

Micropropagation of *Vanda coerulea* Griff ex Lindl.: A Study of Regeneration Competence of Roots *in vitro*

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Abstract - A rapid and efficient procedure is outlined for *in vitro* propagation of *Vanda coerulea* Griff. ex Lindl., an endangered epiphytic orchid. Root segments (0.5-1.0 cm long) from plants growing both *in vivo* and *in vitro* were cultured on MS (Murashige and Skoog, 1962) medium supplemented with auxins [Indole 3-acetic acid (IAA)], cytokinins [6-benzyl aminopurine (BAP), 6-furfuryl amino purine (KN)] and coconut water (CW). The root explants from *in vivo* sources failed to respond whereas those from *in vitro* grown plantlets regenerated protocorm-like bodies (PLBs). An optimum of 16.2 PLBs developed from root explants on medium supplemented with 30 μ M BAP and 15 μ M IAA. The resulting plantlets were successfully transferred to pots containing brick pieces, charcoal chunks and decaying litter (1:1:1) + a top layer of moss. Ninety-five percent of the plantlets survived in the glass house after two months of transplantation.

Keywords-*Vanda coerulea*; conservation; endangered; *in vitro*; *in vivo*

I. INTRODUCTION

Orchids are commercially valuable plants which are marketed both as plants as well as cut flowers and their production has increased magnificently worldwide [1, 2]. However, the orchids are fast depleting from their natural habitats due to deforestation, urbanization, utilization of land for agriculture and over-exploitation for commercial purposes. Tissue culture methods have been extensively exploited, not only for rapid and large-scale propagation of orchids, but also for their *ex situ* conservation. Protocols have been developed for a number of orchid species through *in vitro* culture of various plant parts [3, 4, 5].

Vanda coerulea Griff ex. Lindl. (Fig.1 a), popularly known as the blue Vanda of Asia is a perennial epiphyte growing at an elevation of 1000–1500m in Northeast India and in the northern ranges of Thailand and Burma. Due to its clear blue flowers it has progenerated a vast variety of floriculturally significant hybrids and it has been bred for such qualities as flower size, floriferousness, vigour and cold tolerance in modern vandaceous hybrids. The species is also important ethnobotanically. The juice from its leaves is used to cure diarrhoea, dysentery, and dermal disorders [6]. Habitat destruction and overexploitation are the two important factors threatening its survivability in India [7]. It is listed in the Appendix I of the Committee for International Trade in Endangered Species of Wild Fauna and Flora. It is

also included in the list of Threatened Plants of India published by the International Union for Conservation of Nature and Natural Resources. Therefore, there is an urgent need to conserve this endangered taxon. The present paper describes a successful protocol devised for the regeneration of *Vanda coerulea* Griff ex. Lindl. from root explants so as to obtain true-to-type plantlets for conservation.

II. MATERIALS AND METHODS

A. Plant Material

The young and actively growing roots (1.5-2.0cm long) from mature greenhouse-grown plants (*in vivo*) and 5-months old *in vitro* cultures were harvested. The roots were then segmented to prepare 0.5-1.0 cm long explants.

B. Culture Medium

Murashige and Skoog [8] medium supplemented with growth regulators like 6-benzyl aminopurine (BAP), indole 3-acetic acid (IAA) and 6-furfuryl amino purine (Kinetin, KN) were added to the medium singly and in combinations. Besides, the effect of organic supplement, coconut water incorporated in MS basal medium was also tested in various combinations. The medium was incorporated with 3% (W/V) sucrose and solidified with 0.8% (W/V) agar. The pH of the media was adjusted to 5.8 ± 0.02 using 1N NaOH prior to autoclaving at 121°C.

C. Culture Conditions

Cultures were maintained at $25 \pm 2^\circ\text{C}$ for 12h photoperiod under $50\mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity. There were 10 replicates for each treatment and experiments were repeated thrice.

D. Statistical Analysis

The data was subjected to statistical analysis using one way Anova and comparisons between the mean values of treatment were made by Fisher's LSD test [9].

E. Acclimatization of plantlets

The plantlets with well-defined roots (2-3 cm in length) were transplanted into pots containing brick pieces, charcoal chunks and decaying litter (1:1:1) + a top layer of moss. The percentage survival of plants was calculated after two months of transfer.

