melo* var. *flexuosus*) | 24 | Pantnagar (U.P.) | Yellowish with mottling | Club shaped | Pale yellow | Poor | Less sweet | No |
| CS52 | Snap melon (*C. melo* var. *momordica*) | 24 | Cochin (Kerala) | Yellow | Oblong | White | Poor | Less sweet | Yes |

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Table 2. Crossability index (CI) among five botanical varieties of Cucumis melo.

| | O | M | L | F | S |
|---|---|---|---|---|---|
| O | --- | *** | **** | *** | *** |
| M | ** | --- | ** | * | *** |
| L | *** | * | --- | ** | * |
| F | *** | ** | ** | --- | **** |
| S | ** | ** | * | *** | --- |

* - CI < 50% (Generally crossable)
** - CI > 50% < 60% (moderately crossable)
*** - CI > 60% < 70% (highly crossable)
**** - CI > 70% (Perfectly crossable)
O - Oriental pickling melon, M - muskmelon, L - long melon, F - snake melon, S - snap melon.

Table 3. Genetic distance ($D^2$) among the five botanical varieties of Cucumis melo.

| Parents | O | M | L | F | S |
|---|---|---|---|---|---|
| O | ---- | 5.29 | 2.62 | 3.88 | 3.40 |
| M | 5.29 | ----- | 9.16 | 14.49 | 8.79 |
| L | 2.62 | 9.16 | ---- | 2.94 | 0.38 |
| F | 3.88 | 14.49 | 2.94 | ---- | 1.58 |
| S | 3.40 | 8.79 | 0.38 | 1.58 | ---- |

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RESEARCH NOTES

III. Watermelon

# Reactions of Accessions of *Citrullus colocynthis* from Nigeria to Zucchini Yellow Mosaic Virus and other Cucurbit Viruses.

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Recently (3), we reported that two accessions of Egusi (*Citrullus colocynthis*) from Nigeria were tolerant to zucchini yellow mosaic virus (ZYMV). However, the reaction of Egusi to this virus appeared to be influenced by temperature and perhaps light intensity. When grown in the greenhouse during the winter months, plants responded to ZYMV infection with small systemic chlorotic spots, which slowly enlarged, coalesced, and turned necrotic, causing premature leaf death. Conversely, during the summer months, plants were completely resistant, being free of systemic infection. Similarly, in Egypt, Egusi was the only *Citrullus* species highly resistant to ZYMV.

Previously, an accession of *C. colocynthis* (Webb's 'Egun') had been found to be resistant to watermelon mosaic virus 1 (WMV-1) (1) and watermelon mosaic virus 2 (WMV-2) (4). Since *C. colocynthis* is genetically compatible with *C. lanatus*, the former species can be regarded as a very valuable source of viral resistance for the enhancement of watermelon.

An opportunity to test additional lines of *C. colocynthis* was provided by a new collection of this species, which was made available in 1985 by the Plant Introduction System. These lines were originally collected in Nigeria by T. Eadra of the National Horticultural Research Institute of Ibadan. Viral tests were conducted during the summer of 1985 in a greenhouse kept at 30 C, using cucumber mosaic virus (CMV), WMV-1, WMV-2, and ZYMV (3). Two commercial watermelon cultivars were included as controls. The results are summarized in the following table.

Table 1. Reaction of plant introduction of *Citrullus colocynthis* from Nigeria to four cucurbit viruses.

| Accession No. | CMV | WMV-1 | WMV-2 | ZYMV-CT |
|---|---|---|---|---|
| PI494527 | R | S | S | S |
| PI494528 | R | S | T | R |
| PI494529 | R | S | S | S |
| PI494530 | R | S | S | S |
| PI494531 | R | S | S | S |
| PI494532 | R | S | T | R |
| Sugar Baby | R | S | S | S |
| Crimson Sweet | R | S | S | S |

S = Susceptible (moderate to severe leaf mosaic); T = Tolerant (mild foliar mottle, and vigorous growth); R = Resistant (local infection, but free of systemic symptoms).

These data revealed that: a) all lines were resistant to CMV, but susceptible to WMV-1; and b) two lines were tolerant to WMV-2 and resistant to ZYMV. Thus, it is evident that among accessions of *C. colocynthis*, there are basic differences regarding their reaction to WMV-2 and ZYMV. This differential reaction should be considered in breeding for resistance.

The finding also offers the opportunity to determine the inheritance of resistance to ZYMV and tolerance to WMV-2 in accessions of the same wild species. A similar situation was exploited to elucidate the inheritance of resistance to WMV-1 in Cucumis metuliferus (2). Crosses have been made between Egusi plants of resistant and susceptible lines for further studies.

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# Segregation of glabrous male-sterile in an Autotetraploid Line of Citrullus lanatus

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Love, et al. (4) reported the transfer of a nuclear male-sterile (ms) gene, glabrous male sterile (gms), from a diploid to a tetraploid watermelon line. We noted that this male-sterile tetraploid line could theoretically be maintained by crossing the genotypes gmsgmsgmsgms (female) x +gmsgmsgms (male). Melons harvested from the ms plants would produce seeds that segregate 1:1 for glabrous (male sterile) and hairy (male fertile) plants. The ms tetraploid would serve as the female parent, when planted adjacent to a diploid pollinator, to produce triploid seed for seedless watermelons.

If random chromosome assortment (1,2) is routine in this autotetraploid, the male-fertile simplex genotype +gmsgmsgms, can pollinate the male-sterile nulliplex genotype, gmsgmsgmsgms, to maintain a 1:1 segregation of male-fertile:male-sterile plants (mf:ms). If random chromosome assortment does not occur, segregations may differ widely from this ratio (1,2,3), making it difficult to maintain a ms tetraploid line.

Two plants from an autotetraploid line segregating for gms were selfed and $F_1$ plants were progeny tested (Table 1). In the first family, the testcross between the nulliplex plant 1 and plant 3 resulted in 5:1 mf:ms progeny, indicating a ++gmsgms genotype form plant 3. Selfing of plant 3 also indicated this genotype. The testcross between plant 2 and plant 4, as well as the selfing of plant 4, indicated a +gmsgmsgms genotype for plant 4. Selfing of plant 4 also indicated this genotype. In field isolation, the ms nulliplex plants and mf simplex plants from the 2 x 4 cross produced seed segregating 1:1 and 3:1, respectively.

In the second family, some segregation ratios did not fit the hypothesis of random chromosome assortment. The 1:1 segregation from the testcross 3 x 6 suggests that the genotype of plant 6 is +gmsgmsgsm. In another testcross, 4 x 6, there were no ms plants among 23 progeny. Ms plants were also absent from the progeny when the plant was selfed. In the next generation, $F_2$ ms plants pollinated by $F_2$ mf plants produced progeny segregating closer to 2:1 then1:1 mf:ms. Again, the ms class was low. The isolated, open-pollinated mf $F_2$ plants produced progeny that poorly fit a 3:1 mf:ms ratio. Seed number in melons from ms plants in both families varied from none to 100; seed number in melons from mf plants varied from 100 to over 200.

In summary, analysis of the segregation of gms in autotetraploid watermelon indicated random chromosome assortment in progeny from one parent. From a sister parent, an $F_1$ plant was found that produced a reduced number of gmsgms gametes. Investigations are continuing with larger populations.

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Table 1. Ratios of male-fertile (mf) to male-sterile (ms) plants in families from 2 sister autotetraploid plants of Citrullus lanatus.

| | Observed mf:ms | Expected mf:ms | Chi-Square | P | Putative Genotype |
|---|---|---|---|---|---|
| Sister 1 selfed | 5:2 | 3:1 | 0.05 | 0.50 | +gmsgmsgms |
| ms(1) x mf(3) $F_1$ | 25:6 | 5:1 | 0.03 | 0.9-0.8 | gmsgmsgmsgms x ++gmsgsm |
| mf(3) selfed | 31:1 | 35:1 | 0.18 | 0.7-0.5 | ++gmsgms |
| ms(2) x mf(4) $F_1$ | 22:14 | 1:1 | 1:36 | 0.3-0.2 | gmsgmsgmsgms x +gmsgmsgms |
| mf(4) selfed | 23:9 | 3:1 | 0.04 | 0.9-0.8 | |
| ms(2) x mf(4) $F_2$[z] | 16:17 | 1:1 | -0- | 0.99 | |
| ms(2) x mf(4) $F_2$[y] | 27:9 | 3:1 | 0.04 | 0.9-0.8 | |
| Sister 2 selfed | 8:4 | 3:1 | 0.11 | 0.8-0.7 | +gmsgmsgms |
| ms(3) x mf(6) $F_1$ | 12:13 | 1:1 | -0- | 0.99 | gmsgmsgmsgms X +gmsgmsgms |
| ms(4) x mf(6) $F_1$ | 23:0 | 1:1 | 21.04 | 0.001 | gmsgmsgmsgms X +gmsgmsgms |
| mf(6) selfed | 28:0 | 3:1 | 8.05 | 0.01-0.001 | +gmsgmsgms |
| ms(3) x mf(6) $F_1$[z] | 21:1 | 1:1 | 16.41 | 0.001 | |
| ms(3) x mf(6) $F_2$[y] | 28:7 | 3:1 | 0.24 | 0.7-0.5 | |

[z] $F_2$ from glabrous $F_1$ plants.

[y] $F_2$ from hairy $F_1$ plants.

