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In vitro regeneration of *Begonia rubrovenia* var. *meisneri* C.B. Clarke—A rare and endemic ornamental plant of Meghalaya, India

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In vitro plant regeneration from leaf and petiole explants of *Begonia rubrovenia meisneri* C.B. Clarke, an endemic and rare plant of Meghalaya, has been established. Multiple shoots were induced on Murashige and Skoog (MS) medium supplemented with various concentrations of 6-benzyl aminopurine (BAP) and thidiazuron (TDZ). *In vitro* produced leaf and petiole sections (secondary explants) exhibited enhanced shoot proliferation in MS medium supplemented with either BAP or TDZ in different concentrations (0.05-5.0 mg/L). A maximum, 65 shoots/petiole were formed on MS+0.1 mg/L TDZ. The *in vitro*-raised shoots formed roots in MS medium containing 0.1 mg/L indole 3-acetic acid (IAA), with a maximum of 13.8 roots/shoot. About 73.33% of the 500 plantlets transferred acclimatized successfully within 4 wk in a glasshouse. On being transferred to the field, all the acclimatized plantlets survived after 8 wk.

Keywords: *In vitro* techniques, leaf and petiole sections, shoot multiplication, threatened plant

Introduction

The Northeastern region of India has a reservoir of floristic diversity. Over-exploitation and habitat destruction are serious threats to the biodiversity of this region. In consequence, several species of the region have been kept under the list of threatened species by IUCN[1]. To facilitate the distribution, conservation and storage in '*In Vitro* Gene Banks' of these threatened plant species, application of tissue culture has been widely applied[2-4]. In India, application of tissue culture in *ex situ* conservation has been restricted to economically important plant species[4]. However, attempts should be made to conserve all the threatened plant species.

Genus *Begonia* (Family: Begoniaceae) comprises of around 1000 species and many of these are valuable ornamentals and important medicinal plants[5]. *Begonia rubrovenia meisneri* C.B. Clarke is a herbaceous, endemic, rare and threatened species of Meghalaya, India[1]. The plant has attractive foliage with small white flowers and can be utilized for horticultural purposes. The tender shoots of this species are also edible[6]. The shrinkage of forest cover, rapid changing ecosystems, natural calamities and ruthless collection for ornamental purposes have led to the depletion of this plant species. *In vitro* regeneration and micropropagation of Begonia *via* organogenesis, tissue culture and transverse thin cell layer technology have been investigated by various workes[2,7-12]. However, report on *in vitro* propagation of *B. rubrovenia meisneri* was not available despite its commercial importance. Therefore, this paper deals with the application of *in vitro* techniques for multiplication of *B. rubrovenia meisneri*. The present protocol provides high frequency of shoot multiplication from petiole and leaf explants with successful acclimatization in the greenhouse. The protocol can also be used for conservation and propagation of this plant species.

Materials and Methods

Plant Material and Initiation of Aseptic Cultures

The plants of *B. rubrovenia meisneri* were collected from the forest area of Jarain (Meghalaya) in April 2009 and were maintained in the glasshouse of Plant Biotechnology Laboratory, Department of Botany, North Eastern Hill University, Shillong, India. Leaves and petioles, harvested from the plants growing in the glasshouse, were used as explants. These were thoroughly washed with water for 1 h,

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treated with 2% Labolene (v/v) for 10 min and finally rinsed 4 to 5 times with sterile water. Then they were surface sterilized with 10-15% sodium hypochlorite (4% active chlorine content) for 10 min. The disinfected plant materials were rinsed with sterile water several times and then dissected out to make explants of appropriate size. The leaf sections measuring about 1.0 cm^2 and petioles about 1.0 cm long were cultured on MS medium[13] containing 3% (w/v) sucrose supplemented singly with various concentrations (0.01-0.1 mg/L) of 6-benzyl aminopurine (BAP). Nutrient medium was gelled with 0.8% (w/v) agar and *p*H was adjusted to 5.8±0.02, prior to autoclaving at 1.06 kg cm^{-2} pressure at 121°C for 15 min. The cultures were incubated at 25±2˚C and 12 h photoperiod with an irradiance of 60-62.2 µmoles m^{-2} s^{-1}, provided by cool white fluorescent tubes. MS medium containing 3% (w/v) sucrose without any growth regulators was used as control.

Multiplication and Rooting of Shoots

For multiplication of shoots, the leaf sections and petioles were excised from the *in vitro* raised microshoots and further subcultured in MS medium supplemented with different concentrations (0.05-5.0 mg/L) of BAP and thidiazuron (TDZ). Later, 2-3 cm long *in vitro* shoots were transferred on MS medium supplemented with α-naphthalene acetic acid (NAA), indole 3-acetic acid (IAA) or indole 3-butyric acid (IBA) in varying concentrations (0.05-0.5 mg/L) for rooting purpose.