III. RESULTS AND DISCUSSION

In vitro micropropagation using root tips has been successfully used for propagation of a number of orchids either for conservation or for commercial production [10, 11]. Tissue culture production of ornamental plants in general and orchids in particular forms the basis for the entire horticultural industry. Several workers using various culture media introduced tissue culture methods for regeneration of orchids and different explants have been used for *in vitro* orchid propagation, the most common being shoot tip and axillary buds. Attempts to produce protocorm-like bodies (PLBs) from roots have not been common in orchids. Unlike to shoot meristems, the root apices are considered to be highly recalcitrant in terms of bud formation both *in vivo* and *in vitro* [12]. In our study, root segments from *in vivo* plants failed to respond whereas those from *in vitro* grown plantlets regenerated PLBs. The differential responses of the explants from mature and juvenile roots under identical nutritional conditions seems to indicate the importance of their source and physiological age of explants for *in vitro* regeneration [13, 14]. Often *in vitro* regeneration in orchids is achieved through either callus or PLBs formation. Since many orchid species require auxins and/or cytokinins for PLB formation and plantlet development; the combinations, concentrations and the ratio between them are usually critically important [15]. In the present investigation, the root segments directly developed into the PLBs and the frequency of the PLBs produced was markedly influenced by the quality and combination of growth hormones in the nutrient medium. A combination of 30 μ M BAP and 15 μ M IAA in MS medium (Table 1) proved to be the best treatment for explants response (98.2%) wherein 16.2 \pm 0.37 PLBs were formed with an average of 15.8 \pm 0.37 shoots emerging out of these (Fig. 1 b). The similar stimulatory effect of auxins and cytokinins on roots for protocorm multiplication as well as shoot formation in *Cymbidium* hybrid has been reported [16]. BAP at 30 μ M in MS medium also showed good explants response (96.8%) in terms of PLBs formation and shoot formation. Increased concentrations of both auxins and cytokinins in the medium were inhibitory for response as indicated by the decrease in multiplication and proliferation of PLBs and shoots. Medium supplemented with coconut water (CW) as well as KN also induced PLBs regeneration to plantlets but the response was quite low comparatively (Table 1). The emerging shoots developed roots in 3-4 weeks (Fig.1 c) on being transferred from treatment medium to basal medium. However, plantlets were also able to root eventually in all BAP and IAA combinations. The rooted plantlets were transplanted to pots containing brick pieces, charcoal chunks and decaying litter (1:1:1) + a top layer of moss (Fig. 1 d), and 95% of plantlets were successfully grown in the greenhouse. The layer of

moss on top proved to be beneficial due to higher retention of moisture content. Feeding the plantlets with diluted (10 times) MS nutrient salt solution fortnightly proved beneficial for the healthy growth as it provided essential nutrients to the developing plantlets [17].

TABLE I. INFLUENCE OF DIFFERENT GROWTH REGULATORS AND COCONUT WATER (CW) ON DEVELOPMENT OF PROTOCORM-LIKE BODIES (PLBs) FROM ROOT EXPLANTS OF VANDA COERULEA

Growth regulators (μ M)	Concentrations	Percentage (%) of responsive explants	Number of PLBs	Number of shoots
Control	0	0	0	0
	05.0	0	0	0
	15.0	63.2 \pm 0.58 ^b	05.4 \pm 0.50 _b	04.4 \pm 0.24 _b
	30.0	96.8 \pm 0.37 ^a	09.8 \pm 0.37 ^a	09.0 \pm 0.37 ^a
IAA	50.0	31.2 \pm 0.58 ^c	02.4 \pm 0.24 ^c	02.2 \pm 0.20 ^c
	05.0	52.4 \pm 0.81 ^b	02.4 \pm 0.24 ^c	01.2 \pm 0.37 ^c
	15.0	55.4 \pm 0.60 ^b	03.6 \pm 0.24 _b	03.0 \pm 0.26 _b
	30.0	78.0 \pm 0.54 ^a	07.4 \pm 0.37 ^a	06.8 \pm 0.31 ^a
KN	50.0	33.6 \pm 0.50 ^c	02.6 \pm 0.26 ^c	01.0 \pm 0.24 ^c
	05.0	38.6 \pm 0.40 ^c	01.8 \pm 0.37 ^c	01.0 \pm 0.37 ^c
	15.0	42.2 \pm 0.37 ^b	03.0 \pm 0.31 _b	02.4 \pm 0.24 _b
	30.0	59.2 \pm 0.37 ^a	05.4 \pm 0.24 ^a	05.0 \pm 0.20 ^a
BAP + IAA	50.0	26.4 \pm 0.50 ^d	01.4 \pm 0.24 ^c	01.0 \pm 0.24 ^c
	05.0 + 05.0	50.8 \pm 0.37 ^c	07.6 \pm 0.24 ^c	07.2 \pm 0.20 ^c
	15.0 + 15.0	53.8 \pm 0.50 ^c	12.0 \pm 0.31 _b	12.4 \pm 0.31 _b
	30.0 + 15.0	98.2 \pm 0.37 ^a	16.2 \pm 0.37 ^a	15.8 \pm 0.37 ^a
BAP + KN	30.0 + 30.0	63.2 \pm 0.32 ^b	05.4 \pm 0.24 ^c	04.9 \pm 0.24 _d
	05.0 + 15.0	0	0	0
CW (%v/v)	15.0 + 30.0	26.8 \pm 0.83 ^a	03.4 \pm 0.24 ^a	02.4 \pm 0.24 ^a
	05.0	0	0	0
	10.0	36.4 \pm 0.50 ^b	04.4 \pm 0.24 ^a	04.0 \pm 0.24 ^a
	15.0	39.4 \pm 0.50 ^a	02.4 \pm 0.24 _b	02.0 \pm 0.24 _b

