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Full Paper

A valued Indian medicinal plant – *Begonia malabarica* Lam. : Successful plant regeneration through various explants and field performance

Mathan C. Nisha[1], Sevanan Rajeshkumar[2,*], Thangavel Selvaraj[3] and Modurpalayam S. Subramanian[1]

[1]Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India

[2] Department of Applied Biology, Department of Plant Sciences[3], Ambo University College, Post Box. No. 19, Ambo, Western Shoa, Ethiopia, East Africa

* Corresponding author, e-mail: dhiksha_rajesh@yahoo.co.in

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Abstract: A cost-effective and efficient protocol has been described in the present work for large-scale and rapid in vitro propagation of a valuable medicinal herb *Begonia malabarica* Lam. (Begoniaceae) by shoot auxillary-bud proliferation and organogenesis on MS medium supplemented with 6-benzylaminopurine (BA; 0.0-8.8 mg/l) and indole-3-acetic acid (IAA; 0.0-2.88 mg/l) at different concentrations, either alone or in combinations. Initiation of callus formation from the base of the leaf lamina was observed on MS supplemented with BA, IAA and adenine sulphate. Root induction on shoots was achieved on full strength MS with IAA/ indole-3-butyric acid (IBA) at different concentrations. MS medium with 4.4 mg/l BA and 1.4 mg/l IAA elicited the maximum number of shoots (10 multiple shoots) from nodal explants. Leaf-based callus differentiated into more than 28 shoots on MS with 150 mg/l adenine sulphate. The regenerated shoots were rooted on MS with 1.2 mg/l IBA within ten days. Almost 95% of the rooted shoots survived hardening when transferred to the field. The regenerated plants did not show any morphological change and variation in levels of secondary metabolites when compared with the mother stock. Thus, a reproduction of *B. malabarica* was established through nodal and leaf explants. This protocol can be exploited for conservation and commercial propagation of this medical plant in the Indian subcontinent and might be useful for genetic improvement programs.

Keywords: *Begonia malabarica*, regeneration, explants, field performance

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Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

Introduction

Plants are an important source of medicines and play a key role in world health [1,2]. In almost all regions and cultures of the world, from ancient times till today, plants have been used as medicines [3]. Today's medicinal plants are important to the global economy as approximately 80% of traditional medicinal preparations involve the use of plants or plant extracts [4]. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. An efficient and most suited alternative solution to the problems faced by the phytopharmaceutical industry is development of in vitro systems for the production of medicinal plants and their extracts. In vitro propagation technology has sound and extensive potential for commercial rapid multiplication of medicinal plants and horticultural crops because it is a quick method, allows round-the-year propagation/production of identical plants, and produces plants free from diseases [5]. In vitro propagation has been successfully employed for the conservation of medicinal crop genetic resources, particularly with those crops which are vegetatively propagated [6,7]. Propagation through seed is unreliable due to poor seed quality, erratic germination and seedling mortality as under natural field conditions [8].

Begonia malabarica Lam. (Begoniaceae) is an important medicinal plant whose main secondary metabolites are luteolin, quercetin and β-sitosterol [9]. The leaves are used for the treatment of respiratory infections, diarrhoea, blood cancer and skin diseases [10]. Very few reports on cultivation, breeding and improvement programmes and in vitro studies of *B. malabarica* are available despite its commercial importance. This paper deals with the standardisation of a technique for its micropropagation through multiple shoot formation. The protocol provides early bud-break with a high frequency of shoot multiplication from axillary bud and leaf explants with comparatively reduced requirement for plant growth hormones, as well as successful acclimatisation of plants in the soil. The performance of regenerated plantlets was also evaluated in the field.

