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Micropropagation of Gas Plant (*Dictamnus albus* L.)[1]

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Abstract

Gas plant (*Dictamnus albus* L.) was successfully micropropagated from greenhouse-grown plants capable of flowering. Stems from initial spring growth were surface disinfested and two- node sections were placed horizontally on modified Murishige and Skoog medium with 1 μM (0.2 ppm) benzyladenine (BA). A dose response to BA at 1, 5 and 10 μM (0.2, 1.1, 2.3 ppm) indicated that the greatest number of usable microshoots was observed in cultures from horizontally placed explants treated with 1 μM (0.2 ppm) BA. These cultures initiated between 7.6 and 9.5 shoots per culture with approximately 70% of the shoots greater than 2 cm (0.8 in). Microcuttings rooted poorly in vitro even with indolebutyric acid in the medium. However, microcuttings rooted between 71 and 86 percent under ex vitro conditions. Microcuttings either untreated or treated with indolebutyric acid (5,000 or 10,000 ppm) as a quick dip produced 1.8 to 2.6 roots per rooted cutting after being directly stuck in a peat-lite medium (Metro-Mix or Promix) in cell packs under high humidity. Untreated microcuttings rooted at higher percentages (67 to 88%) in peat-lite media compared to perlite or vermiculite (25 to 58%). Rooted microcuttings were successfully acclimatized to greenhouse conditions by gradually reducing humidity.

Index words: tissue culture, in vitro propagation, herbaceous perennial.

Species to be studied: gas plant (*Dictamnus albus* L.).

Significance to the Nursery Industry

Gas plant (*Dictamnus albus* L.) is an herbaceous perennial plant that has been cultivated for centuries for its medicinal and ornamental value. The availability of gas plant in the nursery trade has been limited because it is difficult to propagate economically. Success with seed propagation has been variable and unreliable. Micropropagation would provide an alternative for the rapid propagation of gas plants. This communication details a protocol for the rapid multiplication of gas plant by micropropagation consistent with techniques used to propagate other herbaceous perennials through tissue culture.

Introduction

Gas plant (*Dictamnus albus* L.) is an attractive, long-lived herbaceous perennial that has been cultivated for centuries (5). White, pink or rose colored flowers are produced in the spring on upright racemes extending above dense, bushy foliage. The common name, gas plant, derives from the flowers and seed capsules having prominent glandular trichomes that produce a volatile exudate that can be ignited without apparent harm to the plant (1). Cultural recommendations for gas plant have not changed substantially in over 200 years (12). Properly grown plants may thrive for decades but do not transplant well once they are established.

Although gas plant enjoys a rich garden tradition, it has limited availability in the commercial perennial trade because it is difficult to propagate. Gas plant has been propagated by seed (2, 8, 15), division (2, 7) and root cuttings (2, 4), but each of these methods is less than ideal for the commercial grower. Seeds require a long stratification treatment and often germinate poorly over several months (2) or not at all (14). Limited quantities of gas plants can be propagated by division or root cuttings. However, these methods are relatively slow and are limited by stock plant availability and multiplication rate.

Micropropagation could produce gas plants more efficiently than division or root cuttings. In an overview of the use of tissue culture for production of herbaceous perennials at Walters Gardens, Zilis et al. (17) reported that gas plants could be multiplied by axillary shoot proliferation and rooted in vitro. However, fewer than 50% of the plants produced by this method were successfully rooted and acclimatized.

The objective of this study was to develop a micropropagation system for gas plant, including investigation of the conditions for rapid shoot multiplication, root formation of microcuttings and subsequent acclimatization.

Materials and Methods

Establishment of Cultures. Dormant gas plants of a flowering size (approximately 3 years old) were potted into 15 cm (5.9 in) containers containing Metro-Mix 360 (Grace Sierra, Cambridge, MA) and placed in a greenhouse in May. Emerging shoots were harvested and leaves removed prior to surface disinfestation. Stems were disinfested in a 20 sec quick dip of 70% ethanol followed by a 10 min agitation in 0.5% sodium hypochlorite (10% Clorox) plus 0.1% Alconox detergent. This was followed by a triple rinse with autoclaved deionized water. The stems were cut into two-node pieces and placed horizontally in autoclaved Magenta jars (Sigma, St. Louis, MO), containing Murashige and Skoog (MS) medium (13) with macro- and micronutrients (Sigma, St. Louis, MO), solidified with Difco Bacto-Agar at 6.6 grams per liter (0.66%) and supplemented with 2% sucrose and 1 μM (0.2 ppm) benzyladenine (BA). The cultures were placed under 40 watt cool white fluorescent bulbs at approximately 40 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (photosynthetically active radiation-PAR) with a 16 hr photoperiod and a temperature of 23 ± 2°C (73.5 ± 3.6°C). Subculturing was performed every 6 weeks aseptically under a laminar flow hood. The subculture medium was the same as the establishment medium.

