Recently, the general term "somaclonal variation" has been applied to the phenotypic variation observed in regenerates from undifferentiated cells (callus, cell suspensions, protoplasts) has been known for 20 years (10, 13). Repeated applications of soil fumigants as they affect commercial chrysanthemum production. Florida Flower Grower. Vol. 6 # 5.


A MICROPROPAGATION PROTOCOL USING FRASER PHOTINIA FOR MUTATION INDUCTION AND NEW CULTIVAR SELECTION

M. E. Kane, T. J. Sheehan, and N. L. Philman
University of Florida, IFAS
Department of Ornamental Horticulture
Gainesville, FL 32611

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Abstract. A micropropagation protocol for Photinia X fraseri Dress is described. This protocol will facilitate future research on the clonal multiplication and screening of phenotypic variants generated in vitro via somaclonal variation and induced mutation. Surface sterilized nodal explants were established on Woody Plant Medium (WPM) supplemented with 2 μM N\(^{-}\)benzyladenine (BA). Shoot multiplication was examined from nodal explants cultured on either WPM or Murashige & Skoog Medium (MS) supplemented with factor combinations of BA (2-16 μM), naphthaleneacetic acid (NAA) [0 and 0.5 μM] and 3 μM gibberellic acid (GA\(_3\)). Optimal shoot proliferation was observed from explants cultured on WPM supplemented with 8 μM BA alone for 35 days. Maximum rooting (66%) of microcuttings was achieved on half-strength MS supplemented with 5 μM indolebutyric acid (IBA). Transplant survival of rooted microcuttings in Vergro soilless potting medium exceeded 80%.

The occurrence of genetic variability in plants regenerated from undifferentiated cells (callus, cell suspensions, protoplasts) has been known for 20 years (10, 13). Recently, the general term "somaclonal variation" has been applied to the phenotypic variation observed in regenerated plants, irrespective of origin in vitro (7). Long thought to be a disadvantage for in vitro vegetative propagation, somaclonal variation is becoming recognized as a valuable tool for cultivar improvement in such crops as sugarcane (6), potato (14) and tomato (5). Somaclonal variation as well as induced mutations are also useful in the development of new commercially valuable cultivars in ornamental crops such as chrysanthemum (2) and geranium (15). However, little progress is being made in using these methods for improving woody landscape plants. Development of techniques to produce new commercially valuable woody plant cultivars for release could have a favorable impact on both the tissue culture and nursery industry in Florida.

Photinia X fraseri (Fraser photinia) is an important woody landscape plant used for hedging and screening in the Southeastern United States (3, 4). Desirable leaf characteristics, pigmentation, rapid growth, and ease of container and field production make Fraser photinia a valuable but often overused nursery crop (3). Somaclonal variation and induced mutation in vitro could be used to provide commercially valuable phenotypic variations possibly including dwarf growth habit and enhanced branching. Prerequisite to further study, it was necessary to determine whether Fraser photinia could be rapidly propagated in vitro via enhanced axillary branching. This micropropagation pathway would facilitate rapid shoot multiplication while preserving genetic stability of recovered variants for screening and field evaluation (12, 13). Consequently, the objective of this study was to develop a protocol for the rapid micropropagation of Fraser photinia via axillary branching.

Materials and Methods

Explant sterilization and culture establishment. In March 1986, 5-cm shoot tip cuttings were taken from actively growing and sexually mature plants of Photinia X fraseri...

Dress in Gainesville, Florida. Shoot cuttings were defoliated, subdivided into stem explants each consisting of two nodes, and rinsed for 1 hr in tap water. Nodal explants were next surface sterilized by successive immersion in 50% (v/v) ethanol for 1 min and 1.0% (v/v) sodium hypochlorite for 12 min, followed by three 5 min rinses in sterile deionized distilled water. Explants were transferred to 25 x 150 mm culture tubes onto 15 ml medium containing Woody Plant Medium (WPM) salts and vitamins (8) supplemented with 58.4 mM sucrose, 2 \( \mu \text{M} \) N6-benzyladenine (BA) and solidified with 0.7% (w/v) TC agar (Hazleton Research Products, Inc., Lenexa, KS). The medium pH was adjusted to 5.7 with 0.1 N KOH before autoclaving at 1.2 Kg cm\(^{-2}\) for 20 min at 121 °C. All cultures and experiments were maintained at 25 ± 2 °C under a 16-hr photoperiod provided by cool-white fluorescent lamps at 90 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \) as measured at culture level. Stock cultures were subdivided and transferred onto fresh medium at 5-week intervals.

