Elimination of In vitro Contamination, Shoot Multiplication, and Ex vitro Rooting of Aglaonema

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Abstract. Elimination of in vitro contamination and shoot multiplication were studied with Aglaonema Schott ‘White Tip’. Apparent contamination was reduced, but explants browned when 200 mg L⁻¹ streptomycin was used as either a pretreatment or incorporated into the medium. Reduced occurrence of contamination and browning was achieved in axillary bud explants excised from the stock plants that had not been watered for 2 months. Six shoots per explant elongated normally in Murashige and Skoog (MS) medium containing 30 μM benzylaminopurine (BA). MS medium containing 20 μM thidiazuron (TDZ) also resulted in six shoots per explant, but these shoots failed to extend beyond a rosette. Only microcuttings from 30 μM BA treatment were used for the ex vitro rooting trial, and indole-3-butyric acid (IBA) at 9.8 or 19.7 mM applied to the base of the microcuttings resulted in 100% ex vitro rooting and the longest roots.

Aglaonema (Araceae) is one of the most popular indoor plant genera due to its attractive foliar variegation and tolerance to drought and low light conditions (Chen et al., 2002). Commercial Aglaonema production almost exclusively starts from cuttings. Cutting propagation, however, may transmit pathogens from stock plants to cuttings. Additionally, some Aglaonema cultivars may host endogenous pathogens in their vascular tissue (Chase, 1997), which could make cuttings a source for carrying and spreading disease.

Tissue culture is preferable for rapid multiplication of healthy plants. However, endogenous microbial contamination is known to be one of the most serious problems in tissue culture of ornamental aroids, including Anthurium Lind. (Kunisaki, 1980), Dieffenbachia Schott (Brunner et al., 1995; Voyiatzi and Voyiatzis, 1989), Philodendron Schott (Fisse and Pera, 1987), Spathiphyllum Schott and Syngonium Schott (Kneifel and Leonhardt, 1992), and Zantedeschia Spreng. (Kritzinger et al., 1998). Conventional disinfection methods appear to be unsatisfactory because the initial explants are damaged beyond a rosette. Only microcuttings from 30 μM BA treatment were used for the ex vitro rooting trial, and indole-3-butyric acid (IBA) at 9.8 or 19.7 mM applied to the base of the microcuttings resulted in 100% ex vitro rooting and the longest roots.

Materials and Methods

Plant material and culture conditions. Stock plants of Aglaonema ‘White Tip’ were grown in a 70% shaded greenhouse with an average noon PPF of 360 μmol·m⁻²·s⁻¹ and mean daily temperature of 26 °C. Stock plants were grown in plastic containers containing 2.1 L of a mix of 4 parts sphagnum peat (Fafard No. 1, Conrad Fafard, Agawam, Mass.):1 part perlite:1 part vermiculite viable by volume). Stem sections 7–10 cm long, each with 10 axillary buds, were taken from the stock plants, and the buds were excised for in vitro culture. All in vitro media were dispensed as 25-ml aliquots in sterilized 9-cm diameter petri dishes. The environment of in vitro culture was maintained at 25 ± 2 °C under a 12-h photoperiod with 45 μmol·m⁻²·s⁻¹ PPF provided from cool-white fluorescent tubes.
Axillary buds were cultured from Aglaonema 'White Tip' in vitro culture: (A) axillary bud of stem section; (B) adventitious shoot formation from axillary bud after culture for 60 d; (C) micropropagated plantlet from axillary bud culture after 4 months; (D) shoot multiplication from stem section cultured in basal medium with 30 μM BA for 45 d; (E) elongated shoots from stem section cultured in basal medium with 30 μM BA; (F) rosette shoots with curved leaves (arrow indicated) from stem section cultured in basal medium with 20 μM TDZ. Abbreviations: a.b. = axillary bud; a.s = adventitious shoot.

Results

Expt. 1: Reducing in vitro contamination with streptomycin. For both pretreatment and medium supplement treatment, the contamination percentage decreased but the browning percentage increased with increasing streptomycin concentration (Table 1). Shoots formed from each explant and elongated normally in the basal medium containing 30 μM BA (Fig. 1D, E). Regression of shoot number and TDZ concentration showed a curvilinear relationship (Fig. 2). In contrast to the BA treatments, high TDZ

14 d. In Expts. 1 and 2, surviving explants were subcultured every 2 months. We observed possible recurrence of contamination through four subculture periods.

Expt. 3: Shoot multiplication and ex vitro rooting. Axillary buds were cultured from stock plants that had not been watered for 2 months, as described in Expt. 2. When the micropropagated plantlets developed from axillary buds had 3–4 leaves, the 1.0-cm-long stem sections were cut from the plantlets and used as explants. The basal medium consisted of MS medium with 1.34 μM NAA, 20 g·L⁻¹ sucrose, 0.1 g·L⁻¹ myo-inositol, and 7 g·L⁻¹ agar. BA (0, 7.5, 15.0, 22.5, or 30.0 μM) or TDZ (0, 0.4, 2, 4, or 20 μM) was supplemented in the basal medium to determine effects on shoot multiplication. Each treatment included eight replicated explants. Shoot numbers were recorded after 60 d of in vitro culture.