Shoot Multiplication. Shoot multiplication experiments were initiated using four-node in vitro developed stems. The explants had leaves removed and were placed in a horizon-

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tal or vertical orientation in Magenta jars containing 40 ml of MS medium supplemented with 1, 5, or 10 μM (0.2, 1.1, 2.3 ppm) BA. There were four explants per jar and four jars per treatment and experiments were repeated. The culture environment was identical to the establishment stage except that the PAR (light intensity) was reduced to 20 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. After the shoots had been removed and counted, the original explants were subcultured on fresh medium at the same BA concentration.

Root Formation. Microcuttings for the rooting experiments were taken from cultures multiplied in vitro on MS medium supplemented with 1 μM (0.2 ppm) BA. All microcuttings used were > 2 cm (0.8 in) long. Cuttings were evaluated for root formation in vitro or under ex vitro rooting conditions. In vitro root formation involved placing single microcuttings in a 25 × 150 mm (1.0 × 5.9 in) culture tube containing 20 ml of ½ strength MS medium supplemented with 2% sucrose and 0, 10, 50, or 150 μM (2.0, 10.2, 30.5 ppm) indolebutyric acid (IBA). The medium was solidified with 6.6 grams per liter (0.66%) of Difco Bacto-Agar. The cultures were then placed at 23 ± 2°C (73.5 ± 3.6°F) under 40 watt cool white fluorescent bulbs at 20 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR and a 16 hr photoperiod.

Microcuttings were treated with a 5-sec quick dip with auxin by delivering IBA at 0, 1000, 5000, or 10,000 ppm in 50% ethanol. Treated microcuttings were transferred directly into ex vitro rooting conditions by placing microcuttings in 13.3 × 13.3 × 5.9 cm (5.3 × 5.3 × 2.3 in) cell packs filled with Metro-Mix 360. In a separate experiment, untreated microcuttings were rooted in cell packs containing Promix (Premier Brands, Yonkers, NY), Metro-Mix 360, Metro-Mix 510, perlite, vermiculite or 50% (v/v) perlite plus vermiculite. The cell packs were placed in flats covered with a clear plastic top and enclosed in a clear polyethylene bag. The flats were placed in a Percival growth chamber with 40 watt cool white fluorescent bulbs at approximately 60 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR with a 16 hr photoperiod and a temperature of 24°C (75°F). Microcuttings were evaluated for root formation after 6 weeks. Rooted microcuttings were acclimatized over a 3 week period in the growth chamber by gradually reducing the humidity.

Results and Discussion

Explants from gas plant readily formed shoots in culture (Table 1, Fig. 1). Regardless of the BA treatment, the number of shoots per explant increased for each subculture (Table 1). An increase in the number of shoots per explant after successive subcultures has been observed for many species.

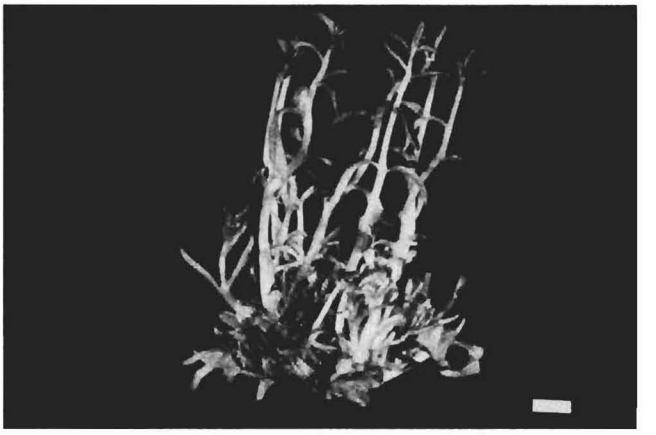

Fig. 1. In vitro shoot formation in gas plant treated with 1 μM (0.2 ppm) BA after six subcultures. Reference bar equals 1 cm.

