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A Simple and Rapid Method for Micropropagation of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'

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Abstract

For efficient regeneration of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn', a simple *in vitro* micropropagation protocol was developed. Axillary bud explants dissected from 30-day-old *in vitro*-grown seedlings sprouted from hybrid seeds were cultured on Murashige and Skoog (MS) medium supplemented with 36 combinations of 6-benzylaminopurine (BA; 0.00, 0.50, 1.00, 2.00, 3.00 and 5.00 mg l^{-1}) and α-naphthaleneacetic acid (NAA; 0.00, 0.10, 0.50, 1.00, 1.50 and 3.00 mg l^{-1}). The BA alone at 0.50 or 2.00 mg l^{-1} was found to be best for shoot length, shoot number, node number, and leaf number than other concentrations of BA and BA in combination with NAA. The highest number of shoots (8.44) and the maximum average shoot length (13.16 cm) were recorded on MS medium supplemented with 0.50 mg l^{-1} BA without NAA. Root length (5.20 cm) and root number (8.77) were the maximum in the medium containing 0.10 mg l^{-1} NAA. The plantlets regenerated in vitro with well-developed shoots and roots were successfully established in pots containing peat and perlite and grown in a greenhouse within 4 weeks with a 100% survival rate. The regenerated plants were morphologically identical with donor plants and did not show any detectable phenotypic variation. Overall, BA at 0.50 or 2.00 mg l^{-1} had a better effect on shoot system than other concentrations of BA and BA in combination with NAA. NAA induced more root formation and root growth than BA.

Keywords: Auxins, Cytokinins, *In vitro* culture, Ornamental plants, Plant growth regulators, Tissue culture.

INTRODUCTION

Ornamental plants are produced primarily for their artistic value; thus, the propagation and improvement of quality attributes and the creation of novel variations are important economic goals for floriculturists (Nazki *et al*., 2018). Petunias (from the family Solanaceae, subfamily Petunioideae) are herbaceous perennials, usually grown as annuals. Petunia is a genus of 35 species of flowering plants and many members of the genus petunia that have 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 the varieties seen in gardens are *Petunia hybrida*. *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn' is a name for the dainty bloomer. Petunia F1 'Opera Supreme Pink Morn' is a heavy continuous bloomer, without deadheading. Petunias with 3 colors are labeled 'morn' types, hence the name 'Pink Morn' (silvery pink flowers with cream centers and a yellow throat).

Plant growth regulators (PGRs), especially auxins and cytokinins (CKs), are vital components required for inducing organogenesis in many micropropagation endeavors (Beyl, 2005; Motte *et al*., 2014). However, the addition of these PGRs into growth media often elicits diverse developmental responses and influences the concentration and type of endogenous PGR pools accumulated in *in vitro* regenerants (Plačková *et al*., 2015; Ċosič *et al*., 2015; Aremu *et al*., 2016). These fluctuations, particularly in the concentration and type of endogenous CKs, remain a crucial factor influencing shoot and root proliferation as well as the occurrence of in vitro-induced physiological disorders in many regenerants (Plačková *et al*., 2015). Therefore, micropropagation endeavors accompanied by hormonal physiology, which often provides important insights on these aforementioned parameters remain pertinent. PGRs have an important role in callus induction and growth. Type of explants plays an important role in the success of callus induction and consequently in micropropagation (Kaviani and Negahdar, 2017). 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 plants (Reuveni and Evenor, 2007). Furthermore, they serve as a useful tool for facilitating the mass production of plant materials to mitigate the declining population of highly-demanded plants. Some researchers have used leaf, stem cutting and anther as explants for micropropagation of *Petunia hybrida* (Abu-Qaoud *et al*., 2010; Clapa and Cantor, 2006). Several studies have focused on micropropagation of *Petunia hybrida* (Abu-Qaoud *et al*., 2010; Clapa and Cantor, 2006). But, relatively few studies have been carried out on the tissue culture of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'. Thus, the aim of this investigation was to evaluate the effect of different concentrations of BA and NAA on shoot multiplication and root induction of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'.