Materials and Methods

The methods of plant tissue culture used were the standard methods as described by Gamborg and Phillips [11]. The nodal explants and leaves of one-year-old plants of *B. malabarica* were collected from the foot-hills of Anamalai around Aliyar Dam, Coimbatore district, Tamilnadu, India. They were washed first under running water (30 min) and treated with 0.2% (v/v) aqueous surfactant (Teepol, BDH, India) for 10 min. followed by repeated rinsing with distilled water. Subsequently, they were treated (20 min) with 0.1% (w/v) carbendenzim (BASF, India). Further sterilisation was done under aseptic condition in a laminar air-flow hood. The plants were surface-sterilised with 50% (v/v) ethanol (1 min) and then by 0.07% (w/v) $HgCl_2$ (3 min). Finally, the plants were washed thoroughly (3-5 times) with sterilised distilled water. The plant nodes were cut into an appropriate size (0.6 cm) and young-leaf laminas with midrib (0.5 cm) were cut and cultured on Murashige and Skoog (MS) medium.

Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

Throughout the experiment a full strength MS medium of 3% (w/v) sucrose and gelled with 0.8% (w/v) agar (Qualigens, India) was used. The pH of all media were adjusted to 5.8 prior to autoclaving (15 min). The cultures were incubated for 16 hr in a culture room at 25±1°C. Photoperiod (50μE/m^2/s) was provided by cool-white fluorescent tubes.

The basal medium was supplemented with 6-benzylaminopurine (BA; 0.0-8.8 mg/l) and indole-3-acetic acid (IAA; 0.0-2.88 mg/l), either alone or in combinations. Initiation of callus formation from the base of leaf lamina was observed on MS medium supplemented with BA, IAA and adenine sulphate (Ads). Root induction on shoots was achieved on full strength MS with IAA/ indole-3-butyric acid (IBA) at different concentrations. Well-developed rooted shoots were removed from the culture vessels, washed gently under running tap water and planted in plastic cups containing a potting mixture of sand, soil and farmyard manure in the ratio of 1:1:1. The plantlets were kept in net house for acclimatisation (2-3 weeks) before their subsequent transfer to the field. All cultures were visually examined periodically. Humidity was maintained by sprinkling water regularly throughout the day [12]. The plants were gradually exposed to the normal conditions and transferred to the medicinal garden of Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

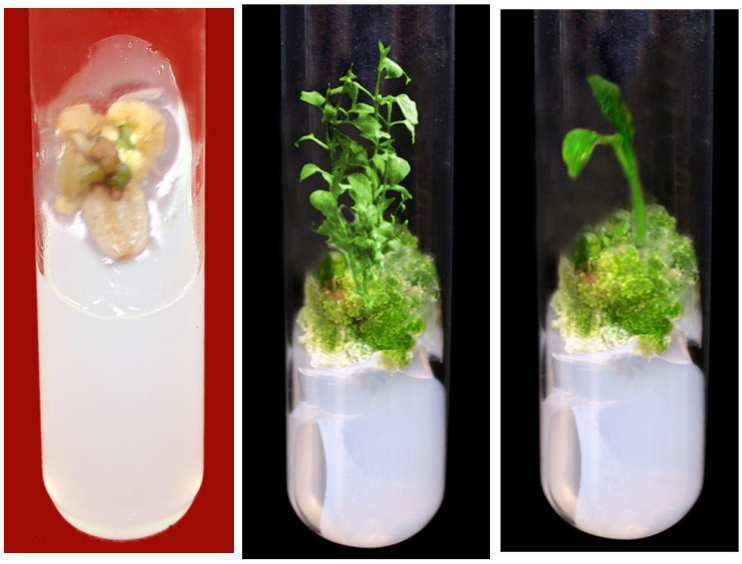
The experiments were set up in a completely randomised design. There were ten cultures per treatment and each experiment was repeated thrice; sub-culturing was carried out at an interval of 4 weeks. Effects of different treatments were quantified on the basis of the percentage of cultures showing responses in regeneration of shoots, mean number of shoots/node, mean-number of roots/shoot and root length, which were statistically analysed. Qualitative analysis of secondary metabolites was carried out by thin layer chromatography. The shade-dried roots and leaves of in vitro raised plants and mother plants were crushed into powder form and were subjected to phytochemical analysis [13].

