IN VITRO PROPAGATION OF POINSETTIA (*Euphorbia pulcherrima* Willd.)

Le Hong Thuy Tien, Nguyen Thi Diep, Nguyen Vu Phong
Nong Lam University, Ho Chi Minh city
Email: nvphong@hcmuaf.edu.vn

ABSTRACT

Poinsettia plants (Euphorbia pulcherrima Willd.) is a flowering potted plant with an high economic value, especially in Christmas and New Year. Conventional propagation of poinsettia by seed and cuttings has several limitations due to genetic variability and a low rate of propagation. This study presents the results of in vitro propagation for poinsettia. Nodal segments with axillary buds were sterilized with javel:distilled water at ratio 1:2 (v/v) in 15 minutes obtained 53.3% cleaned samples. Effect of 6-benzyladenine (BA), naphthalene acetic acid (NAA), gibberellin acid 3 (GA_3), indole 3- butyric acid (IBA) on in vitro shoot and root formation was studied. Full-strength Murashige and Skoog (MS) medium supplemented with 0.5 mg.L^{-1} BA and 0.1 mgL^{-1} NAA was the best for shooting (4.8 shoots per explant). Shoots were transferred onto MS medium adding 0.3 mg.L^{-1} GA_3 for elongation within 4 weeks. The elongated shoots were rooted on half-strength MS medium containing 2 mg.L^{-1} IBA and plantlets were successfully acclimatized with 75% survival rate.

Keywords: *BA, GA_3, nodal segment, poinsettia, regeneration.*

TÓM TẮT

Cây trạng nguyên (Euphorbia pulcherrima Willd.) là loại cây hoa cảnh trồng chậu có giá trị kinh tế cao, rất được yêu thích đặc biệt trong dịp giáng sinh và năm mới. Việc nhân giống theo phương pháp truyền thống bằng hạt hoặc cành giâm gặp hạn chế do cây con biến đổi lớn về di truyền và hệ số nhân giống thấp. Nghiên cứu này trình bày kết quả nhân giống in vitro cây trạng nguyên. Đoạn thân mang chồi ngủ được khử trùng với dung dịch javel:nước tỷ lệ 1:2 trong 15 phút thu được 53,3% mẫu sạch. Mẫu được cảm ứng tạo đa chồi thực hiện trên môi trường cơ bản MS (Murashige and Skoog, 1962) bổ sung 0,5 mg.L^{-1} BA và 0,1 mgL^{-1} NAA tạo được 4,8 chồi/mẫu. Sau 4 tuần nuôi cấy trên môi trường MS bổ sung 0,3 mg.L^{-1} GA_3, chồi con được cảm ứng tạo rễ dưới tác dụng của 2 mg.L^{-1} IBA trên môi trường MS½ không bổ sung đường. Ngoài vườn ươm, 75% cây con tiếp tục sống khi được ươm trên giá thể đất, tro trấu, xơ dừa.

Từ khóa: *BA, đoạn thân, GA_3, tái sinh, cây trạng nguyên.*

INTRODUCTION

Poinsettia (*Euphorbia pulcherrima* Willd.) is one of most favorite ornamental houseplants with the colorful leaves, durability, and having specific meaning Christmas and New Year times in many countries around the world (Clarke et al., 2008). In Vietnam, the dwarf poinsettia with red leaves grows in pot widely used as decoration plant. This cultivar is mainly imported from abroad, therefore the cost is quite high. Poinsettia is propagated by seeds; however, it has low rate of propagation and high genetic variation. The multiplication by stem cuttings is seasonal and takes six to eight weeks for rooting. In addition, the potential pathogenic virus is also easily transmitted from mother plant to seedlings. Previous studies focused on regenerating plants through organogenesis or somatic embryogenesis that are significant to *in vitro* propagation although the rate of propagation is not high (De Langhe et al., 1974; Roy and Jinnah, 2001; Jasrai et al., 2003, Uchida et al., 2004, Pickens et al., 2005; Perera and Trader, 2010). The information of *in vitro* propagation of poinsettia in Vietnam is limited. This study was initiated to develop a protocol for multiplication of *E. pulcherrima* through nodal segments with axillary buds.