MATERIALS AND METHODS

Plant materials and surface sterilization

Hybrid seeds of *Petunia* × *hybrida* F1 ‘Opera Supreme Pink Morn’ were procured from Parmis Co. in Mahallat County of Isfahan province, Iran. The seeds were washed under running tap water and some drops of hand washing liquid for 20 min. After that, the explants were immersed in 30% H_2O_2 and one or two drops of Tween-20 for 10 min. Then, they were rinsed with sterilized distilled water thrice for 1, 3 and 5 min. The explants were disinfected by immersion in 20% sodium hypochlorite (NaClO) with a few drops of Tween-20 for 20 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 modified Murashige and Skoog (1962) medium 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 on MS medium after a week, and the plantlets were produced after 4 weeks. Auxiliary buds were dissected from 4-week-old plantlets to serve as explants. The explants were inoculated on modified MS medium 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 medium was divided to several media containing 0.00, 0.50, 1.00, 2.00, 3.00 or 5.00 mg l^{-1} BA and/or 0.00, 0.10, 0.50, 1.00, 1.50 or 3.00 mg l^{-1} NAA, individually and/or in combination (36 treatments). The medium (30 ml) was dispensed into 250 ml glass dishes. The culture glass dishes containing the media were autoclaved at 121°C for 20 min. The experiments were carried out in three replications, each composed of four explants. 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.

Rooting *in vitro* and plantlets acclimatization

Rooting was done in modified MS medium supplemented with 0.00, 0.50, 1.00, 2.00, 3.00 or 5.00 mg l^{-1} BA and/or 0.00, 0.10, 0.50, 1.00, 1.50 or 3.00 mg l^{-1} NAA, individually and/or in combination. The pH of the media, autoclave time and environmental conditions were the same as shoot production media. For easy acclimatization, the glass dishes containing 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, they were placed in plastic cups (15-cm in diameter) containing a mixture of peat and perlite (1:1). The plantlets were kept in a greenhouse at 27 ± 1°C, 4000–5000 lux light, and 75–80% RH with periodic irrigation.

Measured traits

The recorded traits included shoot length, shoot number, node number, leaf number, root length and root number of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn' that were calculated after 45 days.

Experimental design and data analysis

The experimental design was factorial on the basis of a completely randomized design (CRD). Each experiment was carried out in three replicates and each replicate included four explants. Analysis of variance (ANOVA) was done using the SAS and MSTATC statistical software packages and means were compared using the Least Significant Difference Test (LSD) at the 5% probability level.

RESULTS

Effect of BA and NAA on shoot length

Table 1 and Fig. 1 show that BA at the lowest concentration significantly improved shoot length of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'. The number of shoots in terms of shoot length was improved on BA-containing medium. The highest average shoot length of 13.16 cm per explant (Table 1) was obtained from plants exposed to 0.50 mg l^{-1} BA followed by 12.33 shoots per explant observed in plants exposed to 2.00 mg l^{-1} BA, both without NAA. Media supplementation with BA outperformed NAA for shoot length induction, singularly or in combination (Table 1). BA rates of 2.00 and 5.00 mg l^{-1} in combination with low rates of NAA (0.10 and 0.50 mg l^{-1}) were relatively suitable for enhancing shoot length (10.10 and 10.33 cm/plantlets, respectively) (Table 1). The lowest shoot length (0.44 cm) was measured in plantlets treated with 1.00

or 3.00 mg l^{-1} BA along with 1.50 mg l^{-1} NAA. Our results showed that 1.50 mg l^{-1} NAA + 0, 0.50 or 1.00 mg l^{-1} BA induced the minimum shoot length (Table 1). Concerning shoot length per plantlets, NAA at 0.10 and 1.00 mg l^{-1} induced the maximum length (4.43 and 4.86 cm, respectively). There were significant differences between BA, NAA and interactive effects of BA and NAA in shoot length ($P < 0.01$) (Table 2).