Data for organogenic response and shoot multiplication were recorded after 30 (petiole) and 50 d (petiole & leaf) of culture inoculation. The number and length of roots formed were recorded after 30 d of microshoot transfer. At least 5 replicates were taken for each treatment in an experiment and each experiment was repeated thrice. Statistical analysis was done using one-way analysis of variance (ANOVA). The significant differences among the means were assessed by Tukey's test at 5% probability level (PC version Origin 7.0., Northampton, MA, USA).

Acclimatization and Transplantation

The *in vitro* rooted plantlets were washed thoroughly and potted in small plastic glasses filled with mixture of soil and farmyard manure (3:1). The potted plants were kept inside the glasshouse (25-30°C) and covered for 2-3 wk under polythene bags. These were irrigated regularly and the percentage of plant survival was recorded after a month. Then, the acclimatized and survived plants were transferred to the field.

Results and Discussion

Culture Establishment and Production of Secondary Explants

The leaf and petiole explants collected from the *in vivo* grown plants responded in cultures. The explants exhibited swelling after 2 wk on MS medium with different concentrations (0.01-0.1 mg/L) of BAP. These differentiated into shoot buds in the 3rd wk of culture on MS+0.1 mg/L BAP. The emerging shoot buds were used further and transferred to fresh medium for amplification. Initiation and multiplication of cultures from minimal plant material is always preferred in case of threatened plants for reducing the pressure on the wild plants[14]. A large number of shoots could be obtained within 70-80 d of culture and they were subsequently used as secondary explants.

Adventitious Shoot Formation

Adventitious shoots multiplied directly from the leaf and petiole sections (used as secondary explants), and no callus formation was recorded. Only few shoots emerged when both leaf and petiole sections were cultured on growth regulator-free MS medium. However, incorporation of BAP and TDZ (0.05-5.0 mg/L) in the medium increased the rate of shoot multiplication significantly (Table 1). Over all, the rate of shoot multiplication was higher from petiole explants as compared to the leaf explants incase of both BAP and TDZ. Also, the growth of petiole-derived shoots was faster than the leaf-derived shoots. Further, the number of shoots formed from petiole explants was higher in the medium containing TDZ as compared to the medium containing BAP. However, there was a reverse trend in case of leaf explants (Table 1). Several workers have reported the effectiveness of both cytokinins and auxins on shoot regeneration[5,11,15]. In contrast, in the present investigation, higher number of shoot multiplication was observed in a medium supplemented with cytokinin alone. A maximum of 65 shoots per petiole section was recorded on MS+0.1 mg/L TDZ (Table 1; Fig. 1a) after 50 d of culture, whereas 56 shoots per leaf section was recorded on MS+1.0 mg/L BAP (Fig. 1b). The explants response and number of plants produced were found to be increased with the increase in the concentration of both BAP and TDZ; while at higher concentrations, the number of plants

Table 1—Effect of BAP and TDZ on multiple shoot bud induction from petiole and leaf explants of *B. rubrovenia meisneri*

PGRs (mg/L)	Organogenic response (%)	Petiole (mean shoot no.)		Organogenic response (%)	Leaf (mean shoot no.)
BAP		30 d	50 d		50 d
0.00	53.3±6.6^{b}	6.5±0.95^{a}	7.0±0.57^{a}	26.6±6.6^{a}	4.0±0.0^{a}
0.05	80.0±11.5^{ab}	10.8±0.60^{b}	15.0±0.57^{b}	33.3±6.6^{a}	10.5±0.50^{ab}
0.10	93.3±6.6 ^{a}	33.5±1.43^{e}	43.5±0.99^{d}	53.3±6.6^{ac}	16.0±0.57^{b}
0.50	93.3±6.6^{a}	38.5±0.76^{f}	50.0±0.57^{e}	53.3±6.6^{ad}	38.0±1.52^{c}
1.00	93.3±6.6^{a}	27.5±0.84^{d}	41.5±0.76^{d}	86.0±6.6^{b}	56.0±1.70^{e}
5.00	86.6±6.6^{ab}	19.2±0.60^{c}	34.8±1.32^{c}	66.6±6.6^{bc}	41.2±1.54^{d}
TDZ					
0.00	53.3±6.6^{a}	6.5±0.95^{a}	7.0±0.57^{a}	26.6±6.6^{a}	4.0±0.0^{a}
0.05	86.6±6.6^{b}	38.5±0.92^{c}	42.16±1.40^{c}	33.3±6.6^{a}	10.5±1.0^{ab}
0.10	93.3±6.6^{b}	58.8±1.13^{f}	65.0±0.77^{f}	33.3±6.6^{a}	12.5±0.05^{cb}
0.50	93.3±6.6^{b}	51.3±0.84^{e}	55.5±0.88^{e}	53.3±6.6^{b}	16.0±0.57^{c}
1.00	93.3±6.6^{b}	41.5±1.33^{d}	47.5±1.38^{d}	66.6±6.6^{b}	45.2±1.93^{e}
5.00	93.3±6.6^{b}	16.3±0.49^{b}	21.3±0.49^{b}	53.3±6.6^{ab}	39.0±0.57^{d}

