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Callus Induction and Organogenesis Capacity from Lamina Explant of *Petunia* × *hybrida* F1 Induced by BA and NAA

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Abstract

Plant growth regulators (PGRs) have an important role in callus induction and organogenesis of plant explants cultured *in vitro* conditions. Callus has a proper potential for plant regeneration through indirect organogenesis and embryogenesis as well suspension culture, genetic transformation and production of secondary metabolites. In current experiment, leaf explants of *Petunia* × *hybrida* F1 were cultured on basal Murashige and Skoog (MS) medium supplemented with different concentrations of N6-benzyladenine (BA) (0.25, 0.50 and 1.00 mg l^{-1}) and α-naphthalene acetic acid (NAA) (0.10, 0.20 and 0.30 mg l^{-1}). The maximum fresh weight (5.16 g), dry weight (0.31 g) and volume of callus (24.50 cm^3) was obtained in the medium containing 1.00 mg l^{-1} BA in combination with 0.30 mg l^{-1} NAA. Leaf explants did not produce callus in medium without PGRs. Plantlets were produced on all callus grown on the media containing PGRs. The most plantlets number was produced on callus induced on medium enriched with 1.00 mg l^{-1} BA in combination with 0.30 mg l^{-1} NAA. The plantlets regenerated *in vitro* with well-developed shoots and roots were successfully established in pots containing peat and perlite (1:1) and grown in a greenhouse within 4 weeks with a 100% survival rate. The regenerated plants were morphologically identical with mother plants and did not show any detectable phenotypic variation.

Keywords: *In vitro* culture, Micropropagation, Ornamental plants, Plant growth regulators, Solanaceae.

INTRODUCTION

Petunia (*Petunia* × *hybrida* F1) from the Solanaceae family is an ornamental plant usually grown as annual. *Petunia* is a genus of 35 species of flowering plants and many members of this genus that contributed to the hundreds of hybrids are native to tropical and sub-tropical areas of South America (Maberly, 1990). *Petunia* hybrids are now popular garden and container plants all over the world. Most of today's garden *Petunia* species are produced by hybridization of *P. nyctaginiflora* with white flowers and *P. integriflora* with purple flowers (Abu-Qaoud *et al.*, 2010). Callus is a good source for suspension culture, production of secondary metabolites, clonal propagation through somatic embryogenesis and organogenesis, also, the study of cell division, elongation and differentiation process and genetic transformation (Praveen *et al*., 2010). PGRs have important role in callus induction and growth. Type of explants plays important role in the success of callus induction consequently micropropagation. Micropropagation is the true-to-type propagation of a selected genotype using *in vitro* culture techniques. Different environmental and nutritional factors were found to affect the *in vitro* organogenesis of petunia plant (Reuveni and Evenor, 2007; Qu *et al*., 2009). Some researchers used from leaf, stem cutting and anther as explants for micropropagation of *Petunia* hybrida (Clapa and Cantor, 2006; Abu-Qaoud *et al*., 2010). Several studies on micropropagation of *Petunia* hybrida were carried out using BA, NAA, IBA and TDZ (Clapa and Cantor, 2006; Abu-Qaoud *et al.*, 2010). The purpose of current study was to evaluate the effect of different concentrations of BA and NAA on fresh weight, dry weight and volume of callus and plantlet regeneration of *Petunia* × *hybrid* F1, an ornamental plant.

MATERIALS AND METHODS

Plant materials and surface sterilization

Hybrid seeds of *Petunia* × *hybrida* F1 were brought from Pakan Bazr Co., Isfahan city, Isfahan province, Iran. Seeds were washed under running tap water and a few drops of hand washing liquid for 20 min. After that, explants were immersed in 30% H_2O_2 and one or two drops of Tween-20 for 10 min then rinsed with sterilized distilled water thrice for 1, 3 and 5 min. Explants were disinfected by immersion in 20% sodium hypochlorite (NaClO) with a few drops of Tween-20 for 10 min followed by three rinses in sterile distilled water for 1, 3 and 5 min.

Culture medium and growth conditions for seeds

Surface sterilized seeds were inoculated in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 3% (w/v) sucrose. The medium was solidified with 0.70% agar-agar. The pH of the medium was adjusted to 5.6–5.8. The 30 ml medium was dispensed into each 250 ml glass dish. The culture glass dishes containing the media were autoclaved at 121°C for 20 min. Four seeds were inoculated in each glass dish and plugged firmly. All the cultures were maintained at 20 ±2°C under a 12 h photoperiod at a photosynthetic flux of 50 µmol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps.