Basal medium used is MS. Data recorded after 6 weeks. Values are mean \pm S.E. Means followed by same letter in the column are not significantly different as indicated by Fisher's LSD (p = 0.05)

IV. CONCLUSION

In conclusion, the results clearly indicate that the root segments can be profitably used for micropropagation of *Vanda coerulea*. Since this orchid is endangered in the wild, the root explants acts as an effective alternative to shoot meristem for micropropagation as it does not require the sacrifice of the mother plant, and offers exciting opportunities to raise large numbers of true-to-type plantlets. Thus, this protocol can be used both for restoring the dwindling populations of *Vanda coerulea* in nature and mass propagation for its ornamental or pharmaceutical importance.

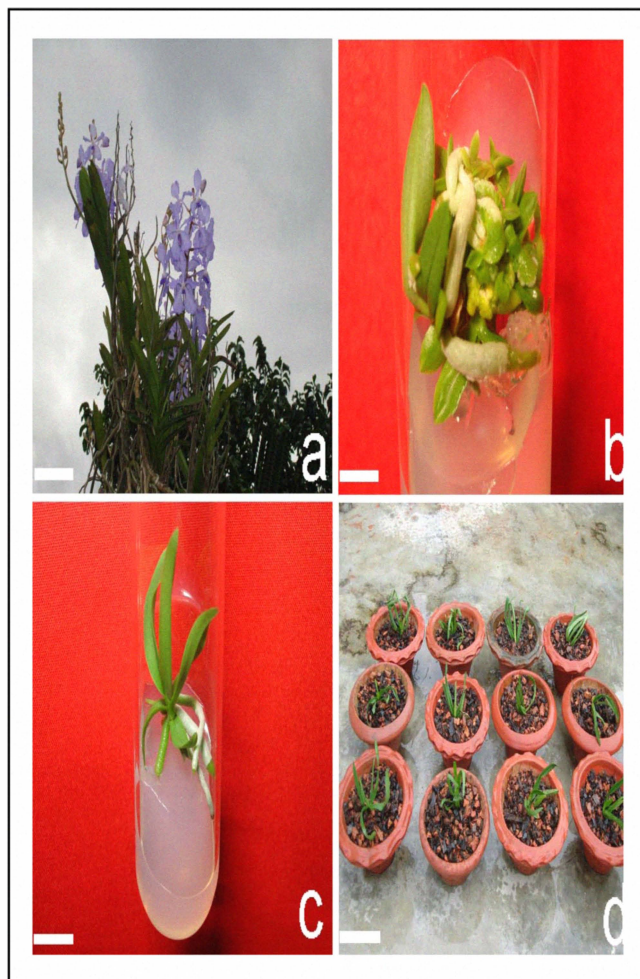


Figure 1. Regeneration competence of root segments of *Vanda coerulea* Griff ex Lindl.: (a) Blooming plants of *Vanda coerulea* (bar = 3cm); (b) Multiple shoots obtained from root segments of *V. coerulea* cultured in MS with BAP (30 μ M) and IAA (15 μ M) (bar = 1cm); (c) Development of roots on MS basal medium after one month (bar = 1 cm); (d) Regenerated plants in pots containing brick pieces, charcoal chunks and decaying litter (1:1:1) + a top layer of moss (bar = 2 cm).

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