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In vitro micropropagation of *Eulophia andamanensis*–A tropical orchid

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Abstract: *Rapid* in vitro *propagation of the tropical orchid* Eulophia andamanensis *was achieved by applying simple and reliable procedure for micropropagation. Pseudobulbs and axenic leaf buds of* Eulophia andamanensis *were placed on MS culture media with high auxin: cytokinin ratio. The incidence of moderate (~ 40%) contamination in cultures was reported due to source of explants. Further the shoot multiplication increased upon subculture on freshly prepared medium. The experiment cycles were repeatable. Media containing the growth regulators as 1 mg/l BAP + 0.5 mg/l NAA, 2 mg/l BAP + 0.5 mg/l NAA, 2 mg/l BAP + 1 mg/l NAA, 1 mg/l Kinetin + 1 mg/l NAA, 2 mg/l Kinetin +1 mg/l NAA, 3 mg/l Kinetin + 0.5 mg/l NAA respectively. While the Pseudobulb explants on media MS + 1mg/l BAP + 0.5 mg/l NAA and 2mg/l BAP + 0.5 mg/l NAA and control only showed good response. Pseudobulb explants performed better than axenic buds. Callusing was not observed in leaf slices and buds explants.*

Keywords: *Pseudobulbs, monopodial orchids, flower stalks, micropropagation, vegetative regenerants*

INTRODUCTION

Eulophia andamanensis is one of the highly evolved terrestrial orchid species, known worldwide for its longest spike length (Recorded in Limca Book, 2013) and long lasting nature. It is called as green pretty orchid. The rare greenish white with maroon stripe flowers has made it exceptionally popular as a cut flower. Plants are commonly propagated from vegetatively by division of Pseudobulbs. To multiply through division, mother plants must be at least 3-4 years old and yield only very few preparative's Pseudobulbs and it grows very slow. To overcome these problems a reliable faster and successful multiplication for large scale commercial cultivation method for Eulophia is necessary. In orchids, shoot tip, meristem, leaf and leaf sections, stem segments, pseudobulbs, floral parts, aerial root etc, has successfully been used for *in vitro* propagation. Among these, pseudobulbs are used as explants for large scale multiplication of commercial important *Eulophia andamanensis* orchids. A large number of growth hormones are used extensively for better regeneration and proliferation in orchids (Hasegawa *et al*., 1985; Kim and Kako. 1982). Hence, the study was undertaken to find out the efficiency of growth hormones for *in vitro* proliferation, differentiation and growth of *Eulophia andamanensis* green pretty orchid.

Orchids are well known for its ornamental importance due its great diversity of flowers and long lasting in nature as well as therapeutic important for it's curing of several aliments by applying compounds of orchids (Vij *et al*., 1984; 2000). The Orchidaceae family is one of the largest of flowering plants and they are terrestrial, epiphytic, or saprophytic herbs with about 1,000 genera and 25,000 species (Pridgeon, 1992; Hamilton, 1998 and Jain, 1980). Orchids are of

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enormous horticultural importance and also play a very important role for stability of the forest ecosystems (Kaushik, 1983). Orchid constitutes an order of monarchs in the world of ornamental plants (Stewart *et al*., 1982). They are one of the most indulge plants and occupy top position among all flowering plants valued for cut flower production as potted plants. The propagation of orchids is naturally very difficult because of low viability of seeds and its different developmental stages to develop as adult plants (Morel 1964; Churchill *et al*., 1973). For the conservation of endangered and medicinally important plant species, it is desirable to establish protocols for rapid clonal propagation (Kaur and Sarma, 1997; Bechtel *et al*., 1980, Murashige *et al*., 1962). The commercial propagation of the orchid very slow; to overcome to this problem in vitro propagation are needed for rapid production of healthy plants.

Eulophia andamanensis is an orchid endemic to the Andaman and Nicobar group of Islands are situated off the eastern coast of India between 6°452 N 93°122 E and 13°412 N 93°572 E. The genus *Eulophia* belongs to the subfamily Vandoideae, tribe Cymbidieae, and subtribe Cyrtopodiinae. *Eulophia andamanensis* is mostly found growing wild in the Little Andaman Islands, where the temperature ranges between 23–30° C and the annual average rainfall is 3473 mm. The growth habit is sympodial. The stem at the base is bulbous, with thick roots. The leaves are short during flowering, linear lanceolate. The bracts are shorter than the pedicel, the sepals 2 cm long, the lip shorter than the sepals. The sepals are linear lanceolate, 3–5 nerved, acuminate; both the sepals and petals are pale green in colour, the lip green at the base and white at the centre with maroon horizontal striations. The flowering period is from November to March, with the florets borne on long spikes (0.6–1.3 m long), and last for about 50 days.

In the present study, different culture media for direct shoot regeneration from nodes of pseudobulbs *Eulophia andamanensis* were tested in order to develop an efficient and rapid propagation method for these orchids.

MATERIALS AND METHODS

The source plants of *Eulophia andamanensis* were maintained in Orchid house of Central Agricultural Research Institute, Port Blair. Juvenile shoots, pseudo bulbs and leaf dormant buds were used as the source of explants. The shoots were excised, defoliated and cut into 5-7 cm long pieced having three or four nodes. Explants were washed thoroughly in running tap water then washed with fungicide followed by surface sterilization under Laminar Air Flow hood with a 0.1% $HgCl_2$ solution for 10 minutes and rinsed thrice in sterile distilled water. The nodal explants of 1 cm length were cut aseptically and placed *in vitro* on MS medium containing 2% sucrose and supplemented with different combination and concentrations of plant growth regulators *viz*., BAP, Kin and IAA. The pH of the medium was adjusted to 5.8 before adding 0.8% agar and autoclaved at 121ÚC for 15 minutes. The cultures were incubated at 25 + 1ÚC under fluorescent light (2000 lux, 16 hr photoperiod).