CGC 9:86 (1986)

RESEARCH NOTES

IV. *Cucurbita* spp.

# Establishment of Seedling Test for Resistance to *Phytophtora capsici* Leonian in *Cucurbita*.

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In Japan, the commercial varieties of pumpkin classified into *Cucurbita maxima* are widely cultivated. *C. maxima* seems to be susceptible to *Phytophtora capsici* compared with *C. moschata* on field observation. It is necessary to transfer *Phytophtora* resistance gene of *C. moschata* to *C. maxima*. The purpose of this study was to establish the method of seedling test to screen *Cucurbita* cultivars for resistance to *Phytophtora capsici*.

Materials and Cultivation. Five varieties were used for this study; *C. maxima*: Utsugiwase-akaguri and Kurokawaguri, and *C. moschata*: Heiankogiku, Hyuga 14 and Bizenchirimen. The seeds after forced sprouting were sown in plastic pot (9 cm diameter) filled with perlite. Experiments were designed for two replications of eight plants per plot.

Preparation of Pathogen and Inoculation. Thirty ml of liquid medium consisted of vegetable juice in 100 ml Erlenmeyer flask was sterilized. Mycelia of *Phytophtora* were inoculated in this medium. Mycelial mat, which was produced by 2-week incubation at 28°C, was rinsed on a Buchner funnel with sterile distilled water and put on the filter paper moisted by sterile distilled water in the petri dish. The petri dish was covered with a Japanese paper lid and placed in a 28°C incubator illuminated with fluorescent lamp. Numerous zoosporangia were formed on the surface of mycelial mat by 20-30 hour incubation. The zoosporangia were collected with a small brush after pouring of sterile distilled water into the petri dish (1). About 50 ml of the zoosporangia suspension was poured into each pot.

Evaluation. At 10-14 days after inoculation, results of the observation were recorded.

Effect of Inoculum Concentration (Table 1). The six kinds of inoculum were prepared as 0, $4\times10$, $2\times10^2$, $1\times10^3$, $5\times10^3$ and $1\times10^4$ zoosporangia/ml. Two weeks after sowing, plants were inoculated. With the inoculation of suspensions above $1\times10^3$ zoosporangia/ml, all of the plants in *C. moschata* were diseased but the percentage of died plants were low. At low inoculum concentrations ($4\times10$ and $2\times10^2$ zoosporangia/ml), the percentage of diseased plants and the percentage of died plants were high *C. maxima* and low in *C. moschata*.

Effect of Seedling Stage (Table 2). Plants were grown to the 8-day, 15-day and 22-day seedling stage before inoculation. Inoculum concentrations were prepared for $2.5\times10^2$ zoosporangia/ml in *C. maxima* and for $1\times10^3$ zoosporangia/ml in *C. moschata*. In *C. maxima*, all of the plants inoculated at all of the seedling stages were diseased and died. In *C. moschata*, the percentage of diseased plants and the percentage of died plants were low compared with those of *C. maxima*, and the percentage of the 15-day seedling was higher than those of the 8-day and 22-day seedling stages.

Effect of Temperature Condition (Table 3). Plants had been kept in 30°C-23°C (day temperature-night temperature), 25°C-18°C and 20°C-13°C during 10 days from 2 days before inoculation. Plants were inoculated with $1x10^3$ zoosporangia/ml at 2 weeks. In C. maxima, the percentage of diseased plants and the percentage of died plants were 100% or nearly 100% at high and middle temperature conditions. In C. moschata, the percentage of diseased plants was very low and the percentage of died plants was 0%, at all of the temperature conditions.

Conclusion. It seems likely that the appropriated zoosporangia concentration may be in the range from $2x10^2$ to $1x10^3$ zoosporangia/ml, because of stability of disease appearance. It was estimated that the uniform seedlings about 2 weeks old may be available to inoculate Phytophtora pathogen. Desirable temperature condition for inoculation and nursery of inoculated seedlings seems to be in the high or middle range; 30°C-25°C at day and 23°C-18°C at night.

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Table 1. Effect of inoculum concentration of Phytophtora capsici on percentage of diseased plants and percentage of died plants.

| Species | Variety | Inoculum Concentration (zoosporangia/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | $4x10$ | $2x10^3$ | $1x10^3$ | $5x10^3$ | $1x10^4$ |
| C. maxima | Utsugiwase-akaguri | 0/0[z] | 100/88 | 100/100 | 100/100 | 100/100 | 100/100 |
| | Kurokawaguri | 0/0 | 88/88 | 100/100 | 100/100 | 100/100 | 100/100 |
| C. moschata | Heiankogiku | 0/0 | 0/0 | 63/13 | 100/25 | 100/38 | 100/38 |
| | Hyuga 14 | 0/0 | 50/25 | 50/25 | 100/63 | 100/50 | 100/38 |

[z]Left; Percentage of diseased plants.
Right; Percentage of died plants.

Table 2. Effect of seedling stage when inoculated on percentage of diseased plants and percentage of died plants.

| Species | Variety | Seedling Stage When Inoculated (Days after sawing) 8 | 15 | 22 |
|---|---|---|---|---|
| C. maxima | Utsugiwase-akaguri | 100/100[z] | 100/100 | 100/100 |
| | Kurokawaguri | 100/100 | 100/100 | 100/100 |
| C. moschata | Heiankogiku | 31/0 | 81/69 | 69/44 |
| | Bizenchirimen | 38/6 | 63/56 | 44/31 |

[z]Left; percentage of diseased plants.
Right; Percentage of died plants.

Table 3. Effect of temperature condition on percentage of diseased plants and percentage of died plants.

| Species | Varieties | Temperature Condition (°C, Day-Night) 30-23 | 25-18 | 20-13 |
|---|---|---|---|---|
| C. maxima | Utsugiwase-akaguri | 100/100[z] | 100/94 | 88/75 |
| | Kurokawasguri | 100/100 | 100/100 | 81/75 |
| C. moschata | Heiankogiku | 19/0 | 19/0 | 6/0 |
| | Bizenchirimen | 13/0 | 6/0 | 31/0 |

[z]Left; Percentage of diseased plants.
Right; Percentage of died plants.

# Inheritance of Internode Length in an Interspecific Cross Cucurbita pepo x C. moschata

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We initiated a breeding program for combining the good flesh quality of C. moschata with the bush plant habit of C. pepo. Under a plastic-film house, bush plants have several advantages such as high-planting density, reduced shading effects and decreased labor. However, there is no germplasm for bush plant habit in C. moschata. Shifriss (3) observed that in the $F_2$ population of C. pepo, the segregation ratio of bush plants to vine plants was 3:1 in the early growth season, but 1:3 in the late season. He attributed this growth habit to the developmental reversal of dominance. Denna and Munger (1) observed a similar phenomenon in C. maxima, and reported that in C. pepo the bush gene is dominant to the vine gene during early growth and incompletely dominant during later growth. Zack and Loy (4) reported that the developmental reversal of dominance can be interpreted as a physiological response rather than a reversal of allelic dominance.

A bush-type C. pepo cv. Zucchini was crossed with a vine-type C. moschata cv. PM 143. PM 143 is a line selected from C. pepo x C. moschata and shows similar characteristics to C. moschata in morphology and interspecific-cross ability (2). Therefore, we temporarily classify 'PM 143' as C. moschata. The cross 'Zucchini' x 'PM 143' produced a few seeds with a fully developed embryo. The $F_1$ plants showed partially self-fertility so we could raise 23 mature plants. The parents, $F_1$ and $F_2$ populations were grown in a plastic-film house and the internode length was measured.

Mean length of sequential internodes of 'Zucchini' (bush), 'PM 143' (vine), and '$F_1$ Zucchini x PM 143' are presented in Figure 1. All three accessions showed a genrally increased internode length although differing in length. In the $F_1$, the first 5 internode lengths were similar to those in the bush parent 'Zucchini' and the internode, internode lengths of $F_1$ was longer than those of the vine parent 'PM 143'. These results are similar to developmental reversal of dominance reported by other workers (1, 3).

A histogram showing the distribution of plant height to the 20th node for parental and filial accessions is presented in Figure 2. The distribution of $F_1$ skewed toward that of the vine parent 'PM 143'. In $F_2$, a wide distribution with transgressive segregants (longer than 200cm) was obtained in the plant height to the 20th node. The long internodes of $F_1$ plants and transgressive plants in $F_2$ may be due to non-allelism or a major gene plus a few modifiers in C. pepo and C. moschata, or the possible influence of hybrid vigor. In the $F_2$ population, plants displaying the bush-type habit were recovered.

These results indicate that it is possible to obtain a bush-type C. moschata. Further studies are under progress for investigating detail inheritance mode of growth habit using BC, $F_1$, $F_2$ and $F_3$ populations.

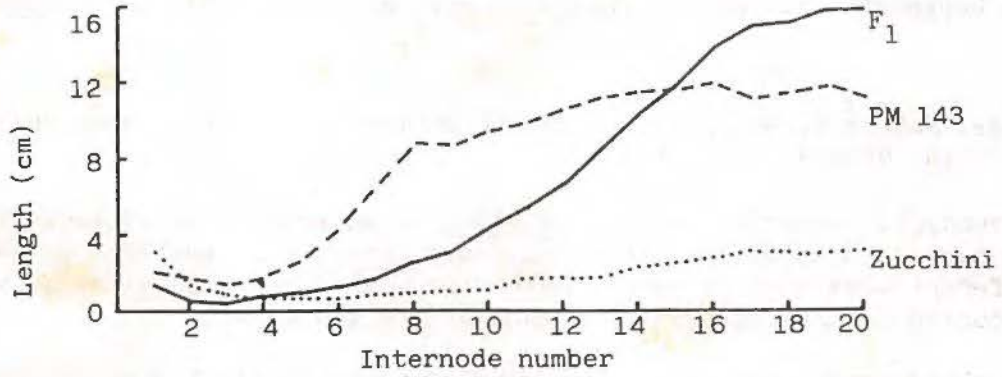


Figure 1. Length of sequential internodes in 'Zucchini'(bush type), 'PM 143'(vine type) and '$F_1$ Zucchini x PM 143'.