Results and Discussion

Bud break on the nodal segments was achieved on MS with 4.4 mg/l BA and 1.4 mg/l IAA (Figure 1a). When MS supplemented with different concentrations of BA and IAA was used, multiple shoots emerged from the nodal explants within two weeks of incubation (Figure 1b). In vitro response varied greatly in various plants depending upon the age of explants [14] as well as on the culture maintenance conditions [15]. Among different concentrations of the growth hormones tested, 4.4 mg/l BA and 1.4 mg/l IAA elicited the maximum number of shoots (10 multiple shoots) from nodal explants (Table 1). Direct shoot regeneration from nodal explants has been reported earlier [16,17] on MS medium with 27.2 mg/l Ads + 2.46 mg/l IBA. Similarly, Verma et al. [18] reported rapid propagation of *Plumbago zeylanica* with a maximum of four multiple shoots per nodal segment with 8.87 mg/l BA and 0.49 mg/l IBA. The present study exemplifies a positive modification on shoot induction efficacy on MS medium with low concentrations of auxin and cytokinin. The elongation of shoots (3-4 cm) was observed on the same proliferation medium within two weeks of incubation

Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

(Figure 1c). Similarly, Chaplot et al. [19] reported rapid propagation of *P. zeylanica* with a maximum of 12 multiple shoots per nodal segment with 4.4 mg/l BA and 1.4 mg/l IAA.



a **b** **c**

Figure 1. Micropropagation through axillary bud proliferation and leaf callus of *B. malabarica*
a. Bud break from nodal explant
b. Multiple shoot formation on MS medium containing BA (4.4 mg/l) and IAA (1.4 mg/l)
c. Shoot formation from callus on MS medium with BA (4.4 mg/l), IAA (1.4 mg/l) and Ads (150mg/l)

Table 1. Influence of different combinations of BA and IAA in MS medium on shoot formation through nodal explants of *B. malabarica*

Concentration (mg/l)		Response (%)	Number of shoots/node* (Mean ± SD)
BA	IAA		
0.0	0.0	0.0	0.0
2.0	0.5	12	1.5 ± 0.2
4.4	0.5	48	4.2 ± 0.4
4.4	1.4	83	10.2 ± 1.4
4.4	2.8	62	5.6 ± 0.4
6.4	1.4	54	4.6 ± 0.6
8.8	1.4	52	4.4 ± 0.3

* Values are from four replications, ten cultures per replicate, and scored after three weeks.

Callus initiation was observed from young leaves on MS medium supplemented with BA (0.0 - 8.8 mg/l), IAA (0.0-2.8 mg/l) and Ads (150 mg/l). Callus formation from the explant leaf of *B. malabarica* is in agreement with results obtained by Chaplot et al. [19] for *P. zeylanica*, although the hormonal combinations were different. The best callus (nodular) formation was observed on MS

Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

medium containing 4.4 mg/l BA, 1.4 mg/l IAA and 150 mg/l Ads. The higher number of shoots from the callus was observed on MS with 4.4 mg/l BA, 1.4 mg/l IAA and 150 mg/l Ads (Table 2). An average of 28 shoots was recorded in callus cultures through organogenesis. Present results are consistant with earlier reports on *P. zeylanica* [19] and Ashwagandha (*Withania somnifera*) [20] in that cytokinin and auxin influence shoot bud regeneration.

Table 2. Influence of different combinations of growth regulators in MS with 150 mg/l Ads on shoot bud regeneration from leaf callus of *B. malabarica*

Concentration (μM)		Response	Number of shoots/node*
BA	IAA	(%)	(Mean ± SD)
0.0	0.0	-	-
2.0	1.4	10	1.04 ± 0.2
2.0	2.8	26	2.08 ± 0.4
4.4	1.4	92	28.04 ± 1.4
4.4	2.8	84	22.06 ± 1.2
6.4	1.4	72	15.04 ± 1.2
6.4	2.8	70	12.8 ± 1.1
8.8	1.4	64	6.34 ± 1.2

* Values are from four replicates, ten cultures per replicate, and scored after three weeks.