This may be the result of stabilization of the cultures (11) or increased adventitious bud formation (3).

There was no difference in the number of shoots induced by any of the BA concentrations tested (Table 1). However, by the third subculture there was a trend for more but shorter shoots produced in the 10 μM BA cultures. For cultures maintained for six subcultures, explants produced on a medium with 1 μM BA had 70.5% of shoots greater than 2 cm in length. This contrasted to less than 50% for 5 and 10 μM BA cultures.

Explants placed horizontally on the medium produced more shoots than explants in an upright orientation (data not shown). This response has been observed in pear (9); lilac (6); french tarragon (10) and apple (16). The horizontal orientation may influence apical dominance of developing shoots allowing for additional axillary shoot formation.

Microcuttings rooted less than 20% under in vitro conditions. However, microcuttings rooted between 71 and 86% under ex vitro rooting conditions (Table 2). IBA at 5,000 and 10,000 ppm increased slightly the number of roots per cutting, but decreased the percentage rooting by 13 to 15%. Two roots per cutting is a relatively low number for most micropropagated crops; however, these roots assumed the role of a primary tap root which is typical of gas plant and plants developed an extensive root system after 1 year (Fig. 2). Rooted cuttings acclimatized at greater than 80% and appeared to grow normally in the greenhouse.

Zilis et al. (17) reported that micropropagation of gas plant was limited because fewer than 50% of the microcuttings

Table 1. Effect of benzyadenine (BA) and subculturing on in vitro shoot formation in gas plant.

BA [μM][z] Concn.	Subcultures 1	2	3
1	3.8 ± 0.9[y]	6.0 ± 1.3	7.6 ± 2.7
5	3.6 ± 1.0	5.2 ± 1.2	8.7 ± 2.3
10	2.4 ± 0.8	5.7 ± 1.7	10.4 ± 2.1

[z]BA concentration of 1, 5 and 10 μM is 0.2, 1.1 and 2.3 ppm, respectively.

[y]Mean number of shoots per explant ± 95% confidence interval.

Table 2. Root formation in gas plant microcuttings treated with a quick dip of indolebutyric acid (IBA) under ex vitro rooting conditions.

IBA [ppm]	Mean roots per rooted cutting[z]	Percentage rooted
0	1.8 ± 0.3	86
1,000	1.9 ± 0.4	82
5,000	2.6 ± 0.5	73
10,000	2.4 ± 0.5	71

[z]Mean ± 95% confidence interval (N = 34). Data collected after 6 weeks.

Fig. 2. Micropropagated gas plants after one-year of growth in the greenhouse. Reference bar equals 2 cm.

rooted under in vitro rooting conditions. The current study confirms the difficulty this species has for rooting in vitro. To judge the impact media might have on ex vitro rooting, untreated microcuttings were rooted in six different media (Table 3). The results show that the highest percentage rooting occurred on peat-lite media (Metro-Mix or Promix) with the poorest rooting in perlite.

The results from this study indicate that gas plant can be micropropagated successfully following procedures consistent with other perennials propagated in vitro. In summary, optimal shoot multiplication was found for horizontally placed explants on MS medium containing 1 μM BA. Microcuttings root 6 weeks after being stuck into a peat-lite medium and maintaining near 100 percent humidity. IBA (5000 ppm) as a quick dip may increase the number of roots formed per cutting but was not essential for root formation. Rooted cuttings can be acclimatized to ambient conditions by gradually reducing humidity over a 3-week period. Plants appear to be growing normally in the greenhouse, but have not yet reached a flowering size.

Table 3. Root formation in gas plant microcuttings rooted in six different media.

Medium	Mean roots per rooted cutting	Percentage rooted
Metro-Mix 360	1.8 ± 0.4[z]	83
Metro-Mix 510	1.9 ± 0.4	67
Promix	1.6 ± 0.3	88
Perlite	1.5 ± 0.4	25
Vermiculite	1.2 ± 0.2	58
Perlite:Vermiculite (50:50)	1.3 ± 0.5	33

[z]Mean ± 95% confidence interval (N = 24). Data collected after 6 weeks.

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