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Research article

INVITRO MICROPROPAGATION OF *EULOPHIA NUDA LIND* AN ENDANGERED TERRESTRIAL ORCHID THROUGH PLB' (PROTOCORM LIKE BODIES)

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ABSTRACT: An efficient and reproducible protocol developed for micropropagation of *Eulophia nuda L.* an endangered terrestrial orchid; using 4mm section of axillary bud segment has been developed. Axillary bud section were cultured on Murashige and Skoog (MS) medium supplemented with different concentration of harmones (BAP), 5,1.0,2.0,mg/L, [α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), 0.5, 1.0, 2.0 and 5.0 µM] and Casein hydrolysetes (CH: 5, 10 and 15%).,2,4-D,.2,.5,1.0, 2.0.The explants developed protocorm like bodies (PLBs) within 6–8 weeks on the growth medium. MS+2 mg/l 2,4-D supplemented medium was found best for the induction of PLBs. Upon subculture on basal MS medium, the PLBs differentiated plantlets within 6–8 weeks with in vitro tuber and roots. This simple protocol will be useful for large-scale propagation of *Eulophia nuda lind.*

Key words: Endangered, Orchid, PLB, *Eulophia nuda lind*

INTRODUCTION

Eulophia belongs to family Orchidaceae, which is one of the largest and highly evolved families of monocotyledons. More than 15,000 species of orchids are found distributed throughout the world. A majority of them are herbs either epiphytic or terrestrial. The Genus *Eulophia* was first described by John Lindley in 1821[1]. The name "Eulophia" was derived from the Greek words "eu" (well) and "lophos" (plume), referring to the crested ridges of the labellum (lip) in most species. These are usually terrestrial or ground orchids, although some are epiphytes, and rarely, lithophytes. They are distributed in shady rainforests or in open scrub or woodland in the tropics and subtropics of Africa, India, Asia, Queensland (Australia), and the Americas, although most are found in Africa. Many can survive the dry season through their large bulbous 'corms'. [4] The inflorescence arises from the base, as raceme, supporting as many as 50 flowers; the sepals and the petals are alike. The lip usually has three lobes. As for most orchids, there are two pollinia for each flower. *Eulophia nuda Lindl* is a common terrestrial orchid found in the forests of India. It has become endangered due to over exploitation for its medicinal value and rapid decline in the forest cover. The corms are used in traditional medicine by the trade name of "Amarkand' or "Bansinghada". The plant is exploited for several of its ethno-medicinal properties like antidote for snake bite , as antihelmintic, against tumors , cases of broncritis , Scrofulous affection of the glands of the neck and in disease of the blood, [15] the plant is also claimed to be useful in tuberculosis [6]. In vitro multiplication has been an effective technique for rapid multiplication of the plants. [2, 3, 5, 7, 8, 11, 12, 13-17]. A protocol for rapid multiplication of *Eulophia nuda lindl* has been developed in this paper.

MATERIALS AND METHODS

The plant material was collected from Forest of Kesla, Madhya Pradesh. The explants were sterilized by standard procedures using different concentration of Sodium hypochlorite and ethanol. Explants were thoroughly washed under running tap water for 10 min. The explants were washed again after removing leaves with distilled water containing few drops of laboline. After 5 min. the explants were washed three times with distilled water. After that they were surface sterilized with 0.1% bavistin (w/v in lukewarm distilled water) solution for 15 min and thoroughly washed with distilled water. Then the explants were surface sterilized in the LAF with 70% methanol for 2 min and washed three times with Double Distilled Water (DDW), followed by surface sterilized with 0.1% mercuric chloride for 2, 5 and 10 min and again washed with DDW for three times.

Salts of MS medium and vitamins supplemented with various combination and concentration of different growth regulators namely I-NAA, 2-4D, BAP, Kinetin, 6(Y dimethylallylamino-purine, 2-Ip, gibberelic acid and sucrose were used for regeneration studies. The PH was adjusted to 5.8 before autoclaving at 15 lbs. for 15 mins. All the cultures were maintened at 26-28Ċ with 24 hrs light, and 32%relative humidity.

