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# REGENERATION COMPETENCE OF *DENDROBIUM NOBILE* LINDL. THROUGH PSEUDOBULB SEGMENTS: A STUDY *IN VITRO*

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**Abstract**

Regeneration competence of pseudobulb segments of *Dendrobium nobile* Lindl., procured from *in vitro* grown cultures, was assessed on Mitra *et al*. (1976, M) medium with and without growth additives [yeast extract (2 $gl^{-1}$); auxin (IAA, 1-2 $mgl^{-1}$; NAA, 1-2 $mgl^{-1}$); and cytokinin (KN, 1-2 $mgl^{-1}$; BAP, 1-5 $mgl^{-1}$)], in various combinations. In basal medium, 25% basal explants showed a bud break within 24.5 ± 2.02 days, turned brown within 55 days and subsequently perished. Incorporation of YE in the medium also induced regeneration response in 25% basal explants via shoot bud formation within 22 ± 1.78 days and shoot buds developed into healthy shoots within 190 ± 1.78 days. Early plantlets were formed in IAA (1 $mgl^{-1}$) containing combination. Plantlets were transferred to clay pots containing potting mixture (brick-bats, charcoal, moss) in ration of 1:1:1. Nearly 60-65% of plantlets survival was recorded.

## Introduction

*DENDROBIUM NOBILE*, an epiphytic species with large and showy flowers is widely distributed within an altitudinal range of 1000-1700 m in Assam, Khasia hills, Manipur, Sikkim, Bhutan, Nepal, Thailand, and China. The plants bear erect stem like pseudobulbs, and coriaceous, oblong to ovate leaves, beautiful, fragrant, waxy, and long lasting white flowers (1-3) tinted with amethyst having funnel shaped lips borne on short racemes arising from the upper nodes of old leafless pseudobulbs. The species is floriculturally important due to its magnificent, fragrant, and long-lasting flowers. It is also used as a tonic, stimulant, and a soothing agent for burns (Arditti, 1992) and to cure malignant and digestive ailments; its root preparation is used to treat general debility. Reckless collections for commercial purposes and habitat destruction have, however, detrimentally jeopardized the size and frequency of its natural populations. Earlier, though the regeneration potential of various explants (seeds, stem, root *etc.*) have been tested *in vitro* in different species (Arora *et al*., 2014, 2016; Bhattacharjee *et al*., 2015; Borah *et al*., 2015; Chauhan *et al*., 2015; Hoque *et al*., 2016; Kaur and Pathak, 2014; Pathak *et al*., 2001, 2016; Sibin and Gangaprasad, 2016; Sibin *et al*., 2014; Verma *et al*., 2013; Vij *et al*., 1989, 1994) so as to develop protocols for their *in vitro* propagation, the data is meager in terms size of the orchid family. Therefore, efforts were made to develop an efficient *in vitro* propagation system for the present species, using pseudobulbs, as explants.

## Materials and Methods

### *Culture Media and Incubation Conditions*

The regenerative competence of pseudobulbs (0.5-0.8 cm long) procured from 48 wk old *in vitro* raised seedlings were assessed on Mitra *et al.* (1976, M ) medium and its different combinations of growth additives [YE (2 $gl^{-1}$)]; auxin [IAA (1-2 $mgl^{-1}$), NAA (1-2 $mgl^{-1}$)]; and cytokinin [KN (1-2 $mgl^{-1}$), BAP (1-2 $mgl^{-1}$)]. The cultures were maintained at 25±2 $^0$C temperature and exposed to 12 hr, illumination of 3500 lux intensity. These were sub-cultured at regular intervals.

### *Acclimatization*

Healthy plantlets with 2-3 well grown leaves and 1-2 roots were gradually hardened *in vitro*, by sequential elimination of growth additives, vitamins, sucrose and minor salts from the nutrient matrix at 15 days interval. The hardened seedlings were washed thoroughly with lukewarm water to remove agar and potted in clay pots, using charcoal, moss, brick-bats (1:1:1) as the potting media.