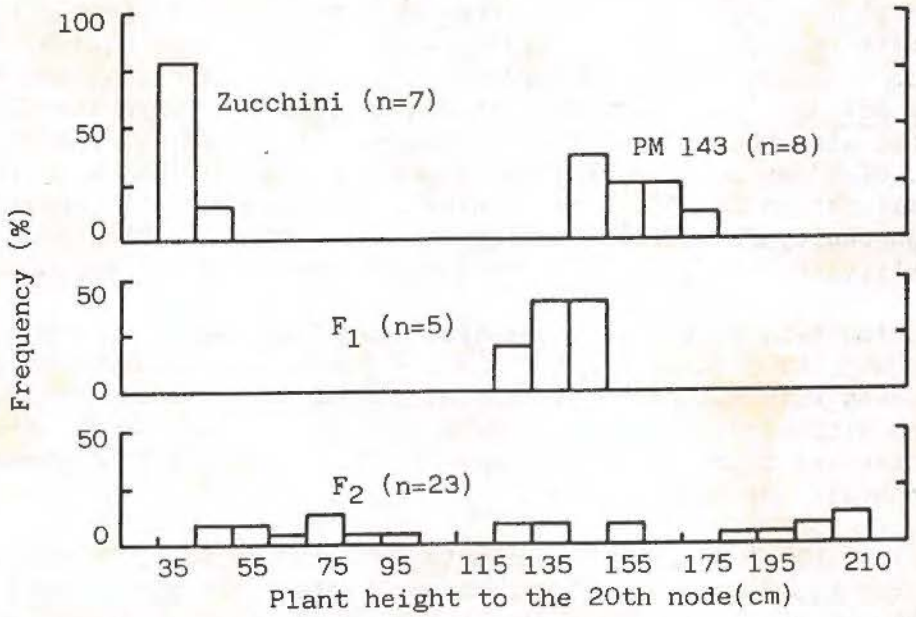


Figure 2. Frequency histogram of plant height to the 20th node in 'Zucchini', 'PM 143', $F_1$ and $F_2$ populations.

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# Age Dependence for Organogenesis of Seed Explants from Four Cucurbita Accessions

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A genotypic comparison in the in vitro organogenesis of explants from seeds was made on 4 accessions from the genus Cucurbita. Explants of seeds of different ages were tested to determine what developmental stage was most condusive to organogenesis for each of the genotypes.

Cucurbita pepo L. explants from cotyledons of germinated seeds have previously (1,2) been reported to produce callus capable of differentiating into embryoids, shoots, and roots. As of yet, no known information has been reported concerning possible genotypic variation of organogenesis from seed cotyledons of varying developmental stages. Seed was collected from fruit harvested at 10, 17, and 45 days after pollination (DAP) from 3 varieties of Cucurbita pepo ('Early Prolific Straight Neck' summer squash, 'Table Queen' acorn squash, and 'Black Beauty' zucchini squash) and one variety of Cucurbita maxima ('Improved Hubbard' winter squash). Once sterilized in 95% ethanol with the embryonic apex removed, each seed was placed on edge in 10 ml. of MS media (3) supplemented with 4.0 mg./l. BAP and 0.2 mg./l. NAA and maintained at 25°C under 2 klux light exposure. To provide a sufficient opportunity for callus development and differentiation into roots and shoots cultivars were incubated for 3 months before final evaluation.

A correlation between the developmental age of the seed and the extent of callus growth and organogenesis for all 4 genotypes was observed (Table 1). Seed explants with mature cotyledons displayed the greatest ability to form callus and differentiate into shoots and roots. No differentiation of callus was observed from seeds of 10 and 17 DAP. Explants from these seeds did not contain cotyledon tissue.

As shown in Table 2 nearly all explants from mature fully developed seed of the four genotypes formed callus tissue. Explants of the zucchini variety 'Black Beauty' developed the greatest number of vigorous shoots and roots. Organogenesis in explants from the C. maxima genotype 'Improved Hubbard' squash was restricted to root tissue. Formation of minute embryos or embryoids as reported in studies by Jaleska (1) was not observed.

These results reveal the existance of genotypic variation in callus formation and organogenesis among the 4 genotypes of Cucurbita in this study. The use of explants from mature seed dramatically simplifies experimentation in organogenesis since seeds can be procured commercially. Experiments are currently under way to survey for variation in explant organogenesis among an array of genotypes in the family Cucubitaceae. Genotypes expressing enhanced levels of organogenesis will be used in future studies to develop a model system for the generation of somoclonal variants. In addition controlled hybridizations will be made to investigate the genetic basis of the observed variation in organogenesis.

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Table 1. Relationship of seed age on explant callus formation and organogenesis

| Genotype | DAP | Callus[z] | Shoots[z] | Roots[z] |
|---|---|---|---|---|
| *C. pepo* 'Black Beauty' | 10 | - | - | - |
| | 17 | ++ | - | - |
| | 45 | ++ | ++ | ++ |
| *C. pepo* 'Table Queen' | 10 | - | - | - |
| | 17 | + | - | - |
| | 45 | ++ | + | + |
| *C. pepo* 'Early Profile Straight Neck' | 10 | - | - | - |
| | 17 | + | - | - |
| | 45 | ++ | + | + |
| *C. maxima* 'Improved Hubbard' | 10 | - | - | - |
| | 17 | + | - | - |
| | 45 | ++ | - | + |

[z](-) equals no growth or differentiation while (+) and (++) indicate increasing degrees of callus vigor and tissue organogenesis.

Table 2. Callus development and organogenesis of 45 DAP seed explants among four *Cucurbita* genotypes.

| Genotype | # of Explants | % with Callus | Vigor of Callus | % with shoots | Vigor of shoots[z] | % with roots | Vigor of roots[z] |
|---|---|---|---|---|---|---|---|
| *C. pepo* 'Black Beauty' | 64 | 100 | 3 | 20 | 3 | 88 | 3 |
| *C. pepo* 'Table Queen' | 76 | 99 | 2 | 5 | 1 | 13 | 2 |
| *C. pepo* 'Early Prolific Straight Neck' | 80 | 99 | 2 | 11 | 28 | 38 | 2 |
| *C. maxima* 'Improved Hubbard' | 90 | 100 | 2 | 0 | 0 | 58 | 2 |

[z]Subjective evaluation for vigor where 0 equals no observed growth or development and 3 equals the maximum.

# Occurrence of Zucchini Yellow Mosaic Virus in the United States in 1985

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During the growing season of 1985, in the United States, zucchini yellow mosaic virus (ZYMV) was more widespread than in any previous year. Epidemics occurred in the following states: Arizona, Arkansas, California, Florida, Georgia, Iowa, Minnesota, Pennsylvania, New Jersey, New York, South Carolina, and Texas. Everywhere, cultivars of summer squash, pumpkin (Cucurbita pepo) and melon (Cucumis melo) were most affected by this virus. However, in some states, winter squash (C. maxima and C. moschata) and watermelon (Citrullus lanatus) were also severely damaged. As it was noted during past surveys, the Florida strain (ZYMV-FL) appeared to be the most prevalent. This strain differs from the Connecticut strain (ZYMV-CT) in that it incites symptoms which resemble those caused by watermelon mosaic virus 1 (WMV-1) (3).

In New York, the epidemics generally occurred in the central part of the state, where many fields of summer and winter squash were abandoned, because fruits were unmarketable. Particularly affected was 'Butternut' (C. moschata), which usually is sporadically infected by cucumber mosaic virus (CMV) and watermelon mosaic virus 2 (WMV-2), the two widespread viruses of the region. Work is in progress to incorporate resistance to ZYMV, as well as to other viruses in summer and winter squash, using an accession of C. moschata from Nigeria ('Nigerian Local') (3), which we have demonstrated to be resistant also to CMV, WMV-1, and WMV-2.

The sudden outbreaks of ZYMV infection in different regions of the USA and abroad, would strongly suggest transmission via seed. Studies are in progress to elucidate this important aspect of the epidemiology of this devastating virus, which was characterized only 5 years ago (1, 2).

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# Relationship Between the B Genes of Two Cucurbita Species

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Gene B conditions precocious depletion of chlorophyll in fruits of Cucrubita pepo L. And chlorophyll depletion is followed by precocious yellow pigmentation. In addition, B can affect leaf blades depending upon the genetic background and the environment. A gene of similar expression, tentatively designated by the same symbol, exists in Cucurbita maxima Duch. It is difficult to study the relationship between the 2 B genes by direct breeding tests because the 2 species are isolated from one another by strong genetic barriers. This difficulty was overcome by transferring the B of C. pepo and the B of C. maxima to C. moschata Poir (3).