Media and culture conditions

Four nutrient media were compared for their suitability as germination media and for protocorm development: Murashige and Skoog (MS), half-strength MS, Knudson 'C' (KC), and; Knudson 1946; Malemmerg Orchid culture medim, BM-1 medium). All media were supplemented with 3 % sucrose (w/v) and solidified with 0.8 % agar ('Hi media', India). The pH was adjusted to 5.8 prior to autoclaving at 121 °C, 15 p.s.i. for 15 min. The cultures were maintained at 25 ± 2 °C and 75 % relative humidity under cool fluorescent light at 50 µmol m^{-2} s^{-1} (Philips, India) with a 14-h photoperiod. Seed germination data were recorded 60 days after inoculation of seeds, whereas different developmental stages of protocorms (i.e. percentage of protocorms with vegetative apex, plantlets with 2–3 leaves and 1–2 roots) were recorded every 30 days.

Observation

MS supplemented with different concentrations of auxins and cytokinens BAP, KIN, NAA, 2,4-D, IAA and IBA was used to study the effects of plant growth regulators on the growth of PLB. The weight and length of PLB's were observed for four months. Effect of different hormones on the color of callus, no. of leaves per callus, size and weight were observed.

Table- 1 Optimization of Media for PLB'S Initiation

S. No.	Treatment	Initiation %	Colour	Weight in gm(s) (Mean +SD)
1.	½ MS	25%	Brown	0.57+.13
2.	MS	28%	Brown	0.885+.07
3.	MS+0.5 mg/L 2,4-D	72%	Green	1.723±0
4.	**MS+1.0 mg/L 2,4-D+0.5 mg/L BAP**	**97%**	**Green**	**3.32±0.032**
5.	MS+2.0 mg/L 2,4-D	63%	Light green	1.83±0.002
6.	0.5mg/LBAP	42%	Brown	5.53±.0.38
7.	1 mg/L BAP	55%	White	1.25±.02
8.	MS+.5 KIN	60%	Light green	4.34±0.26
9.	MS+ 1 KIN	67%	Whitish green	2.34±0.22

Table - 2 Optimization of Medium for shoot and root formation from PLB'S

S. No.	Treatments	% of PLB Formation	Number of leaves Mean± SD	Shoot length (in c.m.) Mean± SD
1	MS+.5 mg/L BAP+0.5 mg/L NAA	25%	1.2±0.24	1.2±0.34
2	MS+1.0 mg/L BAP+1 mg/L NAA	37%	2.1± 0.39	1.4±0
3	MS+2.0 mg/L BAP+0.2 mg/L IAA	35%	1.3 ± 0.02	1.2 ±0.02
4	**MS+1 mg/L BAP+1 mg/L IAA**	**79%**	**3.1 ± .24**	**2.3±0.21**
5	MS+1 mg/L BAP+1mg/L IBA	56%	2 ± 1.1	2.14±0.83
6.	MS+2.0 mg/L BAP+.5 mg/L IBA	58%	1.57±0.23	1.3±0.10
7.	MS+1 mg/L Kin+ .5 mg/l IBA	40%	1.1±0	1.7 ±0.21

RESULTS AND DISCUSSION

Then the primary and secondary developed shoots were transferred to concentration MS+1 mg/L BAP+1 mg/L IAA, 3.1±.24 (Shoot no.mean), 2.3±0.21.(Shoot length mean). Statistical analysis of the PLB's their diameter (d) at 30 days and 60 days indicated that the PLB grew gradually with the time, and most of the PLB diameters were between 3 and 5 mm with 2,4-D1.0 mg/L, These shows that 2,4-D could obviously accelerate the growth of *Eulophia nuda* PLB. When PLB were treated with BAP, the number of PLB (d >8 mm) was 12mm at 30 days, and 34mm at 60 days, respectively. At 60 days, the (d > 5mm) had only a slight increase as the culture days increased. With the enhancement of BA level, the number of PLB (d < 3 mm) gradually increased, whereas the number of PLB (d > 6 mm) was in a balanced state at 30 days; but the number of PLB (d < 6 mm) showed little difference and the number of PLB (d > 5 mm) was gradually reduced at 60 days. It is suggested that high concentration of BA may inhibit the growth of plb's. In further tests, each auxin at .5, 1, 2 mg/L was combined with BAP at concentrations of .5, 1or 2 mg/L As before, media containing IAA formed the most shoots directly, with a combination of 1 mg/L BAP and 1mg/l IAA being the most effective. MS+1 mg/L BAP+1mg/L IBA 56% of PLBs were formed. Generally, explants in media containing 2,4-D again formed mostly PLBs, but with some direct production of shoots as well. As before, media containing BAP formed the most shoots directly, with a combination of 15 mM BAP large numbers of PLBs were also formed. Generally, explants in media containing 2,4-D again formed mostly PLBs, but with some direct production of shoots as well. However, when 15 or 30 mM2,4-D was combined with BAP at 15 mM, almost all direct shoot production was channelled into PLB formation. The highest explant response (97.59 %) and PLB conversion (71.48 %) were observed at 15 mM each of BAP and2,4-D together (Table 2; Fig. 1-3). The response of axillary buds was, however, significantly low as compared with the control in the medium with high concentrations of the control in the medium with high concentrations of BAP. A high response (81.2 %) of the explants was recorded in the medium containing 15 mM BAP and 5 mM NAA.