Effect of BA and NAA on shoot number

A maximum multiplication rate of 8.44 shoots per explant (Table 1) was recorded with 0.50 mg l^{-1} BA followed by 7.22 shoots per explant with 2.00 mg l^{-1} BA, both without NAA. Media supplementation with BA was better than NAA, singularly or in combination (Table 1). Shoot number revealed that BA was comparable with NAA for shoot induction. The application of 5.00 mg l^{-1} BA + 0.00 mg l^{-1} NAA was relatively suitable for induction of shoot per plantlet (4.00) (Table 1). Our findings demonstrated that 1.50 mg l^{-1} NAA produced minimum shoot number (0.66) without BA or with 1.00 mg l^{-1} BA (Table 1). Concerning the shoot number per plantlets, NAA was not suitable and induced the lowest number (around 1.00). There were significant differences between BA, NAA and interactive effects of BA and NAA ($P < 0.01$) in shoot number (Table 2).

Fig. 1. Stages of micropropagation of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'. (1) Seeds of *P. hybrida*; (2) plantlets derived from seeds cultured on MS medium without PGRs; (3) auxiliary buds used as explants; (4) explants on MS medium containing PGRs; (5) auxiliary buds proliferation on medium enriched with 0.50 and 2.00 mg l^{-1} BA without NAA; (6) a plantlet grown on medium enriched with 0.50 mg l^{-1} BA ready for rooting; (7) plantlets with roots produced on medium supplemented with 0.01 mg l^{-1} NAA without BA; (8) roots produced at the base of shoot on MS medium containing 0.01 mg l^{-1} NAA; (9) hardened plants. Plants were acclimated in a greenhouse using pots filled with peat and perlite (1:1).

Table 1. Mean comparison of the effect of different concentrations of BA and NAA on shoot length, shoot number, node number, leaf number, root length and root number of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'.