Microcuttings from MS medium supplemented with 30.0 μM BA were distributed in the ex vitro rooting experiment. When the microcuttings had 3–4 visible leaves, they were randomly sampled, transferred to ex vitro, and treated with a basal dip in 0, 2.5, 4.9, 9.8, or 19.7 μM indole-3-butyric acid (IBA; Sigma-Aldrich Chemical Co.) or 0, 3.4, 6.7, 13.4, or 26.8 μM NAA talc, with eight microcuttings per treatment. They were then grown in plastic pots containing a mix of 2 parts sphagnum peat:1 part perlite:1 part vermiculite (by volume) in a growth room at 25 ± 2 °C, 80% to 90% RH, with 130 μmol·m⁻²·s⁻¹ PPF from cool-white fluorescent tubes for 12 h each day. Percentage of rooting, root number, and root length were recorded after transplanting to the growing mix for 1 month.

Experiment design and data analysis. All experiments were arranged in completely randomized designs. Effects of treatments were determined with analysis of variance followed by Tukey's test at P ≤ 0.05 or the general linear models procedure. The percentages of contamination and browning were transformed using an arcsine transformation before statistical analysis.

Results

Expt. 1: Reducing in vitro contamination with streptomycin. For both pretreatment and medium supplement treatment, the contamination percentage decreased but the browning percentage increased with increasing streptomycin concentration (Table 1). Shoots exhibited browning at ≥50 mg·L⁻¹ streptomycin pretreatment or ≥100 mg·L⁻¹ streptomycin medium supplement treatment. A high concentration of streptomycin (400 mg·L⁻¹) eliminated contamination but caused browning in most explants. Medium supplement treatment caused both higher contamination and browning percentages than pretreatment (P < 0.001). Nevertheless, contamination recurred during four subcultures for both treatments.

Expt. 2: Reducing contamination with nonirrigation treatments. Nonirrigation treatments decreased medium water content and leaf water potential but did not significantly affect the leaf Fv/Fm value as compared with the control (Table 2). Nonirrigation treatments reduced the in vitro contamination, with only 6.7% contamination in explants obtained from the stock plants without irrigation for 2 months. Surviving explants did not exhibit browning, and contamination did not recur through four subculture periods.

Expt. 3: Shoot multiplication and ex vitro rooting. Multiple shoots were observed after 2-month axillary bud culture (Fig. 1B). Stem sections were excised from the micropropagated plantlets from 4-month axillary bud culture (Fig. 1C) and cultured on the basal medium with varied concentrations of BA or TDZ. Shoot number increased linearly with increasing BA concentration (Fig. 2). Six shoots formed from each explant and elongated normally in the basal medium containing 30 μM BA (Fig. 1D, E). Regression of shoot number and TDZ concentration showed a curvilinear relationship (Fig. 2). In contrast to the BA treatments, high TDZ
Table 1. Effects of streptomycin pretreatment and medium supplement on in vitro contamination and browning percentages of *Aglaonema* ‘White Tip’ axillary bud culture.

| Streptomycin concn (mg L⁻¹) | Pretreatment | Medium supplement |
|-----------------------------|--------------|-------------------|
|                             | Contamination (%) | Browning (%) | Contamination (%) | Browning (%) |
| 0                           | 86.7 | 0.0 | 83.3 | 0.0 |
| 25                          | 53.3 | 0.0 | 83.3 | 0.0 |
| 50                          | 33.3 | 3.3 | 63.3 | 0.0 |
| 100                         | 23.3 | 23.3 | 56.7 | 16.7 |
| 200                         | 20.0 | 33.3 | 33.3 | 23.3 |
| 300                         | 6.7  | 46.7 | 26.7 | 83.3 |
| 400                         | 0.0  | 66.7 | 0.0  | 100.0 |

Significance: L* Q** L*** Q*** L*** Q***

Table 2. Effects of irrigation treatment on medium water content, leaf water potential and F0/Fm value of stock plants, and in vitro contamination of axillary bud culture of *Aglaonema* ‘White Tip’.

| Treatment | Medium water content (%) | Leaf water potential (MPa) | F0/Fm value | In vitro contamination (%) |
|-----------|--------------------------|---------------------------|-------------|---------------------------|
| Irrigation once per week | 32.9 a* | -0.49 a | 0.81 a | 73.3 a |
| No irrigation for 1 mo. | 13.7 b | -0.81 b | 0.79 a | 53.3 b |
| No irrigation for 2 mo. | 12.8 b | -0.90 b | 0.76 a | 6.7 c |

*Means separation within columns by Tukey’s test at P = 0.05.