A.M. Rhodes made the above transfers, and in the fall of 1980 he generously sent me a few $BC_6$ seed (6 backcrosses to C. moschata) of each transfer. The source of B in C. pepo was a 'Bicolor Spoon' inbred which presumably carries $B^W$ (2). The source of B in C. maxima was P.I. 165558 (1, 3). The recurrent parents used in backcrossing were of somewhat complex lineage and will be described elsewhere.

The BB inbred of C. moschata in which B was derived from C. maxima is designated IL-B. I failed to obtain from Rhodes' material a fertile BB ($B^WB^W$ ?) inbred in which B was derived from C. pepo. However, such a BB inbred of C. moschata was developed in our department from a cross of C. pepo, "Jersey Golden Acorn', BB, as seed parent, and C. moschata, 'Burpee Butterbush', $B^+B^+$. For this transfer we used the pedigree method of breeding in which BB segregates were selected on the basis of taxonomic features and compability with C. moschata as seed parent. Our BB inbred of C. moschata is designated NJ-B. The parents, the $F_1$ and the $F_2$ are described as follows.

$P_1$, IL-B (n = 35). Under field conditions, the stems and portions of the petioles are precociously yellow, becoming golden with the passage of time. The leaf blades are uniformly green, and the fruits are precociously yellow, becoming golden later. Under greenhouse conditions (fall sowing), the lower leaf blades are partially yellow precociously, but otherwise the phenotype is similar to that observed under field conditions. Precocious stem pigmentation in IL-B is a highly stable trait, a distinguishing feature of this inbred. By contrast, it is a highly unstable phenotype in P.I. 165558 (1).

$P_2$, NJ-B (n = 28). This inbred is green in potentially photosynthetic organs except fruits which are precociously yellow at the bud stage.

$F_1$ (n = 25). The hybrids ($P_1$ x $P_2$) of reciprocal crosses are indistinguishable phenotypically. With respect to precocious pigmentation, they are similar to IL-B ($P_1$) except for one deviation. The leaf blades of some $F_1$ plants exhibit a unique pattern of variegation in which precocious yellowing is confined to a stripe along the midrib (Fig. 1). For the past 4 years, 2 perennially grown $F_1$ plants exhibited this pattern regularly in newly differentiated leaves which appear in the fall (November). The leaf blades of the same plants are uniformly green in summer. This cyclic and precise

pattern may be regulated by the phytochrome system in the presence of a particular genotype. There is considerable variation in the expressivity of this midrib pattern among the $F_1$ plants.

$F_2$ (n = 217). This generation is extremely variable and difficult to classify. The difficulty is due to the fact that precocious pigmentation is subject to developmental changes which are different in different $F_2$ segregates. These changes are related to the stage in which they occur, the tissue or group of tissues affected, the extent and intensity of expression, and the environment. The following is a preliminary classification of the $F_2$ into 7 phenotypic groups.

Group #1 (n = 9). All potentially photosynthetic organs, including fruits at the bud stage, are green. -- Group #2 (n = 21). Stems, petioles, and leaf blades are green. Fruits on the same plant are variable: green and bicolor (partial precocious pigmentation). -- Group #3 (n = 19). Stems, petioles, and leaf blades are green. Fruits are precociously yellow (uniformly pigmented). -- Group #4 (n = 23). Stems and petioles are precociously yellow or variably so. Leaf blades and fruits are green. -- Group #5 (n = 82). Stems and petioles are precociously yellow or variably so. Leaf blades are green. Fruits on the same plant are either variable, green and bicolor, or precociously yellow. -- Group #6 (n = 49). Stems and petioles are precociously yellow. Leaf blades are precociously yellow at an early stage of plant development, but later turn to variegated patterns of different kinds. Differences between plants are largely in extent of leaf yellowing. -- Group #7 (n = 14). Lethal and semi-lethal. Seedlings grow very slowly. Cotyledonary leaves are green. Stems and true leaves are either uniformly albino or yellow early in development. Most plants die at the seedling stage. Two plants were rescued by shifting them to a less stressful environment in which they gradually (after several weeks) turned from yellow to variegated leaf blades.

In plants which produce both green and bicolor fruits, the green fruits often appear first during development. Therefore, there is uncertainty about the phenotypes of plants which bore only 1 or 2 green fruits at the time of classification. This uncertainty applies to a few plants of Group #1, but particularly to plants of Group #4.

No firm conclusions can be made about the relationship between the B genes in this cross considering the small size of the $F_2$, the element of uncertainty in classification, and the absence of backcross data. Nevertheless, the data suggest the following ideas which may be tested in future investigations. 1). The 2 B genes which condition precocious chlorophyll depletion in fruits, but which can also affect leaf blades, reside at different loci. Are these genes independent? 2). There exists another gene which conditions precocious depletion of chlorophyll primarily in stems. Is this gene linked to B of C. maxima? 3). The genetic system governing precocious pigmentation is affected occasionally by instability, perhaps through the activity of mobile elements.

The phenomenon of precocious pigmentation in Cucurbita is useful for studies of differential gene expression as related to the fate of chlorophyll during plant development. Potentially, this phenomenon represents a wide range of genetic variations. The great majority is detrimental. But a small minority can be utilized for breeding new edible, ornamental and edible-ornamental cultivars.

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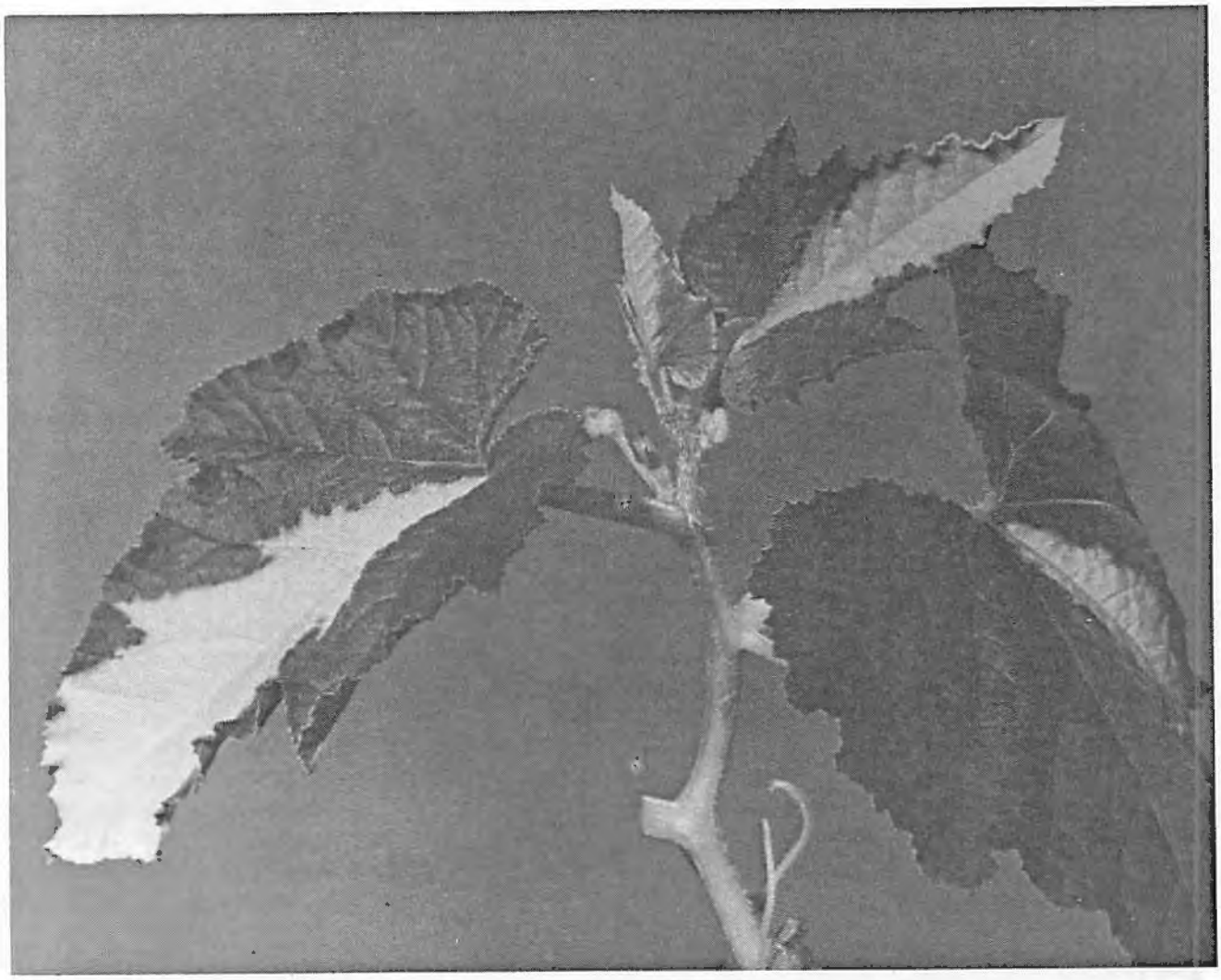

Figure 1. The "midrib pattern" in a hybrid obtained from crossing 2 "precocious" inbreds of C. moschata. Neither inbred exhibits this pattern.