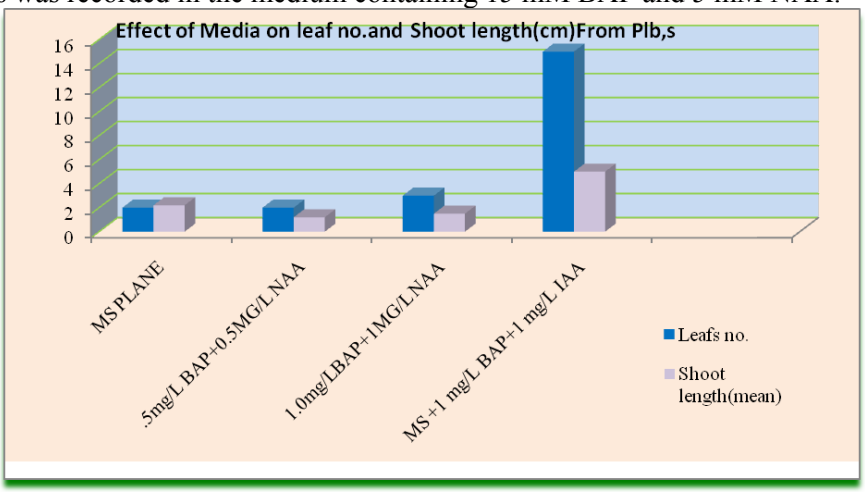

Fig-1.Effect of different media on leaf number and shoot length from PLB's

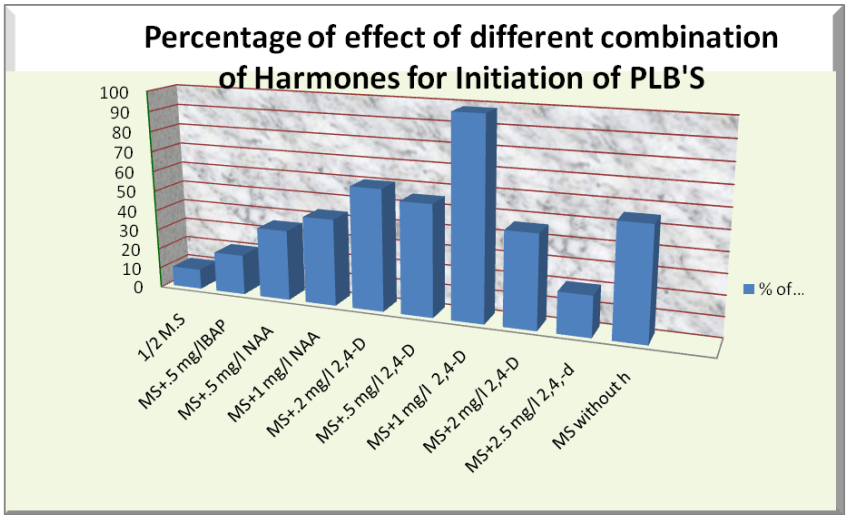

Fig-2. Percentag of different combination of Hormones for Initiation of PLB's

PLB'S induced in various combinations of Auxin and Cytokinin viz MS + (0.5 & 1 mg/L 2, 4 -D), MS+0.5 mg/L 2, 4-D+ (0.1 & 0.5 mg/l BAP). In this study callus initiation was also obtained when two cytokinins (BAP + Kn) were added together in the medium. The best result for callus initiation (98%) was obtained in the concentration MS+1mg/L,2,4-D+0.5mg/LBAP. Callus initiation was also found to be good in combination of cytokinins. The best result for PLB's (98%) was obtained in the concentration MS+1.0 mg/L BAP+1.0 mg/L IAA.

