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Micropropagation of an Important Medicinal Plant, *Begonia fallax* (Begoniaceae)

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Abstract

Begonia fallax A.DC. belongs to the family of Begoniaceae and is an endemic species to Peninsular India. Shoot tip and nodal explants were screened using Murashige and Skoog medium supplemented with BAP, KIN and TDZ individually and combination with IAA, NAA and IBA for multiple shoot initiation. BAP and NAA (0.5+0.5 mg^{-l}) produced 20.8 shoots with up to 4.0 cm shoot length to nodal explants whereas 17.9 shoots with 3.1 cm shoot length to shoot tip explants. For root induction, various concentrations of IAA, NAA and IBA were used individually. The highest rooting frequency was achieved to nodal explants (64.90 roots) on MS medium supplemented with 0.5 mg^{-l} NAA compared to shoot tip explants (31.90 roots). Invariably, root length was up to 2.3 cm to both the explants. The rooted explants were transplanted into plastic cups and showed 70% survival rate in the Environmental Growth Chamber. Nodal explants could be the best for large-scale multiplication and propagation to facilitate *in vitro* conservation and for secondary metabolites production.

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Keywords

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Endemic plant
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Introduction

The family of Begoniaceae is represented by two genera, *Begonia* and *Hillebrandia* (Forrest and Hollingsworth, 2003) and its members are mostly herbs, followed by shrubs, and rarely lianas. Members of Begoniaceae can easily be distinguished from its allies mostly by fleshy stem, stipulate and usually asymmetrical leaves, showy unisexual flowers with petal-like perianth segments, numerous centripetal stamens, and seeds that uniquely have collar cells below an operculum (Clement et al., 2004).*Begonia* is one of the ten largest Angiosperm genera and has about 1,630 species, 53 varieties (The Plant List, 2013) and 15,000 cultivars distributed in tropical and subtropical regions(Clement et al., 2004; Espino et al., 2004).This genus is largely divided into 8 groups on the basis of form such as cane-like, rex-cultorum, rhizomatous, semperflorens, shrub-like, thick-stemmed, trailing or scandent and tuberous (Mendi et al., 2009). *Hillebrandia* is represented by monotypic, *Hillebrandia sandwicensis* Oliv. distributed in Hawaiian archipelago. Sixty species are distributed in the Eastern Himalayas and peninsular India wherein 23 species are endemics (Kumar and Bhattacharyya, 1992) and 8 species are distributed in the State of Tamil Nadu (Ramamurthy, 1983). Generally, *Begonia* species are propagated by stem and leaf cuttings. Their vegetative propagation can be improved by application of

micropropagation techniques (Murashige and Skoog, 1962) to produce more number of adventitious shoots. Simmonds (1984) treated plantlets of *Begonia x hiemalis* with commercial rooting powder to produce much stronger root systems under greenhouse conditions towards adaptation without any loss. MS medium supplemented with different concentrations of BA and IAA combinations facilitated shoot axillary-bud proliferation and organogenesis to produce plantlets in *Begonia malabarica* (Nisha et al., 2009). *Begonia venosa* is a highly valuable ornamental plant of wild origin in Brazil and its tender flower buds produced callus in MS medium with BA and NAA (Pierik and Tetteroo, 1987). Kinetin induced multiple shoots either individually or in combinations in MS medium, for example, 5 µM kinetin produced an average of 8 shoots callus^{-1} in *Begonia x elatior* and 0.5 mg^{-1} kinetin and 2% coconut water to achieve direct somatic embryogenesis from petioles in MS medium in *Begonia gracilis* (Castillo and Smith, 1997). Plant regeneration via organogenesis or protoplasts often leads to more somaclonal variation (Jain, 1997). Physical and chemical factors also influence dwarfing of plants, slow growth, leaf variegation and non-flowering in *Begonia x hiemalis* (Takayama and Misawa, 1982). As there has been no report available on *in vitro* studies of *Begonia fallax* this paper provides information on it here.