MATERIALS AND METHODS

Materials

The dwarf poinsettias with red leaves were collected from flower stores in Truong Chinh street, Tan Binh district, Ho Chi Minh city.

MS (Murashige and Skoog, 1962), and ½ MS (macro nutrient reduces half) supplemented with 8 $g.L^{-1}$ agar, 30 $g.L^{-1}$ sucrose (except rooting experiment) were used as basal media. The growth regulators such as BA (benzyl adenine), NAA (1-naphthaleneacetic acid), IBA (indole-3-butyric acid), and GA_3 (gibberellic acid) (Himedia, India) were added to basal media at various concentrations. The pH of the medium was adjusted to 5.7 ± 0.1 before autoclaving at 121°C at 1.2 atm for 20 min. GA3 is sterilized through the membrane filter and added to the medium after sterilization.

Disinfection of explants

Young nodal explants with axillary buds were washed under running tap water for 60 min then rinsed with 70% (v/v) ethanol for 30 sec. The explants were soaked with commercial Javel solution (My Hao, Vietnam) diluted with distilled water at ratio 1:1 or 1:2 (v/v) for 10, 15, and 20 min. Each disinfected step was followed by a rinsing with sterile distilled water (three times). Finally, the explants were placed on agar-based MS medium. The percentage of clean explants and survival rate were recorded after two weeks of culture.

In vitro shoot proliferation

Induction of shoot proliferation by culturing the clean nodal explants with axillary buds on the MS medium supplemented with 0.1 mg L^{-1} NAA and BA concentrations ranged from 0; 0.1; 0.5; 1.0; and 1.5 $mg.L^{-1}$. The average shoots per explant, height of shoot (cm) and number of leaves (leaf/shoot) were recorded after 4 weeks. Then, the shoots were separated and transferred to MS medium supplemented with GA_3 at 0; 0.1; 0.3; 0.5; 0.7; and 1.0 $mg.L^{-1}$ to stimulate shoot elongation before rooting. Height of shoot (cm) and leaf number (leaf/shoot) were recorded after 4 weeks of culture.

In vitro rooting and acclimatization

The *in vitro* shoots were transferred to either MS basal medium (without sugar) of different strengths (full strength and half strength of macro nutrient) alone or supplemented with IBA at 0,5; 1,0; 1,5; 2,0 $mg.L^{-1}$ for *in vitro* root induction. The percentage of shoots producing roots, the number of root was recorded after 4 weeks.

According to acclimatization and hardening, *in vitro* regenerated plants form 3 -5 cm height having roots were removed from test tubes and agar medium attached was washed off completely. Plants were transferred to plastic pots containing mixture of clean soil : coir : rice husk ash in the ratio of 1: 2: 3. The survival rate of plantlets was recorded after 2 weeks of transfer to plastic pots.

Statistical analysis

The experiments were arranged in a completely randomized, single-factorized manner. Each treatment throughout the experimentation consisted of three replicates and the experimental unit was ten explants per treatment. The data were statistically analyzed, the results were analyzed using ANOVA, the mean and the difference table of the LSD tests.

RESULTS ANS DISCUSSION

In vitro explants sterilization at different time of treatment with javel

The culture media contains sugar, mineral salts and vitamins are very favorable for the growth of bacteria and mold. Microorganisms grow much faster than the growth of plant tissue, which invades both the culture medium leading to the dead of explants. Therefore, plant tissue needs to be cleared of microorganisms to become as a raw material for subsequent trials.

Table 1. Effect of javel on disinfection of nodal explants with axillary buds

Ratio of javel: distilled water (v/v)	Time of treatment (min)	Clean explants rate* (%)	Survival rate* (%)
1: 1	10	20.0^{bc}	13.3^{cd}
1: 1	15	36.7^{abc}	20.0^{cd}
1: 1	20	16.7^{c}	10.0^{d}
1: 2	10	33.3^{abc}	26.7^{bc}
1: 2	15	53.3^{a}	50.0^{a}
1: 2	20	43.3^{ab}	40.0^{ab}

** Values are mean of three replicates. Values sharing common letters do not differ significantly at P < 0.05 according to LSD Test.*

When explants were treated with javel:distilled water at ratio 1: 1 (v/v) at different times, the survival rate was highest at 20%. By reducing the rate of javel (1: 2), the clean explants rate and the survival rate increased significantly (from 25 to 50%), indicating that the concentration of javel significantly affected the disinfection of explants. The highest clean explants rate and survival rate were 53% and 50%, respectively at 15-minute treatment (Table 1). At this concentration of javel, the clean explants rate and survival rate did not differ significantly when the treatment time was up to 20 minutes.