### *Statistical Analysis*

One way analysis of variance was performed with respect to each response (average ± standard error against each additive is mentioned in Table 1). As ANOVA results showed the non significant difference of additives at 5% level of significance, various groups of additives showing identical/similar response were formed statistically. To this end, Tukey Test was performed at 5% level with respect to each response.

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Table 1. Regeneration potential of *in vitro* sourced *Dendrobium nobile* pseudobulb segments on Mitra *et al.* (1976, M) medium.

| Additives | Explants responded (%) | | Time taken in days for initiation of response | Number of meristematic loci in invoked /explant | Number of plantlets obtained /explant | Regeneration pathway | Time taken in days for | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | Apical | Basal | | | | | First leaf primordium | First root primordium | Complete plantlets | |
| - | - | 25 | 24.5 ± 2.02[a] | 1.3 ± 0.25[a] | - | Sb | - | - | - | - |
| YE | - | 25 | 22 ± 1.78[b] | 1.3 ± 0.25[a] | 1.3 ± 0.25[a] | Sb | 48.5 ± 1.71[a] | 78 ± 2.48[a] | 190 ± 1.78[a] | Healthy plantlets |
| YE+$IAA_1$ | - | 50 | 20 ± 1.78[b] | 1.5 ± 0.29[a] | 1.5 ± 0.29[a] | Sb | 45 ± 1.08[b] | 77.5 ± 1.85[a] | 199 ± 1.29[b] | Healthy plantlets |
| YE+$IAA_2$ | - | 25 | 24 ± 2.12[a] | 1 ± 0.00[a] | 1.8 ± 0.48[a] | Sb | 54 ± 1.78[c] | 92 ± 1.78[b] | 198 ± 2.16[b] | Stunted growth |
| YE+$NAA_1$ | 50 | 50 | 19.3 ± 1.55[b] | 1.5 ± 0.29[a] | 4.5 ± 0.65[b] | Sb | 36.3 ± 1.25[d] | 60 ± 1.78[c] | 168 ± 1.71[c] | Healthy plantlets |
| YE+$NAA_2$ | - | - | - | - | - | - | - | - | - | - |
| YE+$KN_1$ | - | - | - | - | - | - | - | - | - | - |
| YE+$KN_2$ | - | - | - | - | - | - | - | - | - | - |
| YE+$BAP_1$ | 75 | - | 17 ± 1.78[c] | 1.3 ± 0.25[a] | 3.3 ± 0.48[b] | Sb | 41 ± 1.78[b] | 72 ± 2.16[d] | 189.3 ± 2.25[a] | Healthy plantlets |
| YE+$BAP_2$ | - | 50 | 13 ± 1.55[d] | 1.5 ± 0.29[a] | 1.5 ± 0.29[a] | Sb | 45 ± 1.47[d] | 76 ± 1.78[a] | 192 ± 1.78[d] | Healthy plantlets |
| YE+$BAP_5$ | - | 75 | 15 ± 2.16[d] | 1.5 ± 0.21[a] | 30 ± 1.08[c] | Sb-PLBs | 31 ± 2.16[e] | 110.5 ± 2.10[e] | 110.5 ± 2.10[e] | PLBs multiplication |