**Optimal shoot proliferation medium.** Stem explants (1 cm length and each bearing two axillary buds with attached subtending leaves) obtained from in vitro produced shoots were transferred into 25 x 150 mm culture tubes containing either 15 ml sterile WPM or Murashige and Skoog (MS) salts and vitamins (9) supplemented with 58.4 mM sucrose and solidified with 0.7% (w/v) TC agar (pH 5.7). Nodal explants were oriented vertically with the basal cut ends embedded ca. 3 mm into the medium. Media were supplemented with factorial combinations of BA (0, 2, 4, 8, and 16 \( \mu \text{M} \)) and naphthaleneacetic acid (NAA) [0 and 0.5 \( \mu \text{M} \)]. The effects of 3 \( \mu \text{M} \) gibberellic acid (GA\(_3\)) on shoot proliferation and length in the presence of 0.5 \( \mu \text{M} \) NAA and BA (0-16 \( \mu \text{M} \)) were also examined. A culture tube containing a single explant served as the experimental unit. Treatments were replicated 10 times using a completely randomized design. Treatment effects on shoot multiplication and length were evaluated after 5 weeks.

**Rooting Studies.** Rooting was attempted in vitro by transferring 8 to 12-mm microcuttings into GA7 vessels (Margenta Corp., Chicago, IL) containing 80 ml sterile agar-solidified (0.8% w/v) medium consisting of half or full-strength MS inorganic salts supplemented with 58.4 mM sucrose and 0.5 \( \mu \text{M} \) indolebutyric acid (IBA). The basal end of each microcutting was embedded ca. 5 mm into the medium. Each GA7 vessel inoculated with 10 microcuttings served as the experimental unit. Each treatment was replicated five times using a completely randomized design. Experiments were performed under the growing conditions described above. Treatment effects on percent rooting, root number, and length were evaluated after 5 weeks. Rooted microcuttings were transplanted into plug trays containing VerGro (Verlite Co., Tampa, FL) soilless potting medium and then covered with clear vinyl propagation domes. After 2 weeks, the propagation domes gradually were removed over 5 days to decrease humidity and acclimate plants for survival under greenhouse conditions.

### Results and Discussion

Nodal explants from *Photinia X fraseri* were established in vitro with less than 5% contamination when the aforementioned surface sterilization procedure was used. Axillary bud release and shoot outgrowth were observed within 3 weeks following initial explantation onto WPM supplemented with 2 \( \mu \text{M} \) BA. Explants displayed no signs of tissue browning. Large calli usually developed from the basal end of each explant by the third week in culture.

Shoot proliferation from *Photinia* nodal explants cultured on either MS or WPM was significantly enhanced by the addition of BA (Table 1; Fig. 1). Shoots produced on MS were more chlorophyllous than those produced on WPM. However, analysis of shoot proliferation data indicated no significant difference between BA responses for explants cultured on either medium. Addition of 0.5 \( \mu \text{M} \) NAA and 3 \( \mu \text{M} \) GA\(_3\) to either medium did not significantly affect BA-enhanced shoot proliferation (Table 1). However, the presence of NAA further promoted formation of callus at the basal end of each explant (Fig. 1). Shoots produced on WPM were significantly longer than those produced on MS (Table 2). Although shoot proliferation was promoted at higher BA levels, explants cultured on either medium supplemented with greater than 8 \( \mu \text{M} \) BA produced fewer shoots exceeding 8 mm in length. This length was arbitrarily selected to separate usable shoots from those too small to subculture or root (Table 2). Neither the addition of NAA alone nor in combination with GA\(_3\) significantly promoted shoot elongation (Table 2). Consequently, maximum production of usable shoots from *Photinia X fraseri* nodal explants was achieved on WPM supplemented with only 8 \( \mu \text{M} \) BA. *Photinia* clonal lines can be efficiently multiplied on this medium when cultured in polypropylene culture vessels (Fig. 2). Maximal in vitro shoot regeneration on media supplemented solely with BA has been similarly reported for other rosaceous plant species (11, 16).