# Reducing Non-Genetic Variability of the Internal Color of Tropical Calabaza

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Cucurbita moschata Duch., known in Spanish as calabaza (Cuba, Florida, Puerto Rico), auyama (Dominican Republic) or ayote sazón (Costa Rica), is an important vegetable throughout the Caribbean. Little breeding work has been reported other than the selection of 'La Primera' in Florida (4). This has a yellow pulp, preferred by people of Cuban heritage. In contrast, Puerto Ricans and Dominicans prefer orange to orange-red calabaza. A thick pulp is widely preferred. We noticed variability within cultivars for pulp color. We wanted to 1) assess the effect of fruit age and position on the vine on internal color; 2) see if reliable harvest maturity indicators existed, to standardize the harvest of samples for color evaluation; and 3) see if genetic differences for pulp color could be easily detectable in a selection program.

Methods. Internal color was evaluated with Plate 10, the orange-yellow transition, of Maerz and Paul (3). This has 12 rows progressing downwards in increasing brightness or value and 12 columns (A - L) progressing from left to right in increasing saturation (colors more pure and less dull). For analysis, column L was given numbers 1-12, column K 0-11, etc. Square A-1 has 66.7% gray reflectance and is labeled "oyster white". L-10, L-8 and K-6 are equivalent to Munsell 5YR 6/12, 7.5YR 7/16 and 10YR 8/14, respectively. L-12 is "Tangier". A color of 4 to 5 is commercially good for Puerto Rico.

In June 1984 and Feb. 1985 'Borinquen', the only existing Puerto Rican cultivar, was planted at Isabela, PR. Borinquen has a good pulp color. Fruits were tagged at anthesis. Data were collected at five day intervals on whether tendrils at the fruit node were green or dry, approximate groundspot color, and change in skin color to yellow-green or to a dusty/opaque sheen. (Borinquen has a dark green skin color.) Sample fruits were harvested at five day intervals from 15 to 75 days in 1984 and 30 to 75 days in 1985, and cut in half for color data. Regressions were done of fruit color on harvest date.

In Feb. 1985, seven $S_1$-derived $S_2$ lines and Borinquen were planted at Isabela and Juana Diaz, PR. Sample fruits were evaluated 50 days after anthesis as above. Distance from fruit to root was measured at Isabela, and a regression done of pulp color on distance. A correlation was done between pulp color and thickness. Variable numbers were sampled per line, but an analysis of variance was possible using four samples per line and location. When more than four fruits were sampled, four were randomly chosen.

Results. Fruit color tended to follow an inverse exponential distribution in the upper right-hand quadrant. The regression of color on distance was non-significant ($P < 0.50$, 111 d.f.). Numerous fruits aborted shortly after anthesis, but a total of 29 non-aborted fruits were available in 1984 and 23 in 1985 for the regression of color on harvest date. This was highly significant in 1984 ($a = -2.20$; $b = 0.13$) and significant in 1985 ($a = 2.73$; $b = 0.05$). In both years an average internal color of 4 - 5 occurred at about 50 days.

A linear increase in red-orange color after fruit harvest has been noted (1,2), attributable to a linear increase in beta-carotene (2). Our results show this commences before harvest, but varies with year in regression slope. Fruit age was reported to obscure varietal differences for characters like soluble solids and sugars (1,4). With uniform sampling at 60 days after anthesis, however, cultivar differences in various quality traits were detectable (1).

We detected genetic differences. Boringuen and La Primera sampled at each of three locations in 1984 had consistent differences in pulp color. In the $S_2$ test, there were highly significant differences among lines for pulp color. Location had an effect at P = 0.06, but there was no line x location interaction. Pulp color and thickness were significantly correlated (r = 0.17; 111 d.f.). Thus selection for pulp color should not decrease pulp thickness, and may even increase it slightly.

Maturity indicator data for Boringuen from all tests at Isabela are summarized in Table 1. All seemed imprecise. Groundspot color changes were difficult to ascertain and this measure was dropped. Many fruits showed no change in skin color by harvest. Dates of color change and dried tendrils were not correlated. We concluded that the best method for evaluating internal color is to harvest tagged fruits at 50 days after anthesis.

Table 1. Means for days after anthesis, (number of fruits and standard deviations) for harvest maturity indicators of 'Boringuen' calabaza.

| | Dried tendrils | Some yellow color on fruit | Opaque, dusty appearance |
|---|---|---|---|
| 1984 | 28.1 (26, 8.2) | not taken | 40.0 (9, 10.6) |
| 1985 | 31.5 (59, 10.4) | 40.4 (45, 6.6) | 44.3 (35, 6.2) |

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# *Cucurbita moschata* Planted at Four Latitudes

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*Cucurbita moschata* Duch. ex Poir. is grown in temperate and tropical zones. 'Ponca' butternut, a vigorous selection in Nebraska (1), was observed to have a dwarfed, unthrifty growth and small fruits in Puerto Rico. Tropical calabaza, however, performs very well in New Jersey, and is a commercial crop there (personal observation). As a first step in understanding these differences, we looked at the effect of latitude on flowering of diverse cultivars.

Methods. Ponca and three other cultivars were planted in mid-May at eight latitudes. 'La Primera' was bred in Florida, 'Borinquen' in Puerto Rico and 'Colombia 34' in Colombia. Locations included Isabela, Puerto Rico, (about 18° N), Clinton, North Carolina (35°), Lincoln, Nebraska (41°) and Decorah, Iowa (43.5°). Data not yet received from other locations will not be discussed. Dates of first male and first female flowers were recorded. Experimental design varied among locations, and will not be described here, but all plantings were replicated.

Results. Figure 1 illustrates flowering data. Ponca showed little difference in flowering response. Any daylength effect within these latitudes on Borinquen and Colombia 34 is not marked. A cooperator in Darwen, England (53.5°N) planted Borinquen in mid-May and grew it past frost in a greenhouse, but no flowering was ever observed. The response of La Primera suggests that some genotypes have photoperiod-sensitive flowering responses. Nitsch et al (3) reported long days slowed flower development in acorn squash (*C. pepo* L.), and that temperature and daylength interacted with each other. The response of La Primera would be consistent with this. Perhaps an interaction of temperature and daylength accounts for the decrease in days to flower of some cultivars at North Carolina.

Ponca showed little change in days to flower, but it again produced small plants and fruits in the south. Ponca in Puerto Rico produces fruits consistently half or less the average weight in Nebraska of 0.8 - 1.3 kg (1). Hall (2) examined photoperiod response of gherkin (*Cucumis anguria* L.), "essentially day-neutral in flowering response", and found that 16 hour days produced larger plants and better growth responses in various characters than eight hour days. Perhaps Ponca responds in a similar manner (Table 1). These data are intriguing, albeit limited, and we hope that complete data and further studies will shed more light.

The cooperation of Dermot Coyne (Nebraska), Glenn Drowns (Iowa), C. H. Miller (North Carolina) and John Wyncoll (England) is gratefully acknowledged.

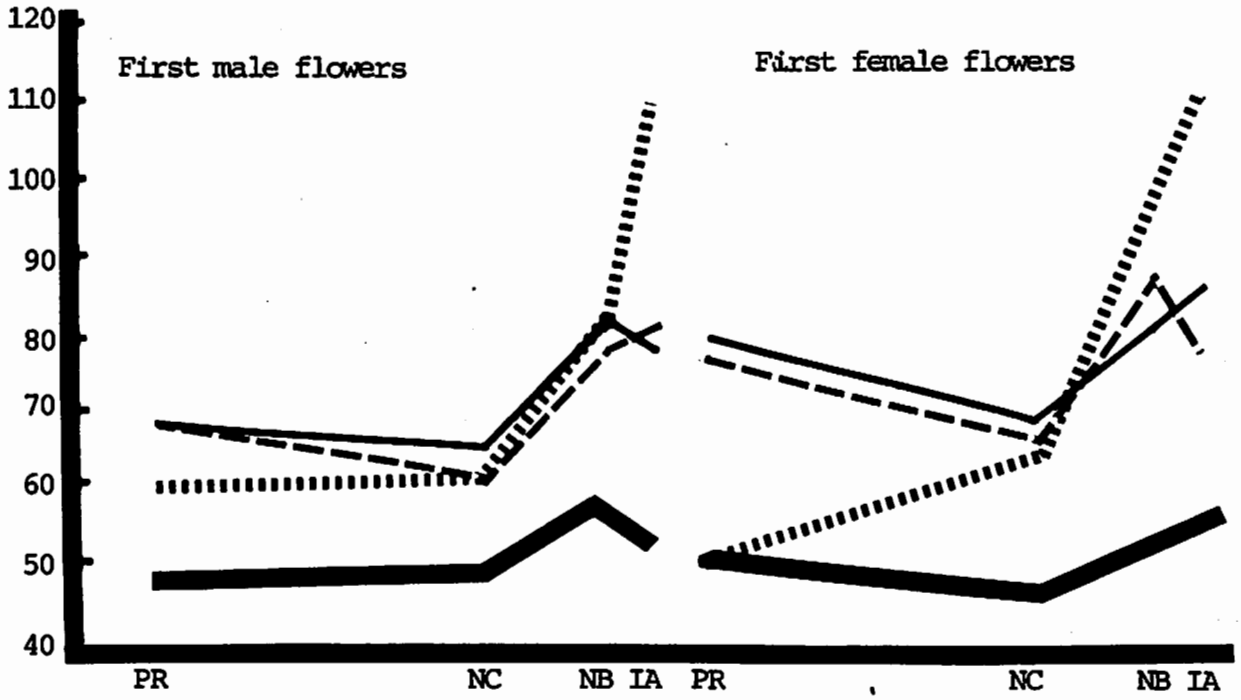


Figure 1. Mean days from planting to first male and female flower, respectively, of four cultivars planted mid-May at four locations.[z]

Key to cultivars: Borinquen, Colombia 34, La Primera, Ponca

z - X-axis is scale of latitude.

