Poinsettia (*Euphorbia pulcherrima*) in Vitro Propagation©

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***Euphorbia pulcherrima* Willd., poinsettia, micropropagation and in vitro proliferation is extensively practiced by poinsettia producers and researchers. Slow growth rate of plantlets, few micro shoots per explant, and slow root growth rate are restrictions of in vitro propagation of poinsettia. Explants (apical buds and axillary buds) obtained from greenhouse-grown plants were placed on Murashige-Skoog (MS) basal medium supplemented with various concentrations (4 µM-12 µM) of 6-benzylaminopurine (BA) and 4 µM of indole-3-acetic acid (IAA) to optimize shoot proliferation and rooting of poinsettia in vitro. Explants placed on media containing only BA and combination of BA and IAA produced red callus at the base of plantlets after 1 month while explants in a medium without any plant growth regulators (PGRs) produced no callus. Subculturing of red callus in a medium with BA produced additional callus and micro buds. Regenerated micro buds produced the greatest number of micro shoots on a medium with BA alone. White callus did not give rise to micro buds or micro shoots. Four-month-old shoots initiated rooting on MS basal medium without any plant growth regulator (PGR); however addition of IAA into the medium increased rooting efficiency in terms of more number of roots at a time and less number of days for root initiation. Incorporation of PGRs into poinsettia micropropagation media at different stages of in vitro plantlet development enhanced rapid callus formation and accelerated shoot and root growth. Optimization of PGR concentrations during poinsettia micropropagation helped resolve previous restrictions of in vitro poinsettia proliferation.**

INTRODUCTION

Euphorbia pulcherrima Willd. ex Klotzsch, poinsettia, belongs to the family Euphorbiaceae and the genus *Euphorbia* consisting of close to 2,000 species (Ecke et al., 2004). Poinsettia is popular during the winter holiday season and it has dominated the ornamental horticulture field. Sales of poinsettia were rated number one among all flowering potted plants in the United States (Pickens et al., 2005). Global production of poinsettia has exceeded hundreds of millions and is still expanding, indicating its economic and market potential for the floral industry (Clarke et al., 2008).

Conventional propagation of poinsettia by cuttings and seed has several limitations. Propagation through seed is difficult since seed lose viability upon storage (Jasrai et al., 2003) and plants originating from seed have genetic variability which is not preferred in producing uniform plants for market demand. Propagation through cuttings has drawbacks because it is seasonal and cuttings take 6–8 weeks to root (Jasrai, 2003). Hence, these propagation methods of poinsettia fail to meet the high demand for quality plants during the winter holiday market. Therefore the objective of this research was to develop an effective and efficient in vitro propagation and proliferation technique for poinsettia.

MATERIALS AND METHODS

Apical buds and axillary buds with 1–2 cm of stem of *E. pulcherrima* 'Prestige Red' were excised from healthy plants grown in the greenhouse. The buds were surface disinfected by placing them in a 1% Tween 20 solution for 5 min, followed by 70% ethyl alcohol for 1 min, and then 20% bleach for 15 min (Pickens et al., 2005). Finally buds were dipped in sterile water thrice for 5 min. Twenty milliliters of basal medium containing Murashige and Skoog (MS) salts (4.4 $g \cdot L^{-1}$), myo-inositol (0.1 $g \cdot L^{-1}$), sucrose (30 $g \cdot L^{-1}$) and agar (7 $g \cdot L^{-1}$) (pH 5.7–5.8) (Pickens et al., 2005) was poured into 100-ml baby food jars and autoclaved at a temperature of 121 ºC at 15 psi pressure for 15 min. Various concentrations of plant growth regulators (PGRs) were incorporated into MS basal medium depending on treatment type. Disinfected buds were placed on the disinfected medium in aseptic conditions under a laminar hood and incubated in a growth chamber with 16-h photoperiod (125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ illumination) at 25 ºC day and 22 ºC night temperatures.

Callus Study. The callus study was composed of two types of treatments: For the first treatment, 6-benzylaminopurine (BA) was incorporated into the medium at concentrations of 4 µM, 6 µM, 8 µM, 10 µM, and 12 µM and for the second treatment; the same concentrations of BA were incorporated into the medium with 4 µM of IAA. For both treatments, the medium without any PGR was used as a control. After a month explants were evaluated for callus weight, number of plants having red callus at the base of the explants, and callus color. Further number of micro buds and micro shoots which are produced by red callus also were noted. After every month explants were subcultured into a new medium with the same treatments up to 4 months.

Rooting Study. Four-month-old in vitro-grown poinsettias were transferred into a rooting media of three treatments: full MS with 28.5 µM IAA (Roy and Jinnah, 2001), ½ MS with 28.5 µM IAA, and BA (4 µM) alone. An experimental control with no PGRs was also included. The plants were evaluated for average number of days taken for root initiation and number of roots initiated per shoot. The experiment was terminated after 5 months.

Each experiment was incorporated with five explants per treatment and each experiment was replicated once. All treatments were arranged in Randomized Complete Block Design (RCBD) and data were analyzed by the Analysis of Variance (ANOVA) using SAS 9.1 (Statistical Analysis Software). Treatment means were separated by Least Significant Difference (LSD) method at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Two types of calluses were identified in poinsettia micropropagation: red callus (Fig. 1A) and white callus (Fig. 1B). With continuous subculturing in a medium with BA, red callus produced more micro buds and micro shoots (data not shown) and finally healthy plants whereas white callus remained unchanged. White callus is a result of recalcitrant cell clumps which are unproductive. Red callus is the most important in poinsettia proliferation: it synthesizes into micro buds and micro shoots. The results observed were consistent with Pickens et al. 2005.