PGRs, Plant growth regulators
Values are mean±SE of 5 replicates and 3 experiments
Mean followed by the same letters are not significantly different by Turkey's test at 5% probability level
Shoot formation from leaf explants occurred only after 50 d of culture

declined and the explants response either declined or remained the same. Moreover, the regenerated shoots were smaller at higher concentration as compared to those produced on lower concentration of growth regulators. This is in consistent with the report of Bowes and Curtis[2] in different species of *Begonia*. The leaf sections from the newly formed shoots could also be used for multiplication of shoots. The shoots obtained from the petiole and leaf explants were sub-cultured within 30 d for elongation on MS medium containing 0.5 mg/L BAP (Fig. 1c). Occasionally, flower bud formation from both petiole and leaf explants was observed after 45 d (Fig. 1d). The emergence of floral buds has been reported in several other species of Begonia from leaf and floral stalk but not from petiole segments[16].

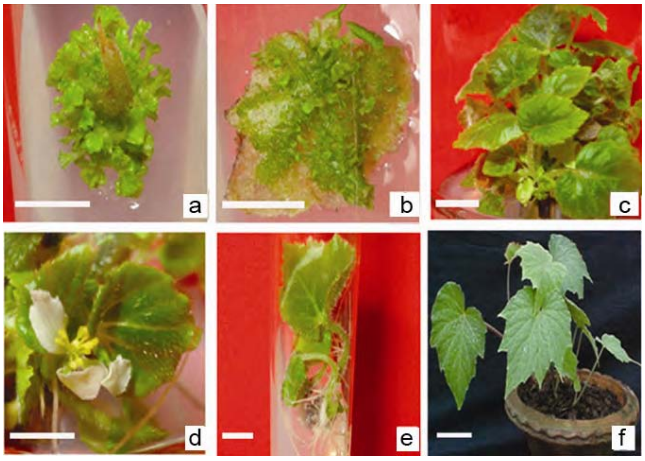

Fig. 1 (a-f)—a. Shoot bud induction from petiole explants on MS medium supplemented with 0.1 mg/L TDZ; b. Shoot bud induction from leaf explants in MS medium supplemented with 1.0 mg/L BAP; c. Elongation of *in vitro* shoots in MS medium supplemented with 0.5 mg/L BAP within 30 d; d. *In vitro* flowering of regenerated shoots after 45 d; e. Rooting of *in vitro* regenerated shoots in MS medium supplemented with 0.1 mg/L IAA after 30 d; & f. Regenerated hardened plantlet transferred to earthen pot after 4 wk. (Bar a-e = 1 cm & f = 5 cm)

Rooting and Plant Acclimatization

About 93.3% shoots rooted with in 2 wk on transfer to MS medium supplemented with either NAA/IAA/IBA (0.05-0.5 mg/L). The highest number (13.8/shoot) of roots was regenerated on the medium supplemented with 0.1 mg/L IAA within 30 d of culture (Table 2; Fig. 1e), while the maximum length (2.5 cm) of roots was recorded with 0.1-0.25 mg/L of IBA. Earlier workers[17-19] had reported lesser number of roots on half-strength MS medium containing IAA and IBA. In the present study, profuse rooting was observed on full strength MS medium supplemented with IAA/IBA. This allowed the higher survivability of the plantlets during their acclimatization. In general, IBA has been documented for *in vitro* rooting promotion attributes. The rooted shoots after transplantation into potting mixture of soil and farmyard manure (3:1) exhibited 73.3% survival and grew normally in the glasshouse (Fig. 1f). These acclimatized plants were then transferred to the field after a month.

