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A Gynoecious Line of B^+B^+ Genotype in *Cucurbita pepo*

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A phenotypically sensitive gynoecious line of *Cucurbita pepo* L., NJ34, has been synthesized recently through crosses of monoecious inbreds and selection (3). NJ34 is 100% pistillate in some environments, but it differentiates a few staminate flowers in other environments. This line is *BB*, in which gene *B* conditions precocious yellow pigmentation of fruit. Since previous observations suggested that *B* can also increase female expression (1), a question arose: Is *B* essential for the synthesis of gynoecism in this species?

There exists another phenotypically sensitive gynoecious line, NJ20, which is closely related to NJ34 and is also *BB*. A mutant was found in 1983 in a small (n = 14) field planting of NJ20. This mutant was female, but unlike females of the 2 gynoecious lines, which normally produce yellow fruit, the mutant plant produced bicolor as well as green fruit. This fact suggested that the mutant was a heterozygote, BB^+, because some known BB^+ hybrids produce fruit of similar color variation (2).

The female mutant was propagated vegetatively in a greenhouse (Fall 1983), and one of its clones was self-pollinated (Summer 1984) following its conversion into monoecism by a gibberellin treatment (3). The offspring and 3 controls—the monoecious cultivar Caserta, NJ20, and NJ34—were planted on 14 Oct. 1984 in a growth chamber (16-hr photoperiod; light of 270 $\mu mol \cdot s^{-1} \cdot m^{-2}$, 95% from fluorescent tubes—Sylvania F96T12CWVHORL—and 5% from incadescent bulbs; 20°C during the light period and 16° in the dark). At about the 5-leaf stage, the plants were transferred into a greenhouse (16-hr photoperiod; supplementary light of 400 $\mu mol \cdot s^{-1} \cdot m^{-2}$ from high-pressure sodium lamps and fluorescent tubes; unrecorded fluctuating temperatures) for further growth and classification.

'Caserta' began to flower at the 2nd leaf axil on the main stem. In contrast, NJ20, NJ34, and the offspring of the mutant began to flower at about the 5th leaf axil. Twenty-five flowering leaf axils of each plant were classified for sex expression. Under the prevailing conditions, 'Caserta' (n = 12) was monoecious, whereas NJ20 (n = 18), NJ34 (n = 18), and the offspring of the mutant (n = 116) were gynoecious. Of the 116 female offspring obtained from the mutant, 62 produced yellow fruit, 51 produced bicolor or bicolor as well as green fruit, and 3 produced green fruit exclusively. One of the 3 green-fruited females was self-pollinated following gibberellin treatment, and its progeny were grown under conditions similar to those described for the offspring of the original mutant. The tested progeny (n = 84) proved to be gynoecious and green-fruited. Clearly, this female progeny was B^+B^+. Therefore, *B* is not essential for the synthesis of gynoecism.

If the original mutant were a normal BB^+ heterozygote, its self-pollinated offspring would have given a monohybrid ratio of 1 *BB* (yellow) : 2 BB^+ (bicolor and green) : 1 B^+B^+ (green). However, the above classification implies that the offspring consisted of 62 *BB*, 51 BB^+, and 3 B^+B^+ plants, a statistically significant deviation ($P < 0.001$) from the expected 1:2:1 ratio. The cause of this deviation is not known, but the mutant could have been a chimeric individual consisting of *BB* and BB^+ cell lineages.

From a practical point of view, the results demonstrate that gynoecious lines can be developed in both the standard (B^+B^+) and the precocious (*BB*) systems of fruit pigmentation.

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A Labor-saving, Nutrient-screening Procedure Using Large-batch Solution Culture

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The identification of exceptional genotypes in a breeding program can involve evaluating the responses of large numbers of plants to environmental stress (2). Major hydroponic systems include aerated standing or flowing-nutrient solution, mist, and nutrient film cultures. Flowing solution culture currently provides the most consistent environment for roots, but it is costly and difficult to maintain (1, 3).

Aerated standing solution cultures in small- or large-batch systems have been used successfully to evaluate large numbers of plants. However, most of these procedures require transplanting after germination and adjusting collar supports around individual plants for long-term evaluations. These steps are labor intensive and can, inadvertently, stress the plants (4).

The objective of this study was to develop a technique using large-batch culture useful in screening large numbers of genotypes rapidly with minimal cost and labor. This method was evaluated by measuring germination percentages and uniformity in 2-week-old cabbage plants and 2nd by examining differential growth response of cabbage to toxic copper solutions.

The basic system consisted of 42-liter vats that supported plastic panels upon which the seeds could be germinated and left intact during the entire screening procedure. The panel system was adapted from a small-scale lettuce germination scheme used by T.W. Tibbitts (personal communication) to produce seedlings for transplanting to solution culture.

A 110 × 30 × 15 cm frame was assembled from construction-grade redwood (2.5 × 15.2 cm). The tops of the frames were leveled to ensure an even solution surface when vats were filled to capacity. The vats were formed by lining the frame with seamless 4 mil black polyethylene film. Plastic

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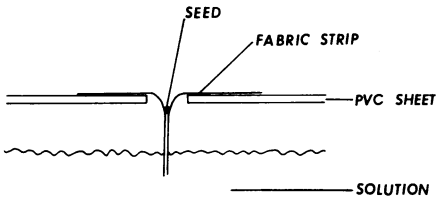

Fig. 1. Setup for placement of cabbage seeds between fabric strips.

film was used because it was disposable, and acid washing thereby was eliminated. Leaks were not a problem.