Table 1. Mean fruit weight (kg) of four cultivars at four locations.

| | PR | NC | NB | IA |
|---|---|---|---|---|
| Ponca | 0.4 | 0.5 | 0.7 | 1.5 |
| La Primera | 5.0 | -[y] | 4.3 | - |
| Borinquen | 2.9 | - | 2.8 | - |
| Colombia 34 | 4.2 | 4.4 | 6.4 | - |

y - denotes no fruit produced at this location.

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# Genetic Analysis of Isozyme Variants in Cucurbita pepo

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Considerable isozyme polymorphism has been identified in cultivars of Cucurbita pepo (1,2,3,4), and a correlation between the particular activity bands and fruit types was noted by Ignart and Weeden (1). We report here a genetic analysis of several enzyme systems previously found to be polymorphic. Two linkage groups were identified. Genetic linkage proved not to be the basis of at least one of the isozyme/morphological correlations reported by Ignart and Weeden.

Three crosses were made between C. pepo cultivars: Senator x Table Queen, Goldrush x Table King, and Blackjack x Early Prolific. Senator, Goldrush, and Blackjack were zucchini types displaying the isozyme phenotypes already described (1). The phenotypes for the acorn squash Table King and Table Queen, have also been described (1). The isozyme phenotypes of Early Prolific, a yellow straightneck type, were similar to those reported in (1) for cultivars of this type, except that PGM had the "A" phenotype. Isozyme analysis was performed as described previously (1,3). Additive expression of the parental banding patterns was observed in the $F_1$ hybrids. Novel bands, representing new heterodimeric forms, also could be seen in the $F_1$ isozyme phenotypes for GPI, AAT, and MDH enzyme systems. Identification of allelic forms was straightforward in systems such as SKDH, LAP, EST, and ALDO, in which one major band was seen in each parent. Genetic analysis of the other enzyme systems was relatively easy once the parental and $F_1$ phenotypes were compared.

The loci identified and their segregation patterns for the appropriate $F_2$ populations are presented in Table 1. In addition to the reported isozyme loci, the Senator x Table Queen $F_2$ segregated for fruit shape (oblong vs. round) and habit (bush vs. vine), the Goldrush x Table King $F_2$ for fruit shape (oblong vs. round) and fruit color (yellow vs. green), and the Blackjack x Early Prolific $F_2$ for fruit color (yellow, light green and dark green) and leaf mottling (Mj). The data on the morphological characters in this last cross was of poor quality due to a severe infection of powdery mildew in the $F_2$ population. Thus, this data is not presented.

In cross 1236 and 1237 (derived from different Goldrush x Table King $F_1$ plants) nine isozyme loci segregated. In both populations the locus coding the "cytosolic GPI-2" isozymes (1), designated Gpi-c2, was linked to Aat-p2, the locus specifying the more slowly migrating chloroplast AAT isozymes (AAT-5 in Ignart and Weeden) (Table 2). The loci Skdh, Aldo-p, and Est also exhibited non-random assortment (Table 2). In both populations the apparent gene order was Skdh - Aldo-p - Est.

In the Senator x Table Queen $F_2$ population six isozyme loci segregated (Table 1), and one linkage group was identified: Gpi-c2 - Aat-p2 (Table 2). There also was a correlation between Skdh and growth habit; however, the highly skewed ratio obtained for the habit character (Table 1) suggested that other factors besides genetic linkage may have contributed to this correlation. Eight isozyme loci segregated in the Blackjack x Early Prolific $F_2$, all displaying phenotypic ratios close to the expected 1:2:1

(Table 1). The loci Skdh and Aldo-p again showed linkage (Table 2). The results also indicated a linkage between the fruit color and the MDH-2 phenotype (data not presented); however, the powdery mildew infestation interfered with the scoring of fruit color, and this linkage requires further verification.

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Table 1. Number of individuals with designated phenotypes in $F_2$ populations.

| Cross | Isozyme or Trait | N | Phenotype: Fast or + | Heterozygous | Slow or - | $\chi^2$ |
|---|---|---|---|---|---|---|
| Senator x Table Queen | | | | | | |
| | AAT-4 | 26 | 6 | 13 | 7 | 0.07 |
| | AAT-5 | 35 | 9 | 17 | 9 | 0.03 |
| | GPI-2 | 35 | 10 | 19 | 6 | 1.2 |
| | LAP | 16 | 3 | 7 | 6 | 1.4 |
| | MDH | 35 | 14 | 14 | 7 | 4.2 |
| | SKDH | 34 | 7 | 22 | 5 | 3.8 |
| | Fruit Shape | 27 | 18 | | 9 | 1.0 |
| | Habit | 35 | 13 | | 22 | 27** |
| Goldrush x Table King | | | | | | |
| 1236 | AAT-4 | 45 | 11 | 27 | 7 | 2.5 |
| | AAT-5 | 43 | 10 | 24 | 9 | 0.6 |
| | ALDO | 44 | 16 | 20 | 8 | 3.3 |
| | EST | 41 | 15 | 17 | 9 | 2.9 |
| | GPI-2 | 45 | 12 | 26 | 7 | 2.2 |
| | LAP | 45 | 7 | 30 | 8 | 5.0 |
| | MDH-3 | 44 | 14 | 22 | 8 | 1.6 |
| | PGM-2 | 45 | 10 | 28 | 7 | 3.1 |
| | SKDH | 45 | 16 | 22 | 7 | 3.6 |
| | Fruit Shape | 38 | 26 | | 12 | 0.9 |
| | Fruit Color | 39 | 34 | | 5 | 3.1 |
| 1237 | AAT-4 | 47 | 11 | 17 | 19 | 6.3* |
| | AAT-5 | 47 | 8 | 22 | 17 | 3.6 |
| | ALDO | 46 | 8 | 26 | 12 | 1.5 |
| | EST | 45 | 10 | 26 | 9 | 1.1 |
| | GPI-2 | 47 | 8 | 27 | 12 | 1.7 |
| | LAP | 46 | 12 | 24 | 10 | 0.26 |
| | MDH-3 | 47 | 13 | 19 | 15 | 1.9 |
| | PGM-2 | 46 | 6 | 28 | 12 | 3.7 |
| | SKDH | 47 | 6 | 24 | 17 | 5.2 |
| | Fruit Shape | 42 | 33 | | 9 | 0.28 |
| Blackjack x Early Prolific | | | | | | |
| | AAT-2 | 53 | 12 | 27 | 14 | 0.17 |
| | AAT-4 | 54 | 9 | 34 | 11 | 3.8 |
| | ALDO | 54 | 15 | 26 | 13 | 0.22 |
| | GPI-2 | 54 | 19 | 27 | 8 | 4.5 |
| | LAP | 46 | 16 | 23 | 7 | 3.5 |
| | MDH-3 | 54 | 8 | 32 | 14 | 3.2 |
| | MDH-4 | 54 | 18 | 22 | 14 | 2.4 |
| | SKDH | 54 | 15 | 25 | 14 | 0.33 |

*Significant at $p \geq 0.05$.

**Significant at $p \geq 0.01$.

Table 2. Joint segregation data for isozyme loci displaying significant deviations from random assortment.

| Loci | N | No. plants with designated phenotype* F/F | F/H | F/S | H/F | H/H | H/S | S/F | S/H | S/S | $\chi^2$ | Recomb. Fract. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Goldrush x Table King 1326 | | | | | | | | | | | | |
| Gpi-s2 : Aat-s2 | 43 | 9 | 1 | 0 | 3 | 19 | 2 | 0 | 4 | 5 | 35.8 | 13 ± 4 |
| Skdh : Aldo-s | 44 | 11 | 5 | 0 | 4 | 15 | 1 | 1 | 1 | 6 | 35.0 | 16 ± 4 |
| Skdh : Est | 41 | 10 | 4 | 1 | 5 | 12 | 2 | 0 | 1 | 6 | 26.7 | 19 ± 5 |
| Aldo-s : Est | 40 | 13 | 2 | 0 | 2 | 14 | 1 | 0 | 0 | 8 | 56.0 | 6 ± 3 |
| Goldrush x Table King 1327 | | | | | | | | | | | | |
| Gpi-s2 : Aat-s2 | 47 | 6 | 2 | 0 | 2 | 20 | 0 | 0 | 5 | 12 | 48.8 | 10 ± 3 |
| Skdh : Aldo-s | 46 | 2 | 6 | 0 | 3 | 16 | 7 | 1 | 1 | 10 | 17.6 | 24 ± 5 |
| Skdh : Est | 45 | 3 | 6 | 1 | 2 | 17 | 7 | 1 | 1 | 7 | 13.8 | 27 ± 6 |
| Aldo-s : Est | 44 | 7 | 3 | 0 | 1 | 23 | 1 | 0 | 0 | 9 | 60.2 | 6 ± 3 |
| Senator x Table Queen | | | | | | | | | | | | |
| Gpi-s2 : Aat-s2 | 35 | 7 | 2 | 0 | 3 | 14 | 0 | 0 | 3 | 6 | 33 | 12 ± 4 |
| Blackjack x Early Prolific | | | | | | | | | | | | |
| Skdh : Aldo-s | 54 | 9 | 3 | 2 | 4 | 16 | 5 | 0 | 7 | 8 | 22.1 | 24 ± 5 |

*Phenotypic designations: F = fast; H = heterozygous; S = slow.