Maintenance of shoot cultures and multiplication

Seeds were sprouted after a week on MS medium and the plantlets were produced after 8 weeks. Leaves were dissected form 8-week-old plantlets and used as explants. Explants were inoculated on MS medium. The medium was divided to several media containing BA (0.25, 0.50 and 1.00 mg l^{-1}) and NAA (0.10, 0.20 and 0.30 mg l^{-1}). Experiments were carried out in three replications and four explants for each replication. All the cultures were maintained at 24 ± 2°C under a 16 h photoperiod at a photosynthetic flux of 50 µmol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps and 75–80% RH.

Plantlets acclimatization

For acclimatization, the glass dishes with plantlets (fully expanded leaflets) were kept open

for 2–3 h after removing the plugs in the culture room itself. Then, the plantlets were removed from the culture media and washed with distilled water; then transferred to plastic cups (15-cm in diameter) containing a mixture of peat and perlite (1:1). Plantlets were kept in a greenhouse at 26 ± 2°C, 4000–5000 lux light and 75–80% RH with periodic irrigation.

Measured characters

Callus fresh weight, callus dry weight, callus volume and plantlets number of *Petunia* × *hybrida* F1 were calculated. Also, the percentage of plants survival was assessed.

Experimental design and data analysis

The experimental design was completely randomized design. Each experiment was carried out in 3 replications and each replication included 4 explants. Analysis of variance (ANOVA) was done using SAS statistical software and means were compared using the Least Significant Difference Test (LSD) at 5% probability level.

RESULTS

Leaf explants were applied for the induction and formation of callus. No callus formation occurred from leaf explants grown on the medium without PGRs (control). Callus induction and formation was observed on leaf explants grown on media enriched with BA and NAA. Results have been shown in Tables 1 and 2.

Callus fresh weight

Current investigation on the effect of PGRs on callus fresh weight revealed that BA plus NAA induced callus formation. Statistical analysis (ANOVA) of the data showed that the effect of PGRs on callus fresh weight was significant ($P<0.01$) (Table 2). The highest callus fresh weight

Table 1. Mean comparison of the effect of different concentrations of BA and NAA on callus fresh weight, callus dry weight, callus volume and plantlet number of *Petunia hybrida*.

PGRs (mg l^{-1})	Callus fresh weight (g)	Callus dry weight (g)	Callus volume (cm^3)	Plantlet number
BA 0.00 + NAA 0.00	0.00^{e}	0.00^{b}	0.00^{e}	0.00^{c}
BA 0.25 + NAA 0.10	0.89^{d}	0.10^{ab}	4.50^{cde}	0.75^{c}
BA 0.25 + NAA 0.20	0.85^{d}	0.17^{ab}	4.12^{de}	2.50^{bc}
BA 0.25 + NAA 0.30	0.66^{de}	0.08^{ab}	2.87^{de}	1.00^{c}
BA 0.50 + NAA 0.10	1.22^{cd}	0.11^{ab}	6.50^{bcd}	5.50^{bc}
BA 0.50 + NAA 0.20	2.18^{b}	0.20^{ab}	10.00^{b}	10.75^{b}
BA 0.50 + NAA 0.30	1.13^{cd}	0.13^{ab}	3.25^{de}	4.25^{bc}
BA 1.00 + NAA 0.10	0.93^{d}	0.11^{ab}	4.00^{de}	5.75^{bc}
BA 1.00 + NAA 0.20	1.78^{bc}	0.14^{ab}	8.62^{bc}	8.00^{bc}
BA 1.00 + NAA 0.30	5.16^{a}	0.31^{a}	24.50^{a}	28.75^{a}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

Table 2. Analysis of variance of the effect of different concentrations of BA and NAA on callus fresh weight, callus dry weight, callus volume and plantlet number of *Petunia hybrida*.

S.o.V	df	Callus fresh weight	Callus dry weight	Callus volume	Plantlet number
Treatment	9	0.725**	0.012**	4.328**	7.566**
Error	30	0.007	0.003	0.061	0.417
CV (%)	-	6.19	7.84	9.81	28.29

**: Significant at $\alpha < 1\%$

(5.16 g) was observed on MS medium containing 1.00 mg l^{-1} BA + 0.30 mg l^{-1} NAA (Table 1). Callus fresh weight (2.18 g) obtained on medium containing 0.50 mg l^{-1} BA + 0.20 mg l^{-1} NAA was relatively high. The lowest callus fresh weight (0.66 g) was calculated on MS medium enriched by 0.25 mg l^{-1} BA + 0.30 mg l^{-1} NAA. NAA concentration was similar in both media that induced the highest and lowest callus fresh weight (0.30 mg l^{-1}) but BA concentration was different (1.00 and 0.25 mg l^{-1}) (Table 1).