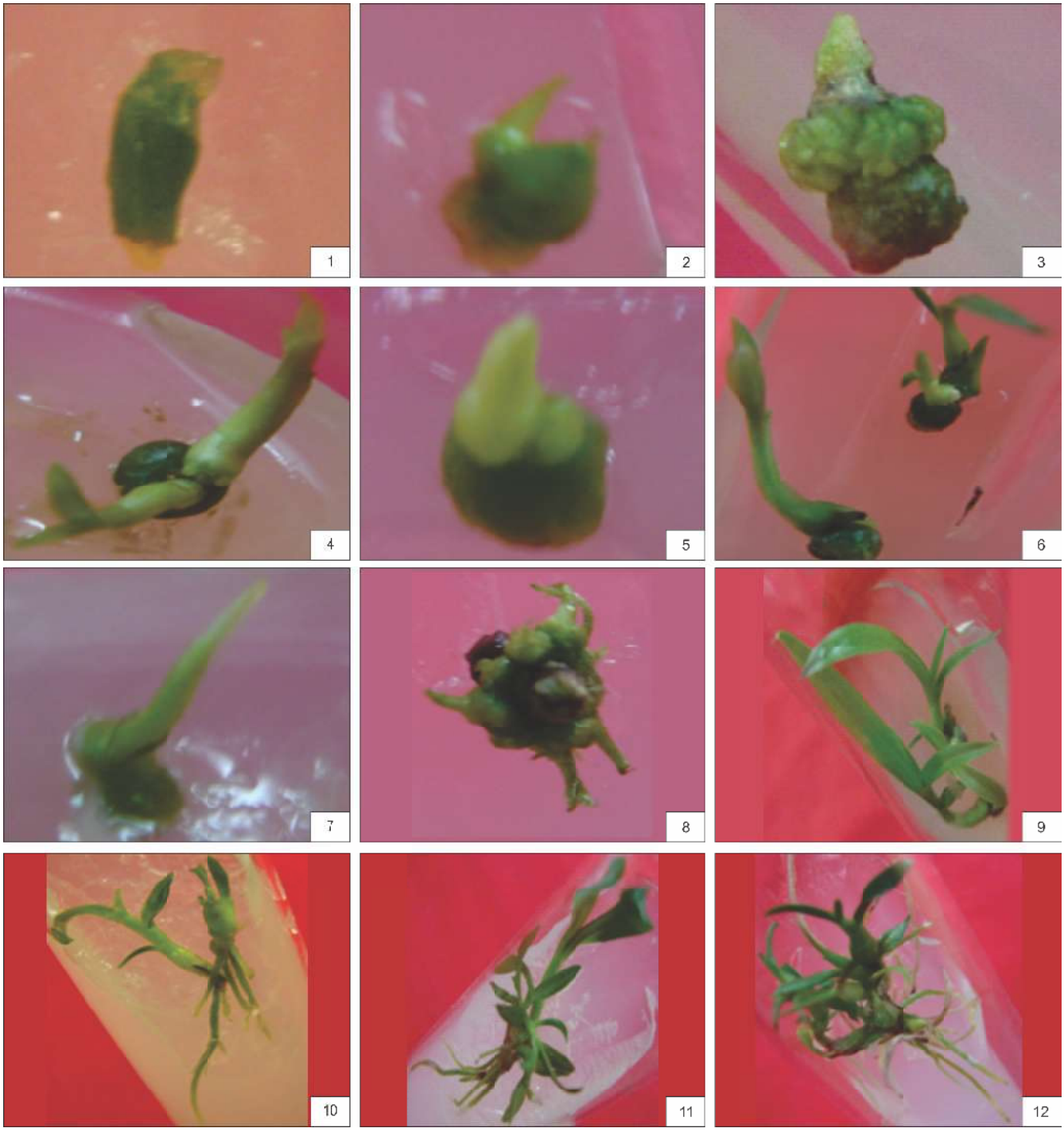
Entries in column no. 4 to 6 and 8 to 10 are Mean ± S.E.; same alphabetical letter in the supercript denotes that the corresponding means in the same group using Tukey test at 5%.

## Results and Discussion

Pseudobulbs (0.5-0.8 cm long) procured from 48 wk old *in vitro* raised seedlings were sliced into apical and basal segments and their regenerative competence was successfully assessed on M medium, with and without growth additives; it was markedly influenced by position and the growth stimulus in the nutrient pool (Figs. 1-12). The explants invariably regenerated by bud break; however, PLBs were also observed in selective combination in the nutrient mix (Table 1). Pseudobulb explants have positively responded to regeneration, earlier in *Bletilla* (Vij and Dhiman, 1997), *Bulbophyllum* (Vij *et al*., 2000), *Cattleya* (Vajrabhaya, 1978), *Changnienia amoena* (Jiang *et al*., 2011), *Coelogyne stricata* (Basker and Narmatha Bai, 2006), *Coelogyne flaccida* (Kaur and Bhutani, 2013), *Cymbidium finlaysonianum* (Islam *et al*., 2015), *Dendrobium* (Vij and Pathak, 1989; Sembi and Vij, 2001; Sunitibala and Kishor, 2009), *Eulophia epidendraea* (Maridass *et al*., 2012), and *Malaxis* (Deb and Arenmongla, 2014; Vij and Kaur, 1998).

