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Interaction between Explant Size and Cultivar Affects Shoot Organogenic Competence of Watermelon Cotyledons

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Abstract. **Organic competence of different explant sizes and locations on watermelon seedlings was determined by calculating the percentage of cotyledon explants that produced adventitious shoots. About 52% (214/412) of explants prepared from the proximal region of cotyledons formed shoots, whereas only ≈6% (24/411) of distal explants did so. Shoot formation was limited to the proximal end of basal explants but was not restricted to any specific region on distal ones. The percentage of explants that produced harvestable shoots was greater from basal halves than basal quarters in 'Sweet Gem', 'Crimson Sweet', and 'Minilee', but explant size did not affect adventitious shoot regeneration of 'Yellow Doll', resulting in significant interaction between cultivar and explant size. This study indicates that cultivars that respond poorly to in vitro procedures may have fewer cells competent for shoot regeneration, requiring special care during explant preparation.**

Adventitious shoot regeneration from cotyledons of diploid watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is the chosen route for obtaining plants through genetic transformation (Choi et al., 1994) and for the production of tetraploid parental lines used to breed seedless watermelon (Compton et al., 1996). Several different types of cotyledonary explants have been used for adventitious shoot regeneration of watermelon, including distal and proximal halves (Choi et al., 1994; Dong and Jia, 1991; Srivastava et al., 1989) as well as proximal halves bisected longitudinally (Compton and Gray, 1993a). The explants in the aforementioned studies were prepared from cotyledons of mature seedlings; similar explants have been prepared from seeds collected from immature fruit 20 d after pollination (DAP; Zhang et al., 1994). However, response varies with cultivar, type of explant, and seed maturity. For example, Compton and Gray (1993a) obtained ≈85% shoot regeneration from cotyledon bases of 5-d-old seedlings from mature seed of 'Mickylee', whereas only ≈19% of cotyledon explants produced shoots when Zhang et al. (1994) used explants prepared from embryos excised from immature fruit of the same cultivar collected 20 DAP. The objective of this study was to compare the effects of explant size and location on adventitious shoot organogenesis from cotyledons of four diploid watermelon cultivars.

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Materials and Methods

Explant preparation and culture procedures. Mature seeds of four watermelon cultivars ('Minilee', 'Crimson Sweet', 'Sweet Gem', and 'Yellow Doll') were used. These cultivars were chosen because they are genetically diverse and distantly related. Mature seed was chosen because a consistent, year-round supply of many cultivars is available. Seeds were surface-disinfested in 50% bleach (2.5% NaOCl plus 0.1% Tween 20®) for 30 min, rinsed three times with sterile-distilled water and imbibed for at least 6 h in sterile-distilled water. Seeds were decoated and embryos surface-disinfested in 25% bleach for 15 min before six rinses with sterile-distilled water. Embryos (nine) were germinated in Magenta GA_7 (Magenta Corp., Chicago) vessels containing 50 mL watermelon embryo germination medium (Compton and Gray, 1993a), and were incubated in darkness at 25 °C during germination (Compton, 2000). Explants were obtained from 5-d-old seedlings from which the cotyledons were removed by making a cut ≈2 mm beyond the point of attachment to the hypocotyl. Explant types tested included distal or proximal halves and distal or proximal quarters of cotyledons. Explants (six/vessel) were cultured abaxial side down in 100 × 15-mm plastic petri dishes that contained 25 mL watermelon shoot regeneration medium (Compton and Gray, 1993a). Explants were subcultured to fresh medium at 3 weeks and remained on regeneration medium for a total of 6 weeks. Explants with harvestable shoots and shoot buds were transferred to Magenta GA_7 vessels containing 50 mL watermelon shoot elongation medium (Compton and Gray, 1993a) and cultured for 4 weeks. All cultures were maintained under a 16-h photoperiod (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent lamps) at 25 °C during shoot regeneration and elongation.

Experimental design and data analysis. Treatments were arranged in a completely randomized design with subsampling. For each treatment 24 distal and proximal half-cotyledon explants and 48 distal and proximal quarter-cotyledons were used. The experiment was conducted twice. The number of explants with harvestable shoots was recorded after 4 weeks on shoot elongation medium. Harvestable shoots were defined as those having a vertical axis (≥1.0 cm) with a discernible apex and at least two nodes. Shoots of this type root best (Compton et al., 1993). Data were analyzed using three-dimensional chi-square contingency tables testing for mutual and partial independence (Zarr, 1984). Treatment means were compared using the standard error of the mean.

Results and Discussion

Chi-square analysis indicated that explant type, size, and genotype were not mutually independent with regard to influence on shoot organogenesis ($P \leq 0.001$). Therefore, additional tests for partial independence were required. One test indicated that the type of explant from the cotyledon (distal vs. proximal) was independent of cultivar and explant size ($P = 0.21$). Therefore, data for cultivar and explant size were pooled for each explant type. About 52% (214/412) of explants prepared from the proximal region of seedling cotyledons formed shoots, whereas only ≈6% (24/411) of distal explants did so (data not shown). Shoot formation was limited to the proximal end of basal sections but was not restricted to any specific region on distal explants. These results differ from those of Choi et al. (1994) that shoot regeneration was greater from the distal than the proximal region of 'Sweet Gem' cotyledons, as well Compton and Gray's (1993a) report that shoot regeneration was restricted to the basal portion of 'Jubilee II' and S86NE cotyledons . However, they parallel reports regarding shoot formation from proximal and distal explants prepared from immature seeds of the watermelon breeding line JXP-91 (Zhang et al., 1994).