In general, the clean explants rate and survival rate were quite low at all treatments similar to the result reported by Perera and Trader (2010). This may be due to poinsettia is a hollow tree that endogenous fungi and bacteria are inhabitable. In addition, young shoots are susceptible to the effects of disinfectants leading to dead or poor regeneration (Figure 1).

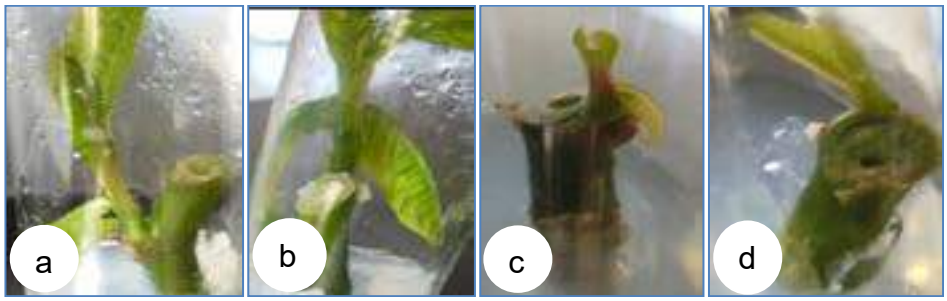

Figure 1. Shoots formed from axillary buds of poinsettia
(*a) and (b) good shoot regeneration; (c) and (d) poor shoot regeneration.*

Effect of BA and NAA on shoot proliferation

Table 2. Shoot proliferation on MS medium supplemented with NAA (0.1 mg.L^{-1}) and various concentration of BA after 4 weeks of culturing

Concentration of BA (mg.L^{-1})	Average shoots per explant (shoot)	Height of shoot (cm)	Leaf number (leaf)
0.0	26^{b}	2.0^{b}	3..5^{a}
0.1	2.7^{b}	0.7^{d}	3.4^{a}
0.5	4.8^{a}	1.8^{a}	2.4^{b}
1.0	1.9^{bc}	1.2^{cd}	2.3^{b}
1.5	1.8^{bc}	0.8^{d}	2.2^{b}

Values are mean of three replicates. Values sharing common letters do not differ significantly at P < 0.05 according to LSD Test.

Figure 2. *In vitro* shoots grow on MS supplemented with various concentration of BA *(a) 0 mg.L^{-1}; (b) 0,1 mg.L^{-1}; (c) 0,5 mg.L^{-1}; (d) 1,0 mg.L^{-1}; (e) 1,5 mg.L^{-1}.*

The results show that shoot regeneration was observed in all the medium compositions used. MS medium supplemented with 0.1 mg.L^{-1} and 0.5 mg L^{-1} BA yields the maximum amount of shoot (4.8 shoot) compared to the other MS media tried. The height of shoots formed on media without BA (Fig. 2a) and BA with 0.5 mg.L-1 (Figure 2c) was highest (2.0 cm and 1.8 cm). Shoots on medium supplemented with BA 0.1 mgL^{-1} or without supplementation had significantly higher leaf numbers (3.5 and 3.4 leaf) than the remaining treatments (1.6 - 2.4 leaf) (Table 2). Similar results reported by Danial and Ibrahim (2016), they obtained 5 shoots/explants on the MS medium contained 0.5 mg.L^{-1}, while the shoot height was relatively low (< 1 cm) with many leaves (11 leaves/ shoot). Picken and co-workers (2005) used 1.5 mg. L^{-1} IAA combined with 0 - 4 mg.L^{-1} BA achieved a maximum of 2.7 shoots per explant with 40% of callus at the site of exposure.