RESEARCH NOTES

V. Other Genera

Plant Regeneration from Explant-Derived Calli of Cucumis anguria L. var longipes.

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Resistances to Tetranychus urticae KOCH, nematodes and cucumber green mottle mosaic virus have been described in Cucumis anguira L. var. longipes. Because of the difficulty in obtaining sexual crosses between this species and C. melo L., we are trying protoplast fusion to achieve hybridization. Basic studies aiming at acquiring knowledge of how in vitro cultured wild species behave have been carried out in our laboratory. In this paper we show the results of some of those experiments devoted to achieving morphogenesis in explant derived calli of C. anguria.

Seeds of C. anguria L. var longipes (kindly supplied by Dr. Jacobs, University of Stellenboch, South Africa) were surface sterilized and germinated on MG medium as previously described (4, 5). When the first true leaf appeared, the shoot apex (1-3 mm length) was excised and aseptically planted in 200 ml bottles containing 40 ml of MB3 solid medium (7). Leaf segments of about 1 $cm^2$ surface as well as internode stem segments of 1 cm length from 15-20 day old axenic plants were used as explants. The explants were cultured in a way similar to that described previously (5) for cotyledon and hypocotyl explants, respectively except that the stem fragments were longitudinally divided in two halves before they were put horizontally onto the solid medium. The culture media consisted of MB3 + 1 mg/l 6-benzylaminopurine + 100 ml/l coconut milk and indole-3-acetic acid (IAA) at the following concentrations: 0.00, 0.01, 0.05, 0.10, 0.50, 1.50, and 3.00 mg/l. All the cultures were incubated for 30 days in a 16 h photoperiod (1,500-1,800 lux) at 27±°C continuous temperature and 60-70% relative humidity.

Most of the growth of the explants on the assayed media was organized tissue. Unorganized proliferation of callus with organized zones was observed only on media with high levels of IAA (1.5 and 3.0 mg/l). Leaf-derived calli were morphologically different from stem derived ones. The former presented numerous, uniformly distributed trichomes, while the latter had fewer trichomes that were in discrete zones.

Table 1 shows the morphogenic responses of both kinds of explants. Calli arising from stem segments produced roots at frequencies higher than leaf-derived calli. The frequencies of shoot-buds and shoots producing calli were greater in leaf calli than in stem ones. Moreover, the number of shoots per callus was also greater in the leaf cslli, where they were practically innumerable.

The influence of the type of explant on the neoformation of shoots observed was similar to the one previously reported in explants of C. melo (5). Nevertheless, regarding the kind of apices developed, there exists a notable difference between both plant species: C. anguria produces well developed shoots which can be easily excised and rooted in MB3 medium to give whole plants; whereas C. melo usually gave few shoots, and, in order to increase the number of individual ones, organogenic pieces of calli must be subcultured in a shoot-developing medium.

Relative to shoot formation, the morphogenic response of primary explants was higher in C. anguria than in C. melo. The nutritional requirements, however, seem to be more restrictive than those of the latter. In preliminary studies (data not shown) media with different IAA and kinetin concentrations but without coconut milk, C. anguria explants did not produce shoots whereas C. melo explants (especially those from cotyledons) did generate shoots.

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## Stocks and Germplasm Desired or for Exchange

### Request from the Gene Curators

CGC has appointed gene Curators for four of the major cucurbit species: cucumber, muskmelon, watermelon and Cucurbita. A curator for species that fall into 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. ALL INVESTIGATORS ARE REQUESTED TO FORWARD SAMPLES OF CURRENTLY HELD GENES STOCKS TO THE RESPECTIVE CURATOR.

#### Cucumber

Curator: Todd C. Wehner, Dept. of Horticulture, North Carolina State University, Raleigh, NC 276695-7609

Approximately 97 mutants have described or brought to my attention. Many of these are not in the collection. Seed increases have been made of the approximately 50 mutants recieved to date. Send any mutant lines that you have or can obtain. The collection is especially weak for those mutants described by Kubicki and by Hutchins. Some of the desired mutants are:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| a | bu | Cm | Fba | H | m-2 | pl | psl | v |
| ap | c | dl | g | I | mp | pm-1 | rc | vvi |
| bl | cd | Es-1 | gb | In-de | ms-1 | pm-2 | T | yp |
| Bt | cla | Es-2 | gi | In-F | P | pm-3 | Tr | |

#### Muskemlon

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

Please send seed of any of the 74 known mutants that you have. Describe the lines as completely as possible, including references.

#### Watermelon

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

Please send seed of any of the 33 known mutants that you have. Describe the lines as completely as possible, including references.

#### Cucurbita

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

Please send seed of any of the 37 known mutants that you have. Describe the lines as completely as possible, including references.

## Gene List for Muskmelon (*Cucumis melo* L.)

Three lists of the genes of *Cucumis melo* have been published previously (12, 13, 52). Following is a complete and updated list of the 74 known genes of muskmelon. We hope to continue this practice, and publish a complete list for muskmelon every 3 years.

The names and symbols of two genes were changed: watermelon mosaic virus 1 is now regarded as a strain of papaya ringspot virus (49) so *Wmv* has been changed to *Prv*. Paris *et al*. (41) proposed that *bush* symbolized *b* be changed to *short internode* (*si*) because it is more descriptive of the phenotypic effect of the gene, and because of a second gene that also affects internode length, *si-2*. This list includes these proposed changes.

Four additional mutants have been described but not tested for allelism with known mutants of similar phenotypes. Three affect leaf characteristics: cut leaf (57), dissected leaves (17) and lobed leaves (19). The fourth is a chlorophyll mutant, flava (42).

| Gene symbol | | | |
|---|---|---|---|
| Preferred | Synonym | Character | Reference |
| *a* | *M* | *andromonoecious*. Mostly $a^+$ staminate, fewer perfect flowers; on $a^-$ plants, pistillate flowers have no stamens; epistatic to *g*. | 47, 53 |
| *ab* | | *abrachiate*. Lacking lateral branches. Interacts with *a* and *g* (e.g. *abab aa* $g^-$ plants produce only staminate flowers). | 22 |
| *Af* | | *Aulacophora foveicollis* resistance. Resistance to the red pumpkin beetle. | 56 |
| *Ag* | | *Aphis gossypii* tolerance. Freedom from leaf curling following aphid infestation. | 4 |
| *Al-1* | $Al_1$ | *Abscission layer-1*. One of two dominant genes for abscission layer formation. See *Al-2*. | 55 |
| *Al-2* | $Al_2$ | *Abscission layer-2*. One of two dominant genes for abscission layer formation. See *Al-1*. | 55 |
| $Ap\text{-}1^1$ | ($APS\text{-}1^1$) | *Acid phospatase-*$1^1$. One of two codominant alleles, each regulating one band. The heterozygote has two bands. See $Ap\text{-}1^2$. | 20 |
| $Ap\text{-}1^2$ | ($APS\text{-}1^2$) | *Acid phospatase-*$1^2$. One of two codominant alleles, each regulating one band. The heterozygote has two bands. See $Ap\text{-}1^1$. | 20 |

| Gene symbol Preferred | Synonym | Character | Reference |
|---|---|---|---|
| Bi | | Bitter. Bitter seedling; dominant to nonbitter. | 32 |
| dc-1 | | Dacus cucurbitae-1 resistance. One of two complementary recessive genes for resistance to the melon fruitfly. See dc-2. | 54 |
| dc-2 | | Dacus cucurbitae-2 resistance. One of two complementary recessive genes for resistance to the melon fruitfly. See dc-1. | 54 |
| Fn | | Flaccida necrosis. Semi-dominant gene for wilting and necrosis with F pathotype of zucchini yellow mosaic virus. | 46, 51 |
| Fom-1 | $Fom_1$ | Fusarium oxysporum f. melonis resistance. Resistance to races 0 and 2, and susceptibility to race 1, 3 and race 1, 2, 3 of fusarium wilt. See Fom-3. | 50 |
| Fom-2 | $Fom_{1.2}$ | Fusarium oxysporum f. melonis resistance. Resistance to races 0 and 1, 3 and susceptibility to race 2 and race 1, 2, 3 of fusarium wilt. | 50, 63 |
| Fom-3 | | Fusarium oxysporum f. melonis resistance. Same phenotype as Fom-1 but segregates independently (15 resistant:1 susceptible in $F_2$) from Fom-1. | 63 |
| g | | gynomonoecious. Mostly pistillate, fewer perfect flowers. Epistatic to a: a$^+$ _ g$^+$ _ monoecious; a$^+$ _ gg gynoecious; aa g$^+$ _ andromonoecious; aa gg hermaphroditic. | 47 |
| gf | | green flesh color. Recessive to salmon. | 26 |
| gl | | glabrous. Trichomes lacking. | 21 |
| gp | | green petals. Corolla leaf-like in color and venation. | 38 |
| gyc | | greenish yellow corolla. | 62 |
| h | | halo cotyledons. Yellow cotyledons, later becoming green. | 40 |
| jf | | juicy flesh. Segregates discreetly in monogenic ratio in segregating generations. | 9 |