Chi-square analysis for partial independence indicated that shoot formation was influenced by an interaction between genotype (cultivar) and explant size ($P = 0.014$). Adventitious shoot production was more efficient from cotyledon bases than from basal quarters in 'Sweet Gem', 'Crimson Sweet', and 'Minilee', but was not affected by explant type in 'Yellow Doll' (Fig. 1). The percentage of explants that produced harvestable shoots was 2.8-, 1.6-, and 1.2-fold as great from basal halves as from basal quarters in the three cultivars ('Sweet Gem', 'Crimson Sweet', and 'Minilee, respectively).

Poorly responding cultivars may have fewer cells competent for shoot regeneration. Therefore, cutting cotyledon bases longitudinally reduces the number of competent cells in an individual explant to below the minimum threshold. Others have shown that the number of competent cells present can influence organogenesis. Cell aggregate size is important in determining organogenic competence in al-

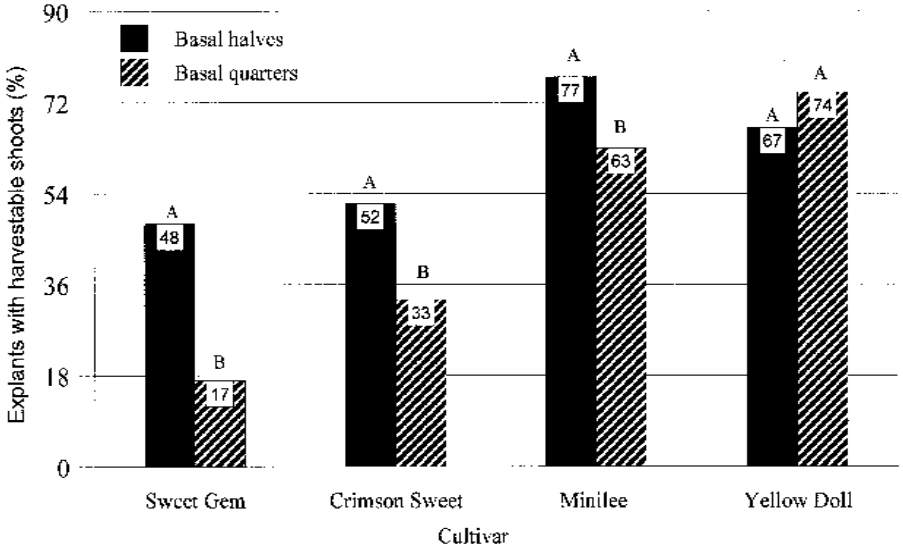

Fig. 1. Influence of explant type (basal halves vs. basal quarters) on the percentage of cotyledonary explants from four watermelon cultivars that produced harvestable shoots. Harvestable shoots were defined as those having a vertical axis (≥1.0 cm) with a distinct apex and at least two nodes. The total number of basal halves and basal quarters were 29 and 48, 42 and 84, 35 and 72, and 36 and 66 for 'Sweet Gem', 'Crimson Sweet', 'Minilee', and 'Yellow Doll', respectively. Mean separation within cultivars by standard error of the mean.

falfa (*Medicago sativa* L.) (Walker, 1979). In addition, thin cell layer explants of *Torenia fournieri* Lind. consisting of the epidermis alone were incapable of organogenesis (Chlyah, 1974). Shoots could be stimulated to form from epidermal tissue only if it was in direct contact with cortical cells. Cell population is also critical for division of plant protoplasts (Bhojwani and Razdan, 1983). Therefore, measures must be taken to use explants of sufficient size to ensure that enough competent cells are present to support cell division and organogenesis, especially in situations that generate cellular stress (e.g., cell culture and genetic transformation).

This is the first published study to report that adventitious shoot regeneration in watermelon is influenced by an interaction between genotype and explant size. Previous studies have either tested fewer genotypes and observed a similar response among the genotypes (Choi et al., 1994; Compton and Gray, 1993a) or examined explants prepared from immature seeds of a single genotype (Zhang et al., 1994). In previous studies of somatic embryogenesis in watermelon, cotyledon explants prepared from immature and mature seed did not respond similarly in vitro (Compton and Gray, 1993b), and 85% of basal explants prepared from seedlings from mature 'Mickylee' seeds formed shoots (Compton and Gray, 1993a), whereas only ≈19% of explants prepared from embryos excised from seeds extracted from immature fruit collected 20 DAP did so (Zhang et al., 1994). Work with melon (*Cucumis melo* L.) demonstrated that embryogenic competence can vary with seed lot (Gray et al., 1993). Based on the above information, sweeping generalizations regarding organogenic competence should not be made when testing a single genotype or seed source. Distantly related cultivars, as used in this study, and several seed sources should be examined before drawing general conclusions.

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