BA (mg l^{-1})	NAA (mg l^{-1})	Shoot length (cm)	Shoot number	Node number	Leaf number	Root length (cm)	Root number
0.00	0.00	3.37^{m} ± 1.30	1.00^{op} ± 0.93	4.27^{m} ± 0.83	9.05^{g-i} ± 0.56	2.17^{f-h} ± 0.36	5.66^{cd} ± 1.63
0.00	0.10	4.43^{k} ± 0.24	1.83^{h-k} ± 0.10	4.66^{l} ± 0.44	11.44^{ef} ± 1.83	5.20^{a} ± 3.39	8.77^{a} ± 4.74
0.00	0.50	1.15^{o} ± 3.52	1.16^{n-p} ± 0.77	2.16^{rs} ± 2.94	5.83^{l-p} ± 3.78	2.94^{d} ± 1.13	3.16^{lm} ± 0.87
0.00	1.00	4.86^{ij} ± 0.19	1.66^{j-l} ± 0.27	1.20^{uv} ± 3.90	5.33^{m-q} ± 4.28	1.49^{ij} ± 0.32	3.88^{i-k} ± 0.15
0.00	1.50	0.61^{p} ± 4.06	0.66^{q} ± 1.27	0.83^{w} ± 4.27	4.00^{qr} ± 5.61	1.056^{j-n} ± 0.76	3.00^{m} ± 1.03
0.00	3.00	3.66^{lm} ± 1.01	1.36^{l-n} ± 0.57	2.61^{pq} ± 2.49	5.83^{l-p} ± 3.78	1.13^{j-m} ± 0.68	4.77^{e-h} ± 0.74
0.50	0.00	13.16^{a} ± 8.49	8.44^{a} ± 6.51	15.10^{b} ± 10.00	26.66^{a} ± 17.05	1.00^{k-n} ± 0.81	4.44^{g-i} ± 0.41
0.50	0.10	5.06^{hi} ± 0.39	2.33^{fg} ± 0.40	6.33^{i} ± 1.23	12.88^{e} ± 3.27	2.33^{f} ± 0.52	5.32^{de} ± 1.29
0.50	0.50	1.22^{o} ± 3.45	1.22^{no} ± 0.71	3.05^{no} ± 2.05	7.38^{i-l} ± 2.23	0.48^{op} ± 1.33	3.44^{j-m} ± 0.59
0.50	1.00	2.22^{n} ± 2.45	2.10^{gh} ± 0.17	3.00^{no} ± 2.10	7.64^{i-k} ± 1.97	0.88^{l-o} ± 0.93	3.66^{j-l} ± 0.37
0.50	1.50	0.47^{p} ± 4.20	1.11^{n-p} ± 0.82	2.33^{qr} ± 2.77	5.44^{m-q} ± 4.17	0.38^{p} ± 1.43	2.05^{n} ± 1.98
0.50	3.00	3.35^{m} ± 1.32	1.00^{op} ± 0.93	2.00st ± 3.10	5.00^{n-q} ± 4.61	0.65^{n-p} ± 1.16	3.04^{ml} ± 0.99
1.00	0.00	6.08^{f} ± 1.41	1.33^{mn} ± 0.60	7.44^{g} ± 2.34	12.88^{e} ± 3.27	1.84^{g-i} ± 0.03	4.44^{g-i} ± 0.41
1.00	0.10	3.79^{l} ± 0.88	1.00^{op} ± 0.93	6.22^{i} ± 1.12	10.66^{fg} ± 1.05	5.00^{a} ± 3.19	4.66^{f-h} ± 0.63
1.00	0.50	0.77^{p} ± 3.90	1.00^{op} ± 0.93	2.77^{op} ± 2.33	6.44^{k-o} ± 3.17	2.46^{ef} ± 0.65	3.33^{k-m} ± 0.70
1.00	1.00	4.55^{jk} ± 0.12	1.56^{k-m} ± 0.37	1.00^{vw} ± 4.10	7.43^{i-l} ± 2.18	0.89^{l-o} ± 0.92	4.88^{e-h} ± 0.85
1.00	1.50	0.44^{p} ± 4.23	0.66^{q} ± 1.27	0.83^{w} ± 4.27	3.10^{r} ± 6.51	2.25^{fg} ± 0.44	0.51^{o} ± 3.52
1.00	3.00	4.20^{k} ± 0.47	1.00^{op} ± 0.93	10.77^{e} ± 5.67	5.00^{n-q} ± 4.61	1.42^{j-k} ± 0.39	5.22^{d-f} ± 1.19