Materials and methods

Collection of plant materials

B. fallax was collected from Tirunelveli district of Tamil Nadu in India. Authentic plant specimen (MBV and CR 856) identified by Prof. M. B. Viswanathan was deposited in the Herbarium of the Centre for Research and Development of Siddha-Ayurveda Medicines (CRDSAM), Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India, for reference.

Sterilization of explants

Wild plants of *B. fallax* were collected and grown in the green house of the Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu, for scientific investigations. Shoot tip and nodal explants were excised with sterile blade and collected from tender plants. The explants were surface-sterilized by washing under running tap water for 15 min, treated with 0.2% (v/v) Tween 20 for 10 min, rinsed with distilled water for 5 times to remove the soap solution, incubated in 0.2% bavistin for 10 min and rinsed with distilled water for 5 or 6 times. The explants were sterilized with 0.06% mercuric chloride solution for 3 min, washed with sterile double distilled water for 5 times, finally sterilized with 30% (v/v) ethanol and then washed in several changes of sterilized distilled water under aseptic condition in a laminar air-flow chamber.

Culture conditions

The pH of the MS medium (Takayama and Misawa, 1982) was adjusted to 5.7, added 0.6% agar to the medium, heated to 80°C for dissolving agar and dispensed in pre-sterilized culture tubes (10 ml/tube). The explants were kept under 16 h light/ 8 h dark day photoperiod at 25±2°C. MS medium was supplemented with combinations of growth regulators wherein culture medium that produced more shoots was used for subsequent subculture.

Statistical analysis

Statistical methods were used for comparison of treatment during optimization of the parameters for *in vitro* regeneration. The data were subjected to analysis of variance (ANOVA) and Duncan multiple range test (DMRT) at 5% probability level (p<0.05), wherever applied (Gomez and Gomez, 1976). The significance was determined using Software SPSS 22 (IBM Corporation Released, 2013).

Results

Shoot induction

Cytokinins such as BAP, KIN and TDZ at different concentrations ranging from 0.1 to 2.0 mg^{-1} were used individually for shoot initiation from shoot tip and nodal explants. The explants differentiated into shoot buds and exhibited variations with respect to number of shoots and their length (Table 1).

Multiple shoot induction

Among them, BAP produced more number of shoots. BAP 0.5 mg^{-1} in MS medium and various concentrations of IAA, NAA and IBA from 0.01-1.0 mg^{-1} were used to study the combined effect on multiple shoot induction. Number of shoots was increased for all but NAA at 0.5 mg^{-1} indicated better cell division and regeneration

capacity and produced a maximum of 20.8 shoots nodal explant^{-1} and 17.9 shoots shoot tip explant^{-1} (Table 2; Figs. 1 and 2). Further increase of BAP and NAA concentration (0.5+0.5 mg^{-l}) suppressed the growth.

Table 1. Individual effects of cytokinins on shoot induction from nodal and shoot tip explants cultured on MS medium.