RESULTS AND DISCUSSION

The regeneration response in the explants was completely dependent to the use of growth regulators in the culture medium (Table 1). Culture initiation was affected by the physiological status of tissues besides the chemical stimulus in the nutrient pool. Explants, pseudobulbs and axenic leaf buds remained recalcitrant to regeneration responded to certain selected media combinations. The cytokinin, BAP (2.0 mg/l) proved to be beneficial for production and proliferation of multiple shoots (Fig1). The relative abundance of phenolics in *Eulophia andamanensis* and the negative effect of the phenolic oxidates on growth and differentiation of tissues in culture as evidenced from extensive browning of the medium and loss of cultures. (Ernst,1974; 1985, 1994; Tanaka *et al*., 1988). Excessive phenolic production is possibly due to increased polyphenol oxidase and catalase activity triggered by certain cultural conditions (Harvais,1982). Phenolic exudations from the pseudo bulbs were observed in all the treatments. Browning of the medium, followed by leaf buds death and blackening of the leaf tips was common

Table 1
Effect of different concentrations of plant growth regulators on in vitro development of Eulophia andamanensis.

Sl. No.	*Media*	*Explant*	*No of explants inoculated*	*No of explants responded*	*% Response of explants*
1.	1 mg/l BAP +	Leaf cuttings	25	Nil	0
	0.5 mg/l NAA	Pseudo bulb	25	6	24
		Axenicbuds	25	2	8
2.	2 mg/l BAP +	Leaf cuttings	25	Nil	0
	0.5 mg/l NAA	Pseudo bulb	25	7	28
		Axenicbuds	25	2	8
3.	2 mg/l BAP +	Leaf cuttings	25	Nil	0
	1 mg/l NAA	Pseudo bulb	25	4	16
		Axenicbuds	25	Nil	0
4.	1 mg/l Kinetin	Leaf cuttings	25	Nil	0
	+ 1 mg/l NAA	Pseudo bulb	25	2	8
		Axenicbuds	25	3	12
5.	2 mg/l Kinetin	Leaf cuttings	25	Nil	0
	+ 1 mg/l NAA	Pseudo bulb	25	3	12
		Axenicbuds	25	2	8
6.	3 mg/l Kinetin	Leaf cuttings	25	Nil	0
	+ 0.5 mg/l NAA	Pseudo bulb	25	3	12
		Axenicbuds	25	3	12
7.	Control MS	Leaf cuttings	25	Nil	0
		Pseudo bulb	25	6	24
		Axenicbuds	25	4	16

Figure 1: A fully matured with flowed spikes of *Eulophia andemanesis*.

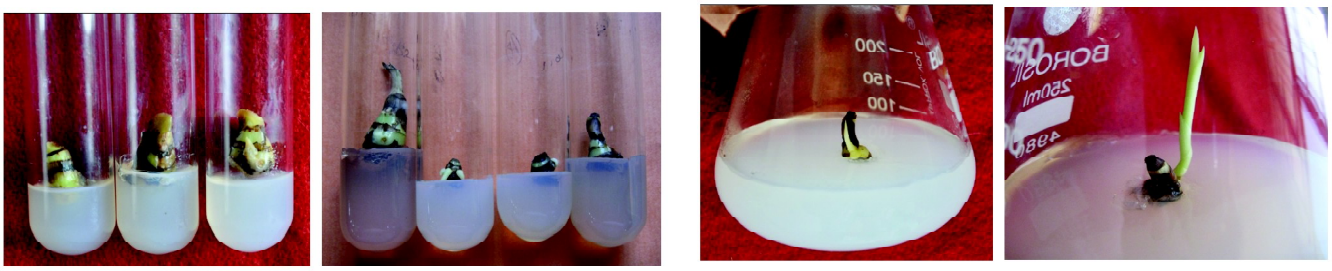

Figure 2: Different stages of in vitro propagation of *Eulophia andemanesis*.

in all the cultures, which affected the survival rate of the plantlets.

Extensive use of growth regulators in the media selectively promotes the growth and proliferation of the explants. Growth regulators depending on type and concentration both inhibit and promote germination in orchids (Arditti *et al*., 1981). The beneficial effects of NAA were in line with the results of several orchids (Chung *et al*, 1983; 1998; 2003). NAA stimulated the seedlings in *Arundina graminifolia* (Kaur and Sarma, 1997) whereas, in *Paphiopedilum spicerianum* and *Dendrobium chrysanthemum* auxins inhibited the growth of shoots and roots (Nayak *et al*., 1997b; Vij and Kaur, 1998; Talukdar, 2001; Temjenasangba *et al*., 2005).

CONCLUSION

The results showed that the average regeneration of plantlets with well developed leaves on culture media. Moreover, medium with NAA/BAP gave rise to plantlets. Generative regenerants only arose directly from dormant buds. The multiplication factor of

generative regenerants on medium more than 50% was found significantly the best among all the treatments used. We managed to obtain direct regeneration without undesirable callus formation on successful culture media and thus to shorten the time needed for formation of first regenerants and reducing the possibility of the occurrence of somaclonal variability.

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