Callus dry weight

Analysis of variance (ANOVA) of the data showed that the effect of BA and NAA on callus dry weight was significant ($P<0.01$) (Table 2). The highest callus dry weight (0.31 g) was calculated on MS medium containing 1.00 mg l^{-1} BA + 0.30 mg l^{-1} NAA (Table 1). Callus dry weight (0.20 g) obtained on medium containing 0.50 mg l^{-1} BA + 0.20 mg l^{-1} NAA was relatively high. The lowest callus dry weight (0.08 g) was observed on MS medium enriched by 0.25 mg l^{-1} BA + 0.30 mg l^{-1} NAA.

Callus volume

Maximum callus volume (24.50 cm^3) was measured with treatment of 1.00 mg l^{-1} BA + 0.30 mg l^{-1} NAA (Table 2). The treatment of 0.50 mg l^{-1} BA + 0.20 mg l^{-1} NAA with callus induction of 10.00 cm^3 was proper, too. Minimum callus volume (2.87 cm^3) was measured with treatment of 0.25 mg l^{-1} BA + 0.30 mg l^{-1} NAA (Table 2). There was significant difference between different concentrations of BA and NAA on callus volume ($P<0.01$) (Table 2).

Plantlet number

Differences of plantlets number in explants grown under BA and NAA was significant ($P<0.01$) (Table 2). Plantlet number was varied with BA and NAA concentrations (Tables 1). Minimum plantlet number (0.75 and 1.00) was recorded in the plantlets produced on media supplemented with 0.25 mg l^{-1} BA + 0.10 mg l^{-1} NAA and 0.25 mg l^{-1} BA + 0.30 mg l^{-1} NAA (Table 1). In these two media, the concentration of BA was similar (0.25 mg l^{-1}). The largest number of plantlet (28.75) was achieved on MS medium supplemented with 1.00 mg l^{-1} BA + 0.30 mg l^{-1} NAA (Table 2). The 10.00 plantlets were produced from callus induced in medium containing 0.50 mg l^{-1} BA + 0.20 mg l^{-1} NAA.

DISCUSSION

The success of *in vitro* culture is related to the correct choice of explants material (George *et al.*, 2008). In current study, leaf explants of *Petunia* × *hybrida* showed relatively suitable potential for production of callus. *In vitro Petunia* leaf explants have been applied for many sorts of studies (Abu-Qaoud, 2012). Thirukkumaran *et al*. (2009) obtained a maximum frequency of shoot regeneration (52.1%) from leaf explants of *Petunia*. It was observed that there are different factors influencing tissue growth and differentiation. The most important factors include explant source and kind and concentration of PGRs (Mihaljevi *et al.*, 2002; Al-Mallah and Salih, 2006). The importance of auxins for callus induction in various plants was demonstrated by many researchers (Jain and Häggman, 2007; Jain and Ochatt, 2010). Current study showed the more importance of BA than NAA for callus induction in *P. hybrida*. Callus induction from explants depends strongly on genotype, too (George *et al*., 2008).

Study of Abu-Qaoud *et al*. (2010) on *in vitro* regeneration of *Petunia* hybrida showed that the highest number of axillary shoot was obtained on medium supplemented with 0.8 mg l^{-1} BA and 0.1 mg l^{-1} NAA. These researchers showed that both of BA and NAA are effective on shoot multiplication. Our study is consistent with this finding. Another study of Abu-Qaoud (2012) on

P. hybrida revealed that the highest shoot percentage (87.5 to 90%) with both explant sources (leaf and shoot) was obtained when TDZ was used singular at levels between 0.5 and 8 µM. Regeneration was reduced significantly when TDZ was combined with 2.7 µM NAA. Contrary to this finding, our work showed the importance of both of auxins and cytokinins on regeneration of shoots. *In vitro* regeneration is mainly regulated by the balance and the interaction between PGRs in the medium and those endogenously produced by the explants (Subotic, 2008; Abu-Qaoud, 2012). Thus, the type of PGRs in culture medium, interaction between auxins and cytokinins and endogenous level of PGRs are important factors for regeneration of shoots and roots from explants (Sriskandarajah *et al*., 2006; Abu-Qaoud, 2012). Many researches showed the effect of hormonal combinations on shoot and root formation (Rui-yue, 2007; Xian-chun, 2010).

CONCLUSION

In conclusion, the present study showed an efficient procedure for callus induction and plantlets regeneration in *P. hybrida*. The maximum callus induction and plantlets regeneration was obtained in the medium containing 1.00 mg l^{-1} BA in combination with 0.30 mg l^{-1} NAA.

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