Concentration of growth regulators	Nodal explant			Shoot tip explant		
	No. of leaves/ explants (Mean±SD)	Mean no. of shoot length (cm) (Mean±SD)	% of response	No. of leaves/ explant (Mean±SD)	Mean no. of shoot length (cm) (Mean±SD)	% of response
BAP						
0.1	5.8±0.38^{d}	2.0±0.04^{d}	87.50^{bc}	5.4±0.16^{de}	2.2±0.02^{c}	78.80^{d}
0.5	10.4±0.16^{a}	2.8±0.03^{a}	93.60^{a}	8.4±0.16^{a}	2.5±0.03^{a}	84.50^{a}
1.0	9.4±0.16^{b}	2.5±0.03^{b}	89.10^{b}	7.9±0.31^{ab}	2.4±0.03^{b}	83.70^{ab}
1.5	7.5±0.16^{c}	2.4±0.06^{c}	84.40^{d}	7.3±0.30^{ab}	2.0±0.01^{d}	81.00^{c}
2.0	5.6±0.26^{d}	1.8±0.04^{e}	80.40^{ef}	7.1±0.45^{b}	2.1±0.04^{d}	74.30^{f}
KIN						
0.1	4.6±0.30^{e}	1.5±0.05^{f}	71.50^{g}	5.2±0.48^{de}	1.3±0.05^{g}	68.60^{h}
0.5	6.0±0.33^{d}	1.8±0.03^{e}	78.40^{f}	5.9±0.45^{cd}	1.6±0.03^{f}	70.60^{g}
1.0	6.3±0.15^{d}	2.1±0.03^{d}	82.90^{d}	6.8±0.32^{bc}	2.3±0.04^{bc}	78.40^{de}
1.5	7.7±0.26^{c}	2.3±0.03^{c}	86.50^{c}	7.8±0.32^{ab}	2.4±0.03^{bc}	82.40^{bc}
2.0	7.3±0.30^{c}	2.1±0.03^{d}	83.60^{d}	7.2±0.48^{ab}	2.1±0.02^{c}	78.40^{de}
TDZ						
0.1	4.6±0.30^{e}	1.5±0.02^{fg}	78.60^{f}	4.8±0.32d^{ef}	1.2±0.07^{gh}	62.60^{j}
0.5	5.6±0.30^{d}	1.8±0.03^{e}	80.90^{e}	4.9±0.45d^{ef}	1.9±0.03^{e}	67.90^{hi}
1.0	4.8±0.32^{e}	1.4±0.03^{h}	72.00^{g}	3.9±0.45^{f}	1.7±0.08^{f}	76.50^{e}
1.5	3.4±0.22^{f}	1.2±0.03^{i}	68.20^{h}	4.2±0.48^{ef}	1.6±0.09^{f}	71.40^{g}
2.0	2.6±0.22^{g}	1.0±0.03^{j}	63.60^{i}	4.2±0.48e^{f}	1.1±0.03^{i}	66.40^{i}

Values represent Mean ±SE. Mean represented with the same alphabets do not differ significantly (Duncan test; p<0.05).

Table 2. Effect of auxins on multiple shoot induction from nodal and shoot tip explants cultured on MS medium fortified with BAP (0.5 mg^{-l}).

Concentration of growth regulators	Nodal explant			Shoot tip explant		
	No. of shoots/explant (Mean±SE)	Mean no. of shoot length (cm) (Mean±SE)	% of shoot responded	No. of shoots/explant (Mean±SE)	Mean no. of shoot length (cm) (Mean±SE)	% of shoot responded
BAP+NAA						
0.5±0.01	07.9±0.10^{g}	2.3±0.15^{def}	74.80^{d}	7.6±0.30^{h}	2.1±0.10^{cd}	74.20^{e}
0.5±0.05	09.8±0.13^{f}	2.6±0.07^{cde}	80.40^{c}	9.0±0.81f^{g}	2.7±0.07^{b}	78.80^{c}
0.5±0.1	15.9±0.90^{d}	3.3±0.15^{bc}	84.90^{b}	14.6±0.33^{b}	2.9±0.02^{ab}	85.90^{a}
0.5±0.5	20.8±0.18^{a}	4.0±0.21^{a}	90.00^{a}	17.9±0.45^{a}	3.1±0.08^{a}	86.60^{a}
0.5±1.0	09.8±0.20^{f}	2.7±0.08^{cde}	79.80^{c}	12.9±0.45^{c}	2.8±0.03^{ab}	83.80^{b}
BAP+IAA						
0.5±0.01	17.9±0.10^{b}	3.3±0.15^{bc}	79.80^{c}	10.6±0.30^{de}	2.7±0.08^{b}	76.20^{d}
0.5±0.05	16.7±0.21^{c}	3.6±0.26^{ab}	74.80^{d}	12.6±0.49^{c}	2.7±0.13^{b}	71.40^{f}
0.5±0.1	13.9±0.10^{e}	2.6±0.06^{cde}	75.00^{d}	9.2±0.13^{fg}	2.3±0.15^{c}	68.60^{g}
0.5±0.5	09.9±0.10^{f}	3.0±0.33^{bcd}	70.60^{e}	7.6±0.40^{h}	1.8±0.03^{ef}	64.00^{h}
0.5±1.0	07.8±0.20^{g}	2.6±0.40^{cde}	59.00^{i}	7.7±0.30^{h}	2.0±0.22^{de}	58.20^{i}
BAP+IBA						
0.5±0.01	05.9±0.23^{h}	1.8±0.02^{f}	54.90^{j}	7.3±0.15^{h}	1.2±0.04^{g}	52.40^{j}
0.5±0.05	07.9±0.10^{g}	2.5±0.27^{de}	64.40^{h}	8.2±0.13^{gh}	1.5±0.04^{f}	58.20^{i}
0.5±0.1	09.7±0.30^{f}	2.2±0.14^{ef}	65.20^{g}	9.6±0.30^{ef}	1.6±0.07^{f}	63.60^{h}
0.5±0.5	13.9±0.31^{e}	2.8±0.35^{cde}	69.80^{f}	11.1±0.58^{d}	1.8±0.03^{ef}	71.50^{f}
0.5±1.0	13.9±0.10^{e}	2.7±0.17^{cde}	69.80^{f}	10.6±0.30^{de}	1.7±0.07^{ef}	68.60^{g}