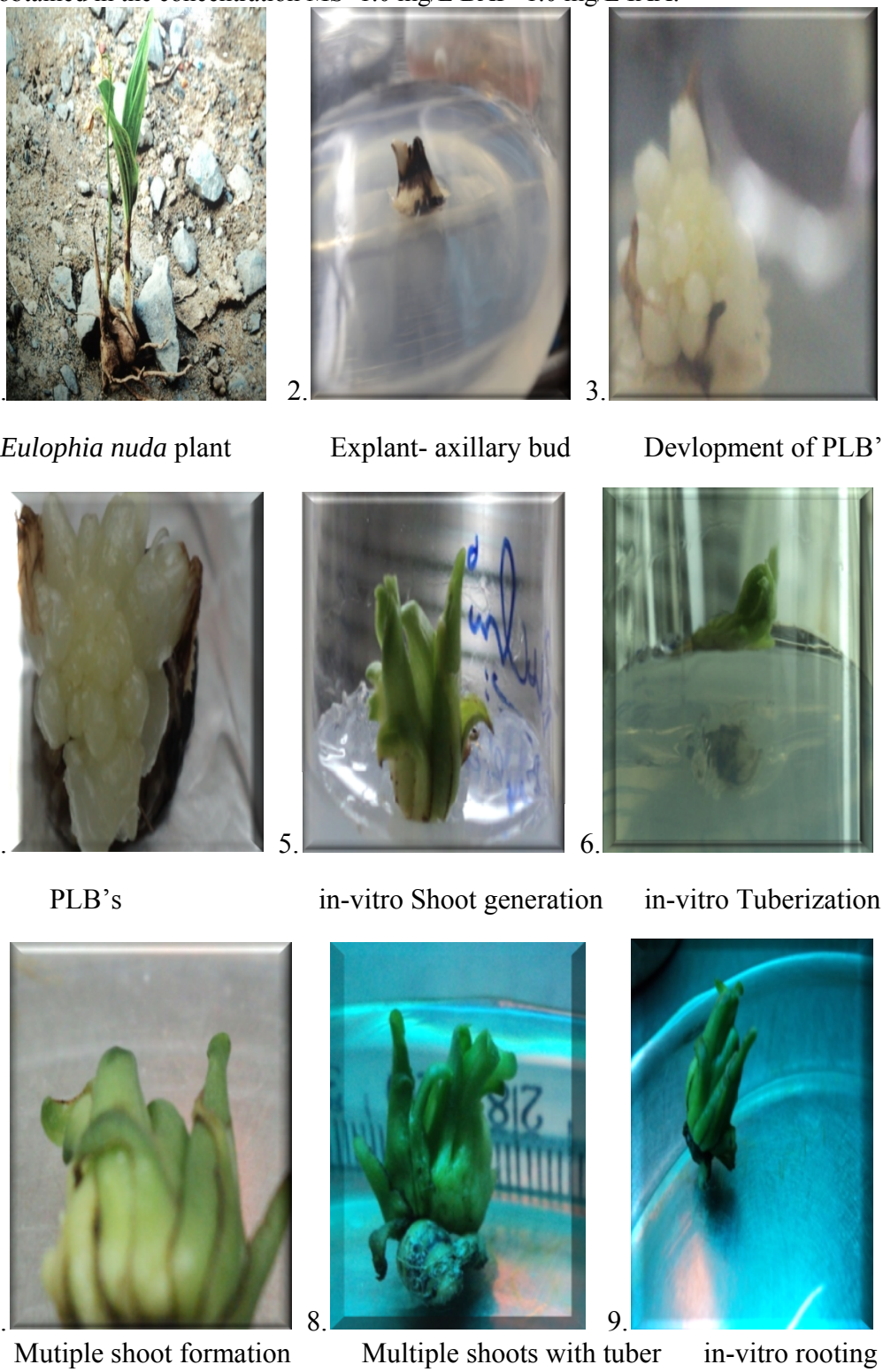

Fig: 3 PLB'S Of *Eulophia nuda* a.Front view b.&c Dorsal view d.Shoot Initiation From PLB'S After 60 days of Culture MS Media fortified with .5MG/LBAP+1mg/L IAA. multiple leaved shoot s and roots at 90 days of culture (Bar 1.5 cm).

CONCLUSION

In vitro multiplication of orchids makes an effective contribution to saving rare species from extinction. The present report gives an efficient protocol for in vitro propagation of the threatened medicinally useful terrestrial orchid *Eulophia nuda lind*. The method uses nodal explants with an auxiliary bud cultured in vitro on MS semisolid medium supplemented with the various concentration of cytokinin BAP and the auxin 2,4-D, each at 15 mM. Regeneration of viable rooted shoots is mediated by combination of direct shoot bud formation and indirectly via PLBs. The shoot bud formation and indirectly via PLBs.

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