In nurseries, Fraser photinia is propagated asexually by vegetative stem cuttings and is considered by growers to be difficult to root (1). Preliminary results showed that tissue culture-derived shoot cuttings could not be rooted directly under mist even when predipped in concentrated IBA or NAA solutions (data not shown). Furthermore, only 18% rooting of microcuttings could be achieved in vitro on WPM supplemented with 2.5 \( \mu \text{M} \) IBA. In contrast, microcuttings were effectively rooted (66%) in vitro in GA7 vessels (Fig. 3) containing agar-solidified half-strength MS supplemented with 5 \( \mu \text{M} \) IBA (Table 3). Root elongation

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**Table 1.** Influence of BA supplementation on shoot proliferation from nodal explants of *Photinia X fraseri* cultured for 35 days on Murashige & Skoog Medium (MS) or Woody Plant Medium (WPM) in the presence or absence of 0.5 \( \mu \text{M} \) NAA and 3 \( \mu \text{M} \) GA\(_3\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>-NAA/-Ga(_3)</th>
<th>+NAA/-Ga(_3)</th>
<th>+NAA+Ga(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA (( \mu \text{M} ))</td>
<td>MS</td>
<td>WPM</td>
<td>MS</td>
</tr>
<tr>
<td>0</td>
<td>1.7</td>
<td>1.2</td>
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<tr>
<td>2</td>
<td>3.4</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>2.3</td>
<td>4.1</td>
</tr>
<tr>
<td>8</td>
<td>5.9</td>
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</tr>
<tr>
<td>16</td>
<td>5.2</td>
<td>6.6</td>
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</table>

**Significance**

<table>
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<th>Medium (M)</th>
<th>Treatment (T)</th>
<th>M x T</th>
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<tbody>
<tr>
<td>NS</td>
<td>***</td>
<td>***</td>
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<td>***</td>
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***, NS; significant at 0.1% level and nonsignificant, respectively.
was inhibited at higher IBA levels (Fig. 4). However, production of plantlets with smaller roots facilitated handling during transplantation since longer roots were more frequently broken. Transplant survival of rooted microcuttings in Vergro exceeded 80%.

Our results indicate that Photinia can be readily propagated in vitro via axillary branching. Although not intended for use in commercial production, this micropropagation protocol will be useful in the multiplication of regenerated plants displaying phenotypic characteristics with potential commercial value. It is clear that Photinia X fraseri displays a high capacity for callus formation in vitro. Further studies are required to determine if this callus is capable of shoot organogenesis.

Literature Cited


CONTAINER SIZE AND POTTING MEDIUM AFFECT GROWTH RATE OF WEEPING FIG AND LOQUAT

DENNIS B. McCONNELL
University of Florida, IFAS
Ornamental Horticulture Department
Gainesville, FL 32611

Additional index words. foliage plant, nursery crop, bulk density, Ficus benjamina, Eriobotrya japonica.

Abstract. Two completely randomized block experiments were established to evaluate the effect of container size and potting medium on plant growth. Rooted cuttings of weeping fig (Ficus benjamina L) were grown in 4, 6, or 8-inch containers in a glass house under 80% light exclusion, and loquat [Eriobotrya japonica (Thunb.) Lindl.] liners in 1, 2, or 3 gal containers on white gravel beds in full sun. Potting medium was either peat:sand:pine bark (1:1:1 by volume) or a commercially prepared medium containing peat, vermiculite, sand, bark ash, and pine bark. Data from four monthly measurements showed weeping fig grew fastest in the 8-inch container and slowest in the 4-inch container, while loquat grew slightly faster in 3-gal than 1-gal containers. Plant growth rate was greater in the commercially prepared mix than the peat:sand:pine bark mix in five of the six container sizes studied.

Growth of ornamental plants has been shown to depend on container size (1, 2, 3, 5, 7) and potting medium (7). However, only limited research has compared plant growth in more than one potting medium in more than one container size (7). It is commonly stated that potting media for small containers should have large pores, and potting media for large containers should have small pores for best drainage (8). When Poole and Conover (7) evaluated four pot sizes (1.75, 2.25, 3.0, and 4.0 inches) and six potting media [peat/perlite (3/1, 5/1, 7/1) and peat/sand (3/1, 5/1, 7/1)], they found best growth in all container sizes with a 7/1 peat/perlite ratio.

The objective of this research was to compare the growth rate of a shade grown plant and a sun grown plant in different container sizes, using potting media with different bulk densities (B.D.).

Materials and Methods

Greenhouse. A completely randomized block experiment was established with three container sizes (4, 6, and 8 in-