Values represent Mean ±SE. Mean represented with the same alphabets do not differ significantly (Duncan test; p<0.05).

Fig. 1: (a-f)-*In vitro* culture of *B. fallax* from nodal explant: a. Shoot initiation from nodal explant; b. Formation of multiple leaves; c. Formation of multiple shoots; d. Induction of roots; e. Fully grown roots; f. Hardened *B. fallax* plant in pot mixture (sand: soil: farm yard manure -1:1:1). Bars = 1 cm.

Root induction

Individual elongated shoots were excised and transferred to the MS medium and tested at various concentrations of IAA, NAA and IBA from 0.01 to 1.0 mg^{-l} for rooting. MS medium supplemented with 0.5 mg/l NAA recorded the highest rooting frequency to nodal explants (64.90 roots) than shoot tip explants (31.90 roots) but root length was up to 2.3 cm in both the cases in the present study (Table 3; Figs. 1 and 2).

Acclimatization

The rooted plantlets were transplanted into plastic cups containing autoclaved sand, soil and farmyard manure mixture (1:1:1). The plants were covered with plastic bags with perforations and grown in the environmental growth chamber at 27±2°C with 85% relative humidity for 2-3 weeks.

Fig. 2: (a-f). *In vitro* culture of *B. fallax* from shoot tip explant: a. Shoot initiation from shoot tip explant; b. Formation of multiple leaves; c. Formation of multiple shoots; d. Induction of roots; e. Fully grown roots; f. Hardened *B. fallax* plant in pot mixture (sand: soil: farm yard manure -1:1:1). Bars = 1 cm.

Discussion

Petiole explants in *B. x hiemalis* NAA 0.1 mg^{-l} and BA 0.5 mg^{-l} produced maximum number of adventitious buds. But lower concentration of BA 0.1 mg^{-l} produced only fewer shoots but shoot growth showed enhancement (Simmonds, 1984). Thereafter, Mendi

et al. (2009) manipulated the culture medium by reducing BA to 0.2 mg^{-1} and maintained same concentration of NAA at 0.5 mg^{-1} to achieve maximum shoot regeneration in *B. elatior* cv. Toran orange. Induction of shoot regeneration was recorded at different combinations of NAA and BA in MS medium such as 0.1+0.1 mg^{-1} in *B. x elatior* and hybrids of *Begonia B. 'tiger'*, 0.1+0.5 mg^{-1} in *B. semperflorens*, and 1.0+1.0 mg^{-1} in *B. rex* (Espino et al., 2004). But, bud break of the nodal segments in *B. malabarica* was achieved at 4.4 mg^{-1} BA and 1.4 mg^{-1} IAA on MS medicum (Nisha et al., 2009). But, in the present study, a combination of BAP 0.5 mg^{-1} and NAA 0.5 mg^{-1} induced multiple shoot production to a maximum of 20.8 shoots nodal $explant^{-1}$ and 17.9 shoots shoot tip $explant^{-1}$ in *B. fallax* (Table 2; Figs. 1 and 2).