2.00	0.00	12.33^{b} ± 7.66	7.22^{b} ± 5.29	17.44^{a} ± 12.34	26.33^{a} ± 16.72	1.84^{g-i} ± 0.03	4.33^{hi} ± 0.30
2.00	0.10	10.10^{c} ± 5.43	3.00^{e} ± 1.07	10.00^{f} ± 4.90	18.64^{c} ± 9.03	2.00^{f-h} ± 0.19	6.88^{b} ± 2.85
2.00	0.50	5.33^{gh} ± 0.66	3.33^{d} ± 1.40	5.22^{jk} ± 0.12	15.00^{d} ± 5.39	2.87^{de} ± 1.06	8.22^{a} ± 4.19
2.00	1.00	6.66^{e} ± 1.99	1.33^{mn} ± 0.60	1.33^{u} ± 3.77	8.42^{h-j} ± 1.19	1.22^{j-l} ± 0.59	5.10^{d-f} ± 1.07
2.00	1.50	7.00^{e} ± 2.33	1.55^{k-m} ± 0.38	5.00^{k} ± 0.10	10.05^{f-h} ± 0.44	1.00^{k-n} ± 0.81	5.00^{e-g} ± 0.97
2.00	3.00	4.50^{jk} ± 0.17	1.17^{n-p} ± 0.76	14.10^{c} ± 9.00	6.50^{k-o} ± 3.11	0.95^{l-n} ± 0.86	4.44^{g-i} ± 0.41
3.00	0.00	4.85^{ij} ± 0.18	1.88^{h-j} ± 0.05	5.33^{j} ± 0.23	12.77^{e} ± 3.16	0.85^{l-o} ± 0.96	4.40^{g-i} ± 0.37
3.00	0.10	1.44^{o} ± 3.23	1.11^{n-p} ± 0.82	4.66^{l} ± 0.44	8.99^{g-i} ± 0.62	4.25^{b} ± 2.44	3.83^{i-k} ± 0.20
3.00	0.50	0.55^{p} ± 4.12	0.88^{pq} ± 1.05	3.11^{n} ± 1.99	4.66^{p-r} ± 4.95	2.16^{f-h} ± 0.35	0.39^{o} ± 3.64
3.00	1.00	1.15^{o} ± 3.52	2.02^{hi} ± 0.09	4.15^{m} ± 0.95	6.50^{k-o} ± 3.11	2.10^{f-h} ± 0.29	0.78^{o} ± 3.25
3.00	1.50	0.44^{p} ± 4.23	1.00^{op} ± 0.93	2.00st ± 3.10	4.83^{o-r} ± 4.78	1.50^{ij} ± 0.31	0.39^{o} ± 3.64
3.00	3.00	6.20^{f} ± 1.53	2.50^{f} ± 0.57	6.78^{h} ± 1.68	7.00^{j-m} ± 2.61	1.10^{j-n} ± 0.71	0.54^{o} ± 3.49
5.00	0.00	10.00^{c} ± 5.33	4.00^{c} ± 2.07	12.44^{d} ± 7.34	20.55^{b} ± 10.94	1.71^{hi} ± 0.10	3.44^{j-m} ± 0.59
5.00	0.10	8.85^{d} ± 4.18	3.00^{e} ± 1.07	10.56^{e} ± 5.46	16.23^{d} ± 6.62	3.44^{c} ± 1.63	4.74^{e-h} ± 0.71
5.00	0.50	10.33^{c} ± 5.66	1.75^{i-k} ± 0.18	1.75^{t} ± 3.35	8.73^{h-j} ± 0.88	2.33^{f} ± 0.52	6.22^{c} ± 2.19
5.00	1.00	5.55^{g} ± 0.88	1.00^{op} ± 0.93	1.20^{uv} ± 3.90	6.22^{k-p} ± 3.39	0.70^{m-p} ± 1.11	4.33^{hi} ± 0.30
5.00	1.50	5.00^{hi} ± 0.33	1.20^{no} ± 0.73	1.10^{uvw} ± 4.00	6.00^{k-p} ± 3.61	0.95^{l-n} ± 0.86	4.00^{ji} ± 0.03
5.00	3.00	4.33^{k} ± 0.34	1.10^{n-p} ± 0.83	1.00^{vw} ± 4.10	6.66^{k-n} ± 2.95	0.72^{m-p} ± 1.09	3.88^{i-k} ± 0.15
LSD		0.374	0.309	0.309	1.750	0.467	0.636