The growing panel, which supported seeds and seedlings, was unplasticized polyvinyl chloride (PVC type I), chosen for its chemical resistance, its ease of cutting, and its opaqueness, which prevented algae growth. The PVC sheets were 4 mm thick (3/32 inch), but the thickness should be increased if larger panels are desired. A rotor was used to make 9 slits (26 × 0.5 cm) in each PVC sheet (40 × 30 cm). Two holes also were drilled in each panel through which glass tubing could be inserted for aeration of the solution below. Three panels covered each vat completely.

Two fabric strips (26 × 4.5 cm) were hung through each slit in the plastic growing panel (Fig. 1). The strips were made from dacron and polyester fabric (Coupe de Ville, Burlington Klopman) selected for its acid resistance, ease of handling, and moisture retention with a low salt buildup. The fabric was rinsed 6 times in distilled water more than 24 hr to remove any substances that might inhibit germination and was cleaned after each use by soaking in 1 M HCl for one to 2 days. The strips then were rinsed and dried in a conventional washing machine and dryer. If the strips were kept saturated with water during their installation, they adhered easily to the surface of the panel. A dish pan filled with distilled H_2O was used to support the panels temporarily while strips were applied and seeds planted.

Each vat was filled with full-strength Hoagland's solution (3) and covered with the plastic growing panels. Seeds (one cultivar per row or 27 cultivars per vat) then were placed between the fabric strips. Proper seed placement (Fig. 1) was critical for acceptable germination. Contact between fabric and solution was maintained throughout the entire procedure. Relative humidity was kept at 50% or higher for even germination.

After germination, seedlings were thinned with blunt-tipped tweezers to 3-cm spacing. When roots reached the solution below, aeration was supplied. The sheets of plants were transferred to the treatment solution soon after the first true leaves emerged. Elemental analysis of the used Hoagland's solutions (determined by inductively coupled plasma emission spectrophotometry) revealed no change in the solution composition after 2 weeks.

The germination percentages of 8 cabbage cultivars grown in this system were comparable to those reported by seed suppliers. Uniformity also was tested by growing a single cabbage cultivar ('A&C #5') for 2 weeks in 3 vats, 3 sheets per vat, and 9 rows per sheet. The average shoot dry weight per plant was calculated by dividing the total row dry weight by the number of plants in that row. Since there were no significant differences at the 5% level between yields (Table 1) in vats or sheets and the coefficients of variation between rows were less than 13%, this method provided extremely uniform growth among cabbage seedlings (Table 1).

The screening procedure was tested by exposing cabbage cultivars to toxic Cu^{2+} solutions and measuring growth responses. Initially, cabbage seeds were germinated in full-strength Hoagland's solution at normal Cu^{2+} levels 0.03 mg·liter^{-1}). After 10–14 days, the cabbage seedlings were transferred to treatment vats 0.03, 1.5, 2.0 mg·liter^{-1} Cu^{+2}). Plants were grown until there was a 50–60% reduction in shoot growth at the highest stress level when compared to the control. Shoot crowding and root entanglement were avoided by harvesting as soon as treatment effects were measurable. After exposure to Cu^{2+} levels of 1.5 and 2.0 mg·liter^{-1} for 7 days, the shoot yields were 60% and 80% of the control, respectively.

Advantages of this method include elimination of transplanting, easily measured germination rates, and quickly changed solutions. Also, entire groups of genotypes can be transferred easily from one treatment to another, since many genotypes grow on each sheet.

Table 1. Test of uniformity in 2-week-old cabbage[z] plants grown using a large-batch solution culture method.

		Shoot		Root	
Vat	Growing panel	Dry wt (mg)[y]	CV[x]	Dry wt (mg)[y]	CV[x]
1	1	26.6	5.8	4.0	12.6
	2	30.4	6.7	4.3	9.2
	3	29.7	4.9	4.7	9.0
2	4	29.3	2.5	4.4	6.0
	5	29.1	6.0	4.2	8.4
	6	31.1	7.0	4.5	8.2
3	7	30.0	7.8	4.3	11.1
	8	29.9	7.3	4.4	8.6
	9	29.6	8.0	4.6	9.4

[z]Cultivar: A&C #5.
[y]Average yield per plant per row.
[x]Coefficient of variation (%), 9 rows per panel.

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Carnation Plantlets from Vitrified Plants as a Source of Somaclonal Variation

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Organ cultures of carnations have been used as a source of virus-free plants (14) and for rapid vegetative propagation through adventitious shoot formation (1, 4, 12). The use of apical meristems has assumed that the new carnation plants remain true to type (10). Some cultured shoot tips develop abnormally as translucent plantlets with short stems and thick, fragile leaves. This phenomenon has been observed in various species and is referred to as "vitrification" (3, 7). These stunted, bushy succulent plants are commonly found in carnation cultures (5, 6, 8, 9, 15, 18) and have been suggested for the propagation and maintenance of pathogen-free carnation tissue under aseptic conditions (5).

The possibility of obtaining new cultivars through somatic variation in culture (soma-

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