Kumaria et al. (2012) reported 13.8 roots $shoot^{-1}$ to IAA 0.1 mg^{-1} on MS medium in *B. rubrovenia* var. *meisneri*. However, in the present study, among the various concentrations of IAA, NAA and IBA from 0.01 to 1.0 mg^{-1} on MS medium for rooting, MS medium supplemented with 0.5 mg^{-1} NAA recorded the highest rooting frequency of 64.90 roots to nodal explants than 31.90 roots to shoot tip explants but root length remained similar that was up to 2.3 cm in both the cases (Table 3; Figs. 1 and 2). After transplantation, the rooted shoots showed 70% survival and grew normally in the Environmental Growth Chamber. Upon growth, the plantlets were transferred to a shadow area for about 30 days and then to earthen pots containing sand, soil and farmyard manure (1:1:1).

Table 3. Effect of auxin on root induction from nodal and shoot tip explants cultured on MS medium.

Concentration of growth regulators	Nodal explant			Shoot tip explant		
	No. of roots/explant (Mean±SE)	Mean no. of root length (cm) (Mean±SE)	% of root responded	No. of roots/explant (Mean±SE)	Mean no. of root length (cm) (Mean±SE)	% of root responded
NAA						
0.01	49.30±0.70^{cd}	1.6±0.31^{c}	74.80^{d}	13.80±0.13^{g}	1.6±0.31^{c}	71.40^{gh}
0.05	49.40±0.60^{cd}	1.6±0.23^{cd}	74.90^{d}	18.90±0.10^{f}	1.6±0.23^{cd}	77.60^{ef}
0.1	59.90±0.09^{b}	1.9±0.1^{e}	79.90^{c}	26.36±0.24^{c}	1.9±0.1^{e}	80.90^{b}
0.5	64.90±0.09^{a}	2.3±0.26^{a}	89.72^{a}	31.90±0.90^{a}	2.3±0.26^{a}	86.50^{a}
1.0	59.80±0.20^{b}	2.0±0.16^{cd}	84.70^{b}	29.50±0.34^{b}	2.0±0.16^{cd}	81.30^{b}
IAA						
0.01	44.90±0.23^{f}	1.5±0.16^{cd}	84.70^{b}	20.90±0.10^{e}	1.5±0.16^{cd}	80.60^{bc}
0.05	45.00±0.14^{e}	2.0±0.13^{b}	84.70^{b}	24.60±0.26^{d}	2.0±0.13^{b}	79.70^{d}
0.1	39.90±0.10^{g}	1.8±0.13^{cd}	74.90^{d}	18.29±0.10^{f}	1.8±0.13^{cd}	78.60^{de}
0.5	34.90±0.10^{i}	1.5±0.16^{cd}	74.90^{d}	13.10±0.23^{g}	1.5±0.16^{cd}	70.40^{h}
1.0	34.90±0.10^{i}	1.5±0.16^{cd}	69.70^{e}	08.29±0.10^{h}	1.5±0.16^{cd}	68.60^{i}
IBA						
0.01	34.90±0.10^{i}	1.2±0.13^{cd}	64.70^{f}	08.80±0.13^{h}	1.2±0.13^{cd}	63.60^{j}
0.05	36.80±1.49^{h}	1.2±0.13^{cd}	69.90^{e}	18.80±0.13^{f}	1.2±0.13^{cd}	68.60^{i}
0.1	39.90±0.10^{g}	1.5±0.16^{cd}	74.80^{d}	20.29±0.10^{e}	1.5±0.16^{cd}	71.60^{g}
0.5	49.90±0.10^{c}	1.8±0.13^{cd}	79.90^{c}	26.90±0.10^{c}	1.8±0.13^{cd}	77.10^{f}
1.0	44.90±0.10^{f}	1.6±0.16^{cd}	74.70^{d}	20.99±0.10^{e}	1.6±0.16^{cd}	78.20e^{f}

Values represent Mean ±SE. Mean represented with the same alphabets do not differ significantly (Duncan test; $p<0.05$).