Means with similar letter(s) in each column were not significant at the 1 and 5% probability level according to LSD test.

Table 2. Analysis of variance (ANOVA) for the effect of different concentrations of BA and NAA, singularly or in combination with each other, on the shoot length, shoot number, node number, leaf number, root length and root number of *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn'.

SoV	df	MS					
		Shoot length	Shoot number	Node number	Leaf number	Root length	Root number
BA	5	92.93**	10.31**	75.82**	141.90**	4.83**	32.52**
NAA	5	79.10**	20.12**	202.10**	441.60**	18.74**	0.98^{ns}
BA × NAA	25	15.30**	5.36**	26.94**	6.59*	1.22**	5.511**
Error	75	0.05	0.03	0.03	1.16	0.08	0.15
CV (%)		4.92	9.84	3.72	11.23	15.78	9.70

*, ** and ^{ns} show significance at the 5 and 1% probability levels and insignificance according to LSD test, respectively.

Effect of BA and NAA on node number

Differences in node number were significant ($P < 0.01$) in explants grown under BA, NAA or a combination of BA and NAA (Table 2). Node number varied with BA and NAA concentrations (Table 1). Minimum node number per explant (0.83) was recorded in the plantlets treated with 1.50 mg l^{-1} NAA + 0.00 or 1.00 mg l^{-1} BA (Table 1). The largest number of nodes per plantlet (17.44 and 15.10) was achieved on MS medium supplemented with 2.00 and 0.50 mg l^{-1} BA, respectively without NAA (Table 1). Among all concentrations of NAA, 0.10 mg l^{-1} induced the largest number of nodes per plantlets (4.66) (Table 1).

Effect of BA and NAA on leaf number

Leaf number varied with BA and NAA concentrations (Tables 1). Differences in leaf number were significant in explants grown under BA ($P < 0.01$), NAA ($P < 0.01$) and combination of BA and NAA ($P < 0.05$) (Table 2). The maximum number of leaf per plantlet (26.66 and 26.33) was obtained on MS media supplemented with 0.50 and 2.00 mg l^{-1} BA, respectively without NAA (Table 1). The minimum leaf number per explant (3.10) was calculated in the plantlets treated with 1.00 mg l^{-1} BA + 1.50 mg l^{-1} NAA (Table 1). The treatment of 1.50 mg l^{-1} NAA without BA was not found to be proper as it resulted in the production of as low as 4.00 leaves per explant. Among all concentrations of NAA, 0.10 mg l^{-1} induced the maximum leaf number per plantlets (11.44) (Table 1).

Effect of BA and NAA on root length

In the case of root length, BA, NAA and interaction effect of these two PGRs had a significant effect ($P < 0.01$) on rooting capacity and the best results (5.20 cm per plantlet) was obtained with shoots treated with 0.10 mg l^{-1} NAA without BA (Fig. 1, Tables 1 and 2). However, 0.10 mg l^{-1} NAA along with 1.00, 3.00 and 5.00 mg l^{-1} BA induced proper root length (5.00, 4.25 and 3.44 cm per plantlet, respectively) (Table 1). Least root length (0.26 cm per explant) was observed in shoots grown on medium containing 0.50 mg l^{-1} BA with 1.50 mg l^{-1} NAA. Our findings showed that NAA at the concentrations higher than 0.10 (0.50–3.00 mg l^{-1}) along with all concentrations of BA (0.50–5.00 mg l^{-1}) reduced root length, completely (Table 1). Root length in the control plantlets (2.17 cm) without PGRs treatments was better than many plantlets grown on media with PGRs (Table 1).

Effect of BA and NAA on root number

The data clearly show that root number was affected by BA, NAA and the interaction of BA and NAA (Table 2). The best results for rooting frequency and mean number of roots were obtained in media enriched with 0.10 mg l^{-1} NAA without BA and 2.00 mg l^{-1} BA along with 0.50

mg l^{-1} NAA (8.77 and 8.22 per plantlet, respectively) (Fig. 1, Table 1). However, 0.10 mg l^{-1} NAA along with 2.00 mg l^{-1} BA and 0.50 mg l^{-1} NAA along with 5.00 mg l^{-1} BA induced good mean number of roots (6.88 and 6.22 per plantlet, respectively; Table 1). With regard to BA concentration, 0.50–3.00 mg l^{-1} induced suitable root number (more than 4.00 per plantlet). However, root number in control plantlets (5.66) was more than these treatments. The lowest number of roots per shoot (0.30–0.54) was observed in plantlets treated with 0.50–3.00 mg l^{-1} NAA along with 1.00 and 3.00 mg l^{-1} BA (Table 1).

The *in vitro* regenerated plantlets with well-developed roots were acclimatized ex vitro and eventually established in a greenhouse with high relative humidity (75–80%). The surviving frequency was 100% four weeks after acclimatization. Micropropagated plants showed good growth and uniformity *ex vitro* and exhibited normal development.