Effect of GA_3 on *in vitro* shoot elongation of poinsettia

Most shoot produce a height of less than 2 cm and the number of leaves per shoot is less than 2-3 leaves. To reach the right size before rooting, GA3 was used to stimulate shoot elongation.

Table 3. *In vitro* shoot elongation of poinsettia on medium contains GA_3

Concentration of GA_3 (mg.L^{-1})	Height of shoot (cm)	Leaf number (leaf)
0.0	2.0^{c}	3.5^{b}
0.1	1.9^{c}	3.5^{b}
0.3	2.2^{c}	4.9^{a}
0.5	3.0^{ab}	4.9^{a}
0.7	3.2^{a}	4.2^{ab}
1.0	2.4^{bc}	4.4^{ab}

Values are mean of three replicates. Values sharing common letters do not differ significantly at P < 0.05 according to LSD Test.

On MS medium supplement with 0.5 and 0.7 mg.L^{-1} GA_3, the height of the shoot reached highest at 3 cm. However, shoots on the medium supplemented with 0.5 mg L^{-1} GA3 produced larger and more uniform shoots (Figures 3c and 3e). On the remaining medium, the shoot height was lower. Average leaf ranged from 3 - 5 leaves (Table 3). Gibberellin is a natural plant growth regulator that affects many developmental processes, especially germination of seeds, development of stem and fruit (Janick, 1979). In micropropagation, GA_3 has an effect on cell division and elongation, which helps to prolong the body. In this study, GA_3 has been shown to be effective in prolonging the shoots and increasing the number of leaf.

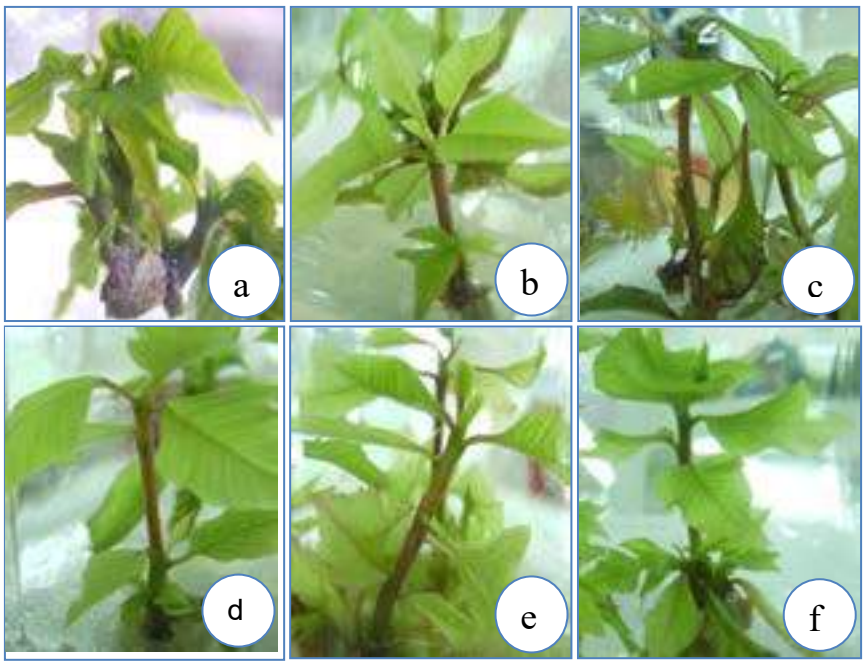

Figure 3. *In vitro* shoots of poinsettia at various GA_3 concentration
(a) 0 mg.L^{-1}; (b) 0,1 mg.L^{-1}; (c) 0,3 mg.L^{-1}; (d) 0,5 mg.L^{-1}; (e) 0,7 mg.L^{-1}; (f) 1,0 mg.L^{-1}.