Conclusion

Multiple shoot production is recorded to both nodal and shoot tip explants and more number of multiples shoots are recorded to the nodal explants in *B. fallax*. Therefore, nodal explants could be the best for large-scale multiplication and propagation not only to facilitate *in vitro* conservation but for the production of secondary metabolites also.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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References

Castillo, B., Smith, M.A.L., 1997. Direct somatic embryogenesis from *Begonia gracilis* explants. Plant Cell Rep.16, 385-388.

Clement, W.L., Tebbitt, C.M., Forrest, L.L., Blair, J.E., Brouillet, L., Eriksson, T., Swensen, S.M., 2004. Phylogenetic position and biogeography of *Hillebrandia sandwicensis* (Begoniaceae): A rare Hawaiian relict. Am. J. Bot. 91, 905-917.

Espino, F.J., Linacero, R., Rueda, J., Vazquez, A.M., 2004. Shoot regeneration in four *Begonia* genotypes. Biol. Plant.48, 101-104.

Forrest, L.L., Hollingsworth, P.M., 2003. A recircumscription of *Begonia* based on nuclear ribosomal sequences. Plant Syst. Evol. 241, 193-211.

Gomez, K.A., Gomez, A.A., 1976. Statistical procedures for agricultural research with emphasis of rice. IRRI, Los Bans, Philippines.

IBM Corporation Released,2013. IBM SPSS Statistics for Windows, Version 22.0.IBM Corp, Armonk, NY.

Jain, S.M., 1997. Micropropagation of selected somaclones of *Begonia* and *Saintpaulia*. J. Biosci. 22, 585-592.

Kumar, K.D., Bhattacharyya, U.C., 1992. Endemic species of the family Begoniaceae of India. J. Econ. Taxon. Bot. 16, 565-568.

Kumaria, S., Kehie, M., Bhowmik, S.D., Singh, M., Tandon, P., 2012. *In vitro* regeneration of *Begonia rubrovenia* var. *meisneri* C.B. Clarke-A rare and endemic ornamental plant of Meghalaya, India. Int. J. Biotechnol. 11, 300-303.

Mendi, Y.Y., Curuk, P., Kocaman, E., Unek, C., Eldogan, S., Gencel, G., Cetiner, S., 2009. Regeneration of *Begonia* plantlets by direct organogenesis. Afr. J. Biotechnol. 8, 1860-1863.

Murashige, T., Skoog, F.A., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

Nisha, M.C., Rajeshkumar, S., Selvaraj, T., Subramanian, M.S., 2009. A valued Indian medicinal plant-*Begonia malabarica* Lam.: Successful plant regeneration through various explants and field performance. Maejo Int. J. Sci. Technol. 3, 261-268.

Pierik, R.L.M., Tetteroo, F.A.A., 1987. Vegetative propagation of *Begonia venosa*Skan*in vitro* from inflorescence explants. Plant Cell Tiss. Organ Cult. 10, 135-142.

Ramamurthy, K., 1983. Begoniaceae. In: Flora of Tamil Nadu (Eds.: Nair, N.C., Henry, A.N.) Series 1: Analysis. Vol. 1. Botanical Survey of India, Coimbatore, Tamil Nadu. 175p.

Simmonds, J., 1984.Induction, growth and direct rooting of adventitious shoots of *Begonia x hiemalis*.Plant Cell Tiss. Organ Cult. 3, 283-289.

Takayama, S., Misawa, M., 1982. Factors affecting differentiation and growth *in vitro*, and a mass-propagation scheme for *Begonia x hiemalis.* Sci. Hortic. 16, 65-75.

The Plant List, Version 1.1. 2013. Published on the Internet; http://www.theplantlist.org/ (accessed 1st January). http://www.kew.org/science-research-data/databases-publications/ plant-names/

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