Kritzinger et al. (1998) also reported that rhizomes of *Zantedeschia aethiopica* Spreng. pretreated with antibiotic mixtures containing streptomycin before in vitro culture could achieve satisfactory disinfection. When both contamination and phytotoxicity were considered, 200 mg L⁻¹ streptomycin supplemented in the culture medium was more appropriate for *Aglaonema* (Table 1). The best dose for supplementary streptomycin in the culture medium was also 200 mg L⁻¹ for *Philodendron* Schott ‘Red Emerald’ (Fisse and Pera, 1987). In the present study, pre-treatment with streptomycin caused lower contamination percentage than did the medium supplement treatment. This is consistent with the result of Geier (1977), who showed that the inclusion of the antibiotics in sterile distilled water instead of culture medium limited the bacterial growth because no nutrients were involved. However, recurrence of contamination was observed through four subculture periods in both streptomycin pretreatment and culture medium supplement.

*Xanthomonas campestris* pv. *dieffenbachiae* (Pammel) Dowson, *Fusarium solani* (Sacc.) Mart. emend. Synd. & Hans, and *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al. have often been found in vascular tissues or bud axils in *Aglaonema* (Chase, 1997) and other Araceae plants (Debergh and Maene, 1981). We used The Sherlock Microbial Identification System (MIDI, Newark, Del.), a well-established, fully automated gas chromatography analytical system (Cho et al., 2002), in an attempt to identify the microorganisms that caused the contamination. This technique showed that the contamination may have been incited by *Xanthomonas*. However, further investigation is needed to verify the identity of the causal organism.

Water stress can delay or inhibit germination of *Fusarium* (Ramirez et al., 2004) and growth of several fungi (Coleman et al., 1989). In stock plants of *Aglaonema* pretreated with 2 months of nonirrigation, the water potential declined, and thus the pathogens probably could not grow or survive well. This may explain why nonirrigation treatments could minimize in vitro contamination without browning in axillary bud explants (Table 2). Another plausible explanation was that the drought-stressed tissue absorbed bleach solution into deeper layers of explant tissue, resulting in a more effective kill of the microorganisms. Nonirrigation treatments also could prevent infections from soil or water when using overhead watering. These nonirrigation pretreatments did cause the oldest 3–4 leaves to yellow but did not reduce the root number increased when the NAA concentration increased to 13.4 mM, and declined when NAA increased to 26.8 mM. Root length was unaffected by NAA concentrations (4 or 20 μM) resulted in rosette (<0.5 cm long) clusters with small and curved leaves (Fig. 1F).

In the ex vitro rooting experiment, all microcuttings from the 30.0 μM BA treatment were successfully acclimatized and rooted when treated with 6.7 or 13.4 mM NAA and 4.9, 9.8, or 19.7 mM IBA after transplanting to the growing mix for 1 month (Table 3). Root number increased when the NAA concentration increased to 13.4 mM and declined when NAA increased to 26.8 mM. Root length was unaffected by NAA concentration. Root number increased with increasing IBA concentration. The 9.8 or 19.7 mM IBA treatments resulted in the longest roots.

**Discussion**

Serious in vitro contamination problems were shown in *Aglaonema* (Tables 1 and 2), similar to other ornamental aroids (Fisse and Pera, 1987; Brunner et al., 1995; Kritzinger et al., 1998). For *Aglaonema*, in vitro visible contamination could be temporarily reduced using a streptomycin pretreatment (Table 1).
Table 3. Effects of NAA and IBA concentration on rooting percentage, number of roots, and root length in ex vitro rooting of Aglaonema ‘White Tip’ microcuttings.

| Auxin concn (mg) | Rooting (%) | Number of roots | Root length (cm) |
|------------------|-------------|-----------------|-----------------|
| NAA              |             |                 |                 |
| 0                | 75.0        | 1.8             | 0.6             |
| 3.4              | 75.0        | 2.8             | 0.6             |
| 6.7              | 100.0       | 3.0             | 0.9             |
| 13.4             | 100.0       | 3.8             | 0.8             |
| 26.8             | 62.5        | 1.5             | 0.8             |
| IBA              |             |                 |                 |
| 0                | 75.0        | 1.8             | 0.6             |
| 2.5              | 75.0        | 2.3             | 0.4             |
| 4.9              | 100.0       | 3.3             | 0.4             |
| 9.8              | 100.0       | 3.5             | 2.4             |
| 19.7             | 100.0       | 4.3             | 2.0             |

Significance

| NAA concn | Rooting (%) | Number of roots | Root length (cm) |
|-----------|-------------|-----------------|-----------------|
| Q*        | Q**         | NS              |                 |
| IBA concn |             |                 |                 |
| Q*        | Q**         | NS              | **              |
| Auxin source |                 |                 |                 |
| NS        | NS          | NS              |                 |
| Concn x source |             |                 | ***             |

- **NS** Non-significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively; L, linear; Q, quadratic.

Terminal cuttings of Aglaonema modestum Schott ex Engler treated with a basal quick-dip in auxins (IBA and NAA) produced more roots than the untreated cuttings (Blythe et al., 2004). The present work also showed that, after the 9.8 or 19.7 mg IBA treatments, all microcuttings rooted and produced maximum root numbers and lengths (Table 3). Thus, ex vitro rooting, compared with in vitro rooting, is of practical value in reducing the time and cost of transplantation.

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