Table 2— Effect of NAA, IAA and IBA on root formation in *in vitro* raised shoots of *B. rubrovenia meisneri*

PGRs (mg/L)	Rooting (%)	Mean root no.	Mean root length (cm)
Control 0.00	33.3±6.6[a]	1.5±0.65[a]	0.21±0.02[a]
NAA			
0.05	93.3±6.6[b]	8.5±0.60[cd]	0.33±0.04[a]
0.10	93.3±6.6[b]	6.8±1.23[c]	0.64±0.12[b]
0.25	93.3±6.6[b]	3.8±0.26[b]	0.55±0.22[a]
0.50	93.3±6.6[b]	CL	-
IAA			
0.05	93.3±6.6[b]	4.5±0.72[b]	0.58±0.12[ab]
0.10	93.3±6.6[b]	13.8±1.30[f]	1.20±0.20[c]
0.25	93.3±6.6[b]	9.6±1.20[d]	1.55±0.44[cd]
0.50	93.3±6.6[b]	1.5±1.00[a]	0.67±0.14[b]
IBA			
0.05	93.3±6.6[b]	6.5±0.52[c]	1.50±0.55[c]
0.10	93.3±6.6[b]	7.8±1.13[cd]	2.50±0.32[de]
0.25	93.3±6.6[b]	10.3±0.84[e]	1.80±0.24[cd]
0.50	93.3±6.6[b]	3.5±1.33[b]	0.72±0.34[b]

PGRs, Plant growth regulators; CL, Callus

Values are mean±SE of 5 replicates and 3 experiments

Data scored after 30 d of transfer of shoots to MS medium supplemented with auxins for rooting

Mean followed by the same letters are not significantly different by Turkey's test at 5% probability level

The present study describes an efficient multiplication system for *B. rubrovenia meisneri* using *in vitro* techniques that can be successfully applied for its *in vitro* conservation and large-scale propagation.

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References

1 IUCN, *IUCN red list of threatened plants* (IUCN Publications Services Unit, Cambridge) 1998.

2 Bowes B G & Curtis E W, Conservation of British national *Begonia* collection by micropropagation, *New Phytol*, 119 (1991) 169-181.

3 Rathore T S, Tandon P & Shekhawat N S, *In vitro* regeneration of pitcher plant (*Nepenthes khasiana* Hook. f.)—A rare insectivorous plant of India, *J Plant Physiol*, 139 (1991) 246-248.

4 Tandon P, Biodiversity: A scientific approach—Agenda for the 21st Century, *Natl Acad Sci Lett*, 26 (2003) 111-118.

5 Mathan C N, Sevanan R, Thangavel S & Modurpalayam S S, A valued Indian medicinal plant—*Begonia malabarica* Lam.: Successful plant regeneration through various explants and field performance, *Maejo Int J Sci Technol*, 3 (2009) 261-268.

6 Vidhan J, Culture and ethnobotany of Jantia tribal community of Meghalaya, Northeast India—A mini review, *Indian J Tradit Knowle*, 9 (2010) 38-44.

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

8 Nakano M, Niimi Y, Kobayashi D & Watanabe A, Adventitious shoot regeneration and micropropagation of hybrid tuberous begonia (*Begonia* × *tuberhybrida* Voss), *Sci Hortic*, 79 (1999) 245-251.

9 Bouman H & De Klerk G J, Measurement of the extent of somaclonal variation in begonia plants regenerated under various conditions. Comparison of three assays, *Theor Appl Genet*, 102 (2001)111-117.

10 Burritt D J & Leung D W M, Adventitious shoot regeneration from *Begonia* × *erythrophylla* petiole sections is developmentally sensitive to light quality, *Physiol Plant*, 118 (2003) 289-296.

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

12 Nhut D T, Hai N T, Huyen P X, Huong D T Q, Hang N T T *et al*, Thidiazuron induces high frequency shoot bud formation from Begonia petiole transverse thin cell layer culture, *Propag Ornam Plants*, 5 (2005) 151-157.

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

14 Pence V C, The application of biotechnology for the conservation of endangered plants, in *Plant conservation biotechnology*, edited by E E Benson (Taylor and Francis Ltd., UK) 1999, 227-249

15 Mendi Y Y, Curuk P, Kocaman E, Unek C, Eldogan S *et al*, Regeneration of begonia plantlets by direct organogenesis, *Afr J Biotechnol*, 8 (2009) 1860-1863.

16 Ringe F & Nitsch J P, Conditions leading to flower formation on excised *Begonia* fragments cultured *in vitro*, *Plant Cell Physiol*, 9 (1968) 639-652.

17 Selvakumar V, Anbudurai P R & Balakumar T, *In vitro* propagation of the medicinal plant *Plumbago zeylanica* L. through nodal explants, *In Vitro Cell Dev Biol-Plant*, 37 (2001) 280-284.

18 Chaplot B B, Dave A M & Jasrai Y T, A valued medicinal plant—Chitrak (*Plumbago zeylanica* Linn.): Successful plant regeneration through various explant and field performance, *Plant Tissue Cult Biotechnol*, 16 (2006) 77-84.

19 Rout G R, Saxena C, Smantaray S & Das P, Rapid plant regeneration from callus cultures of *Plumbago zeylanica*, *Plant Cell Tissue Organ Cult*, 56 (1999) 47-51.