DISCUSSION

The present study revealed that in *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn', *in vitro* propagation could be done efficiently by culturing auxiliary buds from sprouting seeds on MS medium. Shoot multiplication was accompanied by simultaneous rooting of the shoots and the formation of normal plants that could be transferred directly to the soil. We found that BA individually promoted the induction of shoots, nodes and leaves. This induction was done at low concentrations and without the presence of NAA. The cytokinins, especially BA, have been commonly used for the induction of organogenesis in many plants (Mneny and Mantell, 2002; Sujatha and Ranjitha Kumari, 2007; Kaviani, 2015). In agreement with our report, BA has been preferred to other cytokinins in inducing multiple shoots in plants (Sujatha and Ranjitha Kumari, 2007; Kaviani, 2015). BA is most effective for meristem, shoot tip and bud cultures (Sujatha and Ranjitha Kumari, 2007). In the present study, when BA and NAA combinations were examined, the frequency of shoot, node and leaf induction was no better. In agreement with the present investigation, BA was reported to produce more number of shoots in *Eustoma grandiflorum* (Kaviani *et al*., 2014). Contrary to our report, some researchers have revealed that several species may require a low concentration of auxin in combination with cytokinins to increase shoot proliferation (van Staden *et al*., 2008; Kaviani *et al*., 2013). In the present study, the higher concentrations of BA reduced the number of micropropagated shoots. A similar response was also observed by Kaviani *et al*. (2014). Thus, the results suggest that small amounts of BA (0.10 mg l^{-1}) were effective in stimulating *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn' shoot elongation and shoot, node and leaf number. In a study on *Petunia hybrida* cv. Grandiflora, Clapa and Cantor (2006) revealed that the higher frequency regeneration (66-72%) was obtained when the stem cuttings were cultured in the media supplemented with 1 mg l^{-1} BAP. Abu-Qaoud *et al*. (2010) showed that lower regeneration percentage was achieved with leaf explants in *Petunia hybrida*. The highest shoot number (1.7) was obtained from MS medium supplied with 0.4 mg l^{-1} BA and 0.1 mg l^{-1} NAA. However, in case of shoot explant higher shoot number was obtained from 0.8 mg l^{-1} BA and 0.1 mg l^{-1} NAA. These researchers showed that the highest shoot regeneration rate (45%) was observed in MS medium supplemented with 2 mg l^{-1} BA.

Our study demonstrated that NAA at lower concentrations (0.10 mg l^{-1}) and individually is suitable for root induction. In agreement, NAA was reported as a good auxin for rooting of many plants (Kalimuthu *et al*., 2007; Jain and Ochatt, 2010; Kaviani *et al*., 2011; Kaviani, 2014; Kaviani, 2015). The addition of auxin to the medium was not essential to induce rooting in the regenerated shoots. Root initiation occurred on media lacking NAA although the greatest percentage of shoots was rooted on NAA-enriched media. The results presented here showed that BA at various concentrations along with NAA promoted rooting. Some studies have confirmed this finding (Kaviani *et al*., 2011; Kaviani *et al*., 2014; Kaviani, 2014). Increasing the concentration of NAA above 0.50 mg l^{-1} decreased the frequency of root regeneration. Similar findings have been reported by some

workers (Ghaffari Esizad *et al*., 2012; Kaviani *et al*., 2014). In a study on micropropagation of *Petunia hybrida*, Abu-Qaoud *et al*. (2010) showed good rooting of shoots when these shoots were immersed in IBA and were transferred into pots. According to the study done by these researchers, shoots regenerated from leaf explants were transferred onto MS basal medium for three weeks. The shoots with 2 cm length were immersed in 2500 mg l^{-1} IBA powder and transferred into pots containing sterile rooting media (2:1 mixture of sterile sphagnum peat and vermiculite).

CONCLUSION

It can be concluded that *Petunia* × *hybrida* F1 'Opera Supreme Pink Morn' can be well-multiplied, rooted and grown on MS medium supplemented with suitable concentrations of BA and NAA for each process. BA at 0.50 or 2.00 mg l^{-1} had a better effect on shoot system than other concentrations of BA and BA in combination with NAA. NAA induced more root formation and root growth than BA.

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