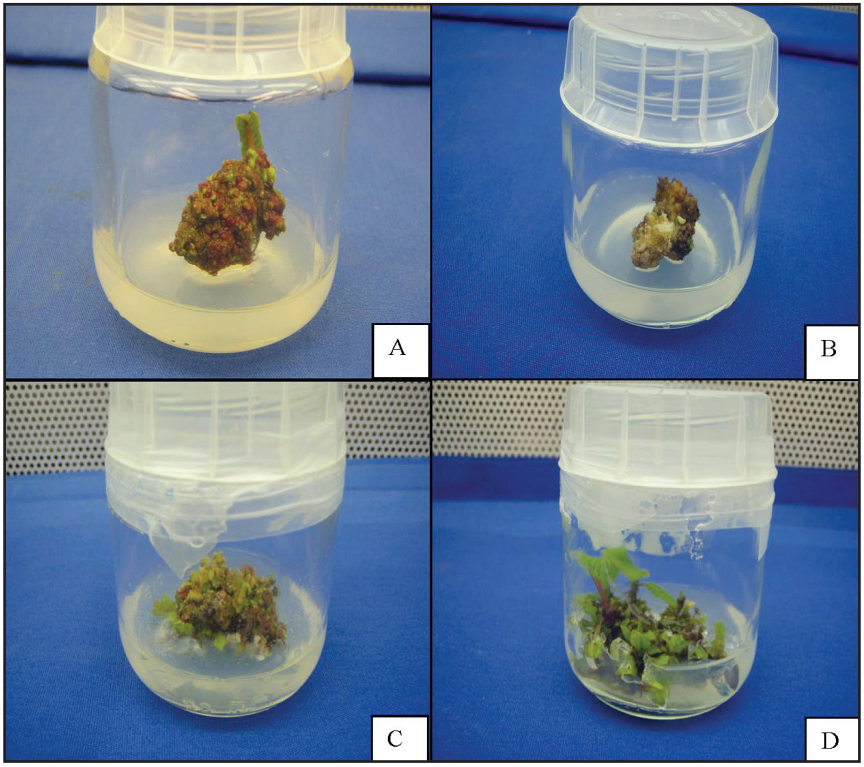

Figure 1. Explants producing red and white calluses, micro buds and micro shoots in poinsettia micropropagation.

Callus Study. Explants grown in a media supplemented with BA produced red callus at the base of the explant whereas explants without BA did not give rise to any callus (Table 1). There is a significant ($\alpha = 0.05$) difference between plants which are treated with BA and without BA. Therefore use of BA in poinsettia micropropagation is effective and it helps producing red callus. BA can be used to generate more red calluses, more micro buds (Fig. 1C), and micro shoots in poinsettia (Fig. 1D).

Explants supplemented with both BA and IAA produced red callus at the base of the plant showing a significant ($\alpha = 0.05$) difference among treatments (Table 2). Continuous growth in a medium supplemented with BA resulted in more micro buds and micro shoots compared to medium supplemented with both IAA and BA. Incorporation of IAA and BA into the medium generated more red calluses but BA/IAA ratio is critical in generating red callus. The greater the BA/IAA ratio, the higher the red callus produced in this study. Equimolar concentrations of cytokinins to auxins are generally used to maintain callus, whereas higher cytokinin to auxin ratios tend to induce shoot development (Pickens et al., 2005).

Rooting Study. Four-month-old poinsettia rooted irrespective of plants being provided with IAA or not, but incorporation of BA had an inhibitory effect on rooting.

Table 1. Red callus initiation from 1-month-old poinsettia supplemented with BA.

Treatments (BA) µM	Plants with a red callus at the base (no.)	Average callus weight gain (g) after one month
4	5 a[z]	0.468 a
6	5 a	0.308 a
8	5 a	0.358 a
10	5 a	0.274 a
12	5 a	0.324 a
No BA	0 b	0.000 b

[z]Treatments with the same letter are not significantly different.

Table 2. Red callus initiation from 1-month-old poinsettia supplemented with IAA + BA.

Treatments	Plants with a red callus at the base (no.)
4 µM IAA and 4 µM BA	4 b[z]
4 µM IAA and 6 µM BA	4 b
4 µM IAA and 8 µM BA	5 c
4 µM IAA and 10 µM BA	5 c
4 µM IAA and 12 µM BA	5 c
No IAA and BA	0 a

[z]Treatments with the same letter are not significantly different.

Table 3. Root initiation from 4-month-old poinsettia supplemented with plant growth regulators.

Treatments	Days taken for root initiation (avg. no.)	Roots initiated (no.)
No PGRs	35 a[z]	2 a
4 µM BA	0 d	0 c
½ MS with 28.5 µM IAA	24 c	3 b
Full MS with 28.5 µM IAA	28 d	3 b

[z]Treatments with different letters are significantly different.

There was a significant ($\alpha = 0.05$) difference among treatments and ½ MS with 28.5 µM IAA is the best medium for poinsettia in vitro rooting.

Findings of this research can be used as an effective and efficient in vitro micro propagation and proliferation technique of poinsettia. Further research is necessary to determine how to prevent white callus production during in vitro poinsettia culture.

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