| Gene symbol | | Character | Reference |
|---|---|---|---|
| Preferred | Synonym | | |
| lmi | | long mainstem internode. Affects internode length of the main stem but not of the lateral ones. | 35 |
| Mc | | Mycosphaerella citrullina resistance. High degree of resistance to gummy stem blight. | 48 |
| Mc-2 | $Mc^1$ | Mycosphaerella citrullina resistance. Moderate degree of resistance to gummy stem blight. | 48 |
| ms-1 | $ms^1$ | male sterile-1. Indehiscent anthers with empty pollen walls in tetrad stage. | 7 |
| ms-2 | $ms^2$ | male sterile-2. Anthers indehiscent, containing mostly empty pollen walls, growth rate reduced. | 5 |
| ms-3 | ms-L | male sterile-3. Waxy and translucent indehiscent anthers,containing two types of empty pollen sacs. | 37 |
| ms-4 | | male sterile-4. Small, indehiscent anthers. First male flowers abort at bud stage. | 33 |
| n | | nectarless. Nectaries lacking in all flowers. | 2 |
| nsv | | necrotic spot virus resistance. | 11 |
| O | | Oval fruit shape. Dominant to round; associated with a. | 58 |
| p | | pentamerous. Five carpels and stamens; recessive to trimerous. | 53 |
| Pa | | Pale green foliage. PaPa plants are white (lethal); $Pa^+Pa$ are yellow. | 36 |
| Pc-1 | | Pseudoperonospora cubensis resistance. One of two complementary incompletely dominant genes for downy mildew resistance in PI 124111. See Pc-2. | 10 |
| Pc-2 | | Pseudoperonospora cubensis resistance. See Pc-1. | 10 |
| $Pgd\text{-}2^1$ | ($6\text{-}PGDH\text{-}2^1$) | Phosphoglucodehydrogenase-$2^1$. One of two codominant alleles that regulate 6-phosphoglucodehydrogenase, each regulates one band. The heterozygote has one intermediate band. See $Pgd\text{-}2^2$. | 20 |

| Gene symbol Preferred | Synonym | Character | Reference |
|---|---|---|---|
| Pgd-$2^2$ | (6-PGDH-$2^2$) | Phosphoglucodehydrogenase-$2^2$. One of two codominant alleles that regulate 6-phosphoglucodehydrogenase, each regulates one band. The heterozygote has one intermediate band. See Pgd-$2^1$. | 20 |
| Pgi-$1^1$ | (PGI-$1^1$) | Phosphoglucoisomerase-$1^1$. One of two codominant alleles, each regulating two bands. The heterozygote has three bands. See Pgi-$1^2$. | 20 |
| Pgi-$1^2$ | (PGI-$1^2$) | Phosphoglucoisomerase-$1^2$. One of two codominant alleles, each regulating two bands. The heterozygote has three bands. See Pgi-$1^1$. | 20 |
| Pgi-$2^1$ | (PGI-$2^1$) | Phosphoglucoisomerase-$2^1$. One of two codominant alleles, each regulating two bands. The heterozygote has three bands. See Pgi-$2^2$. | 20 |
| Pgi-$2^2$ | (PGI-$2^2$) | Phosphoglucoisomerase-$2^2$. One of two codominant alleles, each regulating two bands. The heterozygote has three bands. See Pgi-$2^1$. | 20 |
| Pgm-$2^1$ | (PGM-$2^1$) | Phosphoglucomutase-$2^1$. One of two codominant alleles, each regulating two bands. The heterozygotes has three bands. See Pgm-$2^2$. | 20 |
| Pgm-$2^2$ | (PGM-$2^2$) | Phosphoglucomutase-$2^2$. One of two codominant alleles, each regulating two bands. The heterozygote has three bands. See Pgm-$2^1$. | 20 |
| Pm-1 | $Pm^1$ | Powdery mildew resistance. Resistance to race 1 of Sphaerotheca fuligenea. | 28, 29 |
| Pm-2 | $Pm^2$ | Powdery mildew resistance-2. Interacts with Pm-1. Resistance to races 1 and 2. | 8 |
| Pm-3 | $Pm^3$ | Powdery mildew resistance-3. Resistance derived from PI 124111. | 24 |
| Pm-4 | $Pm^4$ | Powdery mildew resistance-4. Resistance derived from 'Seminole'. | 24 |
| Pm-5 | $Pm^5$ | Powdery mildew resistance-5. Resistance derived from 'Seminole'. | 24 |

| Gene symbol Preferred | Synonym | Character | Reference |
|---|---|---|---|
| *Prv*$^{1}$ | *Wmv* | *Papaya ringspot virus* resistance. Resistance to *W* strain of papya ringspot virus (formerly watermelon mosaic virus 1); dominant to *Prv*$^{2}$. | 44, 59 |
| *Prv*$^{2}$ | | *Papaya ringspot virus* resistance. Derived from PI 180283. Allele at the same locus as *Prv*$^{1}$ but different reaction with some strains of the virus; recessive to *Prv*$^{1}$; dominant to *Prv*$^{+}$. | 30, 44 |
| *Px-1*$^{1}$ | (*PRX-1*$^{1}$) | *Peroxidase-1*$^{1}$. One of two codominant alleles, each regulating a cluster of four adjacent peroxidase bands. The heterozygote has five bands. See *Px-1*$^{2}$. | 20 |
| *Px-1*$^{2}$ | (*PRX-1*$^{2}$) | *Peroxidase-1*$^{2}$. One of two codominant alleles, each regulating a cluster of four adjacent peroxidase bands. The heterozygote has five bands. See *Px-1*$^{1}$. | 20 |
| *Px-2*$^{1}$ | (*Px*$_{2A}$) | *Peroxidase-2*$^{1}$. One of two codominant alleles, each regulating a cluster of three adjacent peroxidase bands. See *Px-2*$^{1}$. | 15 |
| *Px-2*$^{2}$ | (*Px*$_{2B}$) | *Peroxidase-2*$^{2}$. One of two codominant alleles, each regulating a cluster of three adjacent peroxidase bands. See *Px-2*$^{1}$. | 15 |
| *r* | | *red* stem. Red pigment under epidermis of stems, especially at nodes; tan seed coat color. | 3, 36 |
| *ri* | | *ridge*. Ridged fruit surface, recessive to ridgeless. | 55 |
| *s* | | *sutures*. Presence of vein tracts ("sutures"); recessive to ribless | 1 |
| *si-1* | *b* | *short internode-1*. Short internodes. Extremely compact plant habit. | 6, 16, 39, 41 |
| *si-2* | | *short internode-2*. Short internodes from 'birdsnest' melon. | 41 |
| *So* | | *Sour* taste. Dominant to sweet. | 31 |
| *sp* | | *spherical* fruit shape. Recessive to obtuse; dominance incomplete. | 1, 34 |
| *st* | | *striped* epicarp. Recessive to nonstriped. | 23 |

| Gene symbol Preferred | Synonym | Character | Reference |
|---|---|---|---|
| v | | virescent. Pale cream cotyledons and hypocotyls; yellow-green foliage. | 25 |
| v-2 | | virescent-2. | 18 |
| Vat | | Virus aphid transmission resistance. Resistance to the transmission of all the viruses by Aphis gossypii. | 43, 46 |
| w | | white color of mature fruit. Recessive to dark green fruit skin. | 26 |
| wf | | white flesh. Recessive to orange. | 27 |
| Wi | | White color of immature fruit. Dominant to green. | 31 |
| Wt | | White testa. Dominant to yellow or tan seed coat color. | 23 |
| Y | | Yellow epicarp. Dominant to white fruit skin. | 23 |
| yg | y | yellow green leaves. Reduced chlorophyll content. | 60 |
| yv | | yellow virescence. Pale cotyledons; yellow green young leaves and tendrils; bright and yellow petals and yellow stigma; etiolated; older leaves becoming green. | 61 |
| Zym | | Zucchini yellow mosaic virus resistance. Resistance to pathotype 0 of this virus. | 45 |

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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 (11, 12, 13, 40) before choosing a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature were adopted in order to provide guidelines for the naming and symbolizing genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

Gene List Committee:
Cucumber: T. C. Wehner
Muskmelon: M. Pitrat
Watermelon: W. R. Henderson
Cucurbita spp.: T. W. Whitaker
Other Genera: 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 curcurbits, 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. Bemis

J. D. Norton

R. W. Robinson

W. R. Henderson

M. L. Robbins

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. Bemis

J. D. Norton

R. W. Robinson

W. R. Henderson

M. L. Robbins

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. Bemis

J. D. Norton

R. W. Robinson

W. R. Henderson

M. L. Robbins

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. Bemis
(Cucurbits sp.)

W. R. Henderson
(Watermelon)

J. D. Norton
(Muskmelon)

M. L. Robbins
(Cucumber)

R. W. Robinson
(Other genes and species)

R. L. Lower, Chairman

# 1986 MEMBERSHIP DIRECTORY

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# FINANCIAL STATEMENT

31 December 1985

| | | |
|---|---|---|
| Balance on 30 June 1985 | | $2,303.04 |
| Receipts 1 July 1985 through 31 December 1985 | | |
| Dues and Back Issues | 294.00 | |
| Interest | 50.73 | 344.73 |
| Expenditures | | |
| Report #8 (Publishing and Mailing) | 1,019.28 | |
| Reports #1,#2,#7 (Reprinting) | 466.66 | |
| Report #9 (Call for Papers) | 149.39 | |
| Miscellaneous | 20.04 | 1,655.37 |
| BALANCE on 31 December 1985 | | $2,644.19 |