Effect of medium and IBA concentration on root formation of poinsettia

Table 4. Effect of medium and IBA concentration on root formation of poinsettia

Medium	IBA concentration (mg.L^{-1})	Explant with root (%)	Number of root
½ MS	0.0	5.6	0
½ MS	0.5	11.1	2
½ MS	1.0	5.6	2
½ MS	1.5	16.7	2
½ MS	2.0	38.9	3
MS	0.0	5.6	2
MS	0.5	11.1	2
MS	1.0	11.1	2
MS	1.5	11.1	2
MS	2.0	27.8	3

Medium having ½ MS strength with 2 mg.L^{-1} IBA recorded to be the best medium giving 38.9 % root induction with an average number of 3 roots per cultured shoot, followed by MS contained 2 mg.L^{-1} IBA (27.8%) with 3 roots per shoot. The number of shoot was very low at 5 - 17% on the remaining media. In addition, the number of roots produced from 1 to 2 roots/shoots showed that the tested IBA concentrations were not suitable for rooting (Table 4). The results observed were consistent with that of Perera and Trader (2010) achieved 3 roots per shoot after 4 months of culturing. Similarly, the root formation of poinsettia was good with NAA treatment and the remaining auxin like IBA and IAA were ineffective (Danial and Ibrahim, 2016).

In general, in this experiment, the medium and IBA concentrations did not induce good *in*

vitro root formation after 4 weeks of culturing. Additional research is needed to improve the rate of rooting in order to produce complete seedlings before moving to the field.

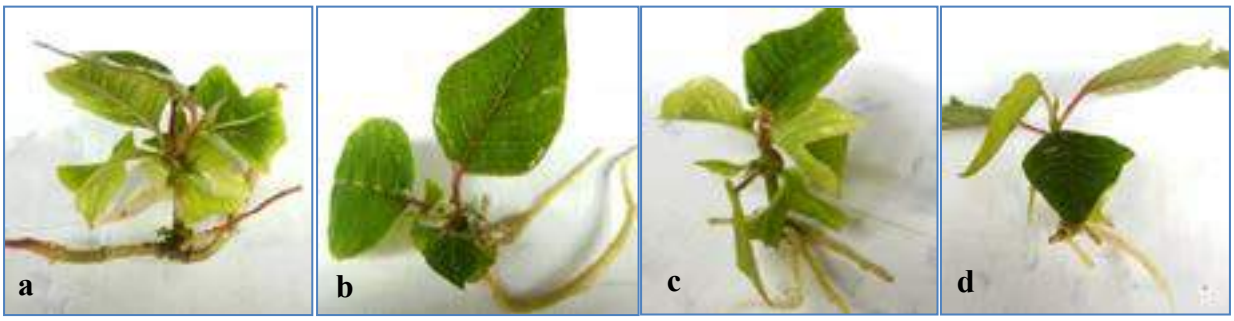

Figure 4. *In vitro* root formation of poinsettia on different media
(a) MS and IBA 1,0 mg.L^{-1}; (b) ½MS and IBA 1,0 mg.L^{-1}; (c) MS and IBA 2,0 mg.L^{-1}; (d) ½MS and IBA 2,0 mg.L^{-1}.

Acclimatized plantlets

The mixture of clean soil : coir : rice husk ash in the ratio of 1: 2: 3 provided the good condition for hardening of micropropagated plantlets. The survival rate was 75 % after 2 weeks of transfer to plastic pots. The seedlings grow and develop normally without morphological changes. It was recommended that the plantlets should be sprayed regularly and placed away from direct sunlight at about 26^{o}C in the garden.

CONCLUSION

The treatment with javel: distilled water at ratio 1: 2 (v/v) for 15 minutes showed the best for disinfection of nodal explants. MS medium supplemented with 0.1 mg.L^{-1} and 0.5 mg L^{-1} BA yields 4.8 shoots per explant. The shoots were then cultured on MS supplemented with GA_3 at 0.3 mg.L^{-1} for shoot elongation and leaf increasing. The suitable medium for rooting was ½MS (sugar free) medium contained 2 mg.L^{-1} IBA. *In vitro* formed plantlets were hardened and transferred to soil with 75% survival. This work was initiated to develop a protocol for multiplication of *Euphorbia pulcherrima* Willd. through nodal explants. Micropropagation through somatic embryogenesis and rooting should be continued in order to increase multiplication or produce poinsettia against viral diseases by genetic engineering.

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