Well-developed shoots (3-4 cm with three nodes) generated through axillary-bud proliferation and leaf callus were excised and cultured on MS medium with different concentrations of auxins for root induction, which was found to be more prominent in the medium containing IAA (0.56 mg/l) or IBA (1.2 mg/l) (Figure 2; Table 3). Earlier workers [17,19,21] had reported smaller numbers of roots on half-strength MS medium containing 0.57 mg/l IAA, 4.92 mg/l IBA and 2 mg/l IBA respectively. While profuse rooting was observed on full strength MS supplemented with IAA or IBA (Table 3), the best result (16 roots) was obtained on MS with IBA (1.2 mg/l) within 10 days. The potency of IBA in root induction has been reported in Petunia [22] and in *P. zeylanica* [18]. In our case, maximum frequency (96%), number of roots/shoot (around 16) and mean root length (13.62 cm) were achieved within 10 days when shoots were cultured on MS with IBA.

The ultimate success of the in vitro propagation lies in the successful establishment of plants in the soil. In this study, an 80% transplantation success of in vitro hardened plantlets in the field was observed in comparison to the 60-90% survival of plantlets recorded in the experiments of previous workers [17,21]. The high survival rate of in vitro *B. malabarica* plants in the present study indicates that this procedure may be easily adopted for large scale multiplication and cultivation. The in vitro propagated plantlets resemble the general growth and morphological characteristics of the donor plants (Figure 3). The in vitro raised plants and the seed-grown plants were then uprooted from the field for root harvesting. A significantly higher number of roots (16.0 ± 0.8) per plant for the former were observed compared to that for the seed-generated stock (4.2 ± 1.2). There was a threefold increase in root biomass on fresh weight basis of the in vitro raised plants (148.3 ± 1.8 g) in

Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

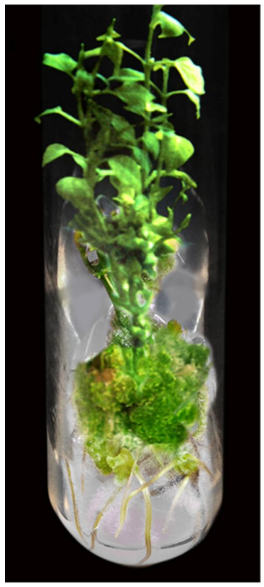


Figure 2. Induction of roots on regenerated shoots on MS medium containing IBA

Table 3. Effect of different auxins in MS medium on root induction from regenerated shoots

Concentration (μM)		Number of roots/shoot* (Mean ± SD)	Root length (cm)
IAA	0.0	-	-
	0.56	13.62 ± 1.2	11.2 ± 0.42
	1.42	6.84 ± 1.4	5.12 ± 0.28
IBA	0.0	-	-
	0.48	4.2 ± 0.42	5.04 ± 0.02
	1.2	16.04 ± 1.2	13.62 ± 0.04
	2.48	8.36 ± 1.4	6.14 ± 0.02

* Values are from four replicates, ten cultures per replicate, and scored after two weeks.

comparison with that of the seed-generated plants (42.4 g ± 1.2 g). Similar observations have been reported by Roja and Heble [23] for in vitro generated plants (*Rauwolfia serpentina*) with thick root stumps (fresh weight 60.56 g) compared to the long slender roots (fresh weight 11.92 g per plant) of the conventionally grown counterparts. Present results are also in agreement with the recent report by Chaplot et al. [19] who obtained a higher number of roots (18.0 ± 0.6) and fresh weight (5.1 ± 1.4 g) in in vitro raised *P. zeylanica* as compared to what were observed in rooted cuttings. Further qualitative analysis of secondary metabolites in roots and leaves of plantlets showed the presence of flavonoids, alkaloids, phenols, saponins and tannins in both the in vitro generated and seed-grown plants without showing any levels of variation.

Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

Figure 3. Hardened *B. malabarica* plant in potting mixture of sand: soil : FYM (1:1:1) ready for transplantation in the field.

Conclusions

A reproduction protocol for *B. malabarica* has been established through nodal and leaf explants. This protocol can be exploited for conservation and commercial propagation of this medicinal plant in the Indian subcontinent and may be useful for genetic improvement programs.

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Maejo Int. J. Sci. Technol. **2009**, *3*(02), 261-268

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