In the basal medium, 25% basal explants showed a bud break within 24.5 ± 2.02 days. The shoot buds however turned brown within 55 days and subsequently perished. Additional use of YE and / or growth regulators induced regeneration depending upon their quality and quantity of growth additives in the medium. Incorporation of YE in the medium also induced regeneration response in 25% basal explants via shoot bud formation within 22 ± 1.78 days. The shoot buds developed into healthy shoots within 190 ± 1.78 days.

When IAA (1 $mgl^{-1}$) was supplemented in combinations containing YE, 50% basal explants regenerated via shoot bud formation within 20 ± 1.78 days. Additional meristematic loci were also activated and healthy plantlets complete with leaves and roots were obtained within 199 ± 1.29 days. The regeneration response was reduced to 25% when the concentration of the above growth regulator was enhanced (2 $mgl^{-1}$). Shoot buds developed in 24 ± 2.12 days showed multiplication at its base and plantlets were obtained within 198 ± 2.16 days. The plantlets, however, showed stunted growth in this combination. When NAA was used at concentration (1 $mgl^{-1}$), both (50% each) apical and basal explants responded via shoot bud formation within 19.3 ± 1.55 days. The regenerated shoots multiplied at the base and an average of 4.5 ± 0.65 plantlets per explant were obtained in 168 ± 1.71 days. Its higher concentration, however, failed to show any regeneration in the explants. NAA induced PLB mediated regeneration in *Dendrobium moschatum* (Vij and Sood, 1982). The benign role of auxins has also been reported earlier in *Bletilla* (Vij and Dhiman, 1997), *Bulbophyllum* (Vij *et al*., 2000), and *Spathoglottis* (Bapat and Narayanaswamy, 1977).




Figs. 1-12, Regenerative competence of pseudobulb explants in *Dendrobium nobile*: 1, Explant at the time of inoculation; 2, Bud break (M); 3, Callusing at the base of the regenerated shoot bud [M+YE (2 $gl^{-1}$)+BAP (5 $mgl^{-1}$)]; 4, Shoot bud mediated regeneration (M+YE (2 $gl^{-1}$); 5, Bud break [M+YE (2 $gl^{-1}$)+BAP (1 $mgl^{-1}$)]; 6, Additional meristematic loci activated at the base of the regenerated shoot [M+YE (2 $gl^{-1}$)+NAA (1 $mgl^{-1}$)]; 7, Bud break [M+IAA (1 $mgl^{-1}$)]; 8, PLB mediated regeneration [M+YE (2 $gl^{-1}$)+BAP (5 $mgl^{-1}$)]; 9, Plantlets [M+YE (2 $gl^{-1}$)+NAA (1 $mgl^{-1}$)]; 10-11, Plantlets with healthy roots [M+YE (2 $gl^{-1}$)+IAA (2 $mgl^{-1}$), M+YE (2 $mgl^{-1}$)+BAP (2 $mgl^{-1}$)]; 12, Multiple shoots [M+YE (2 $gl^{-1}$)+BAP (5 $mgl^{-1}$)].

Amongst the cytokinins used, KN at both the concentrations (1,2 $mgl^{-1}$) failed to induce any regeneration response in the explants. BAP was successfully employed to initiate the regeneration response in the explants; the growth regulator (5 $mgl^{-1}$) also induced multiple PLBs. Vij *et al*. (2000) has also reported a multifold increase in the proliferation potential of the shoot buds on addition of BAP. Roy and Banerjee

(2003) reported that the concentration of BAP higher than 2 $mgl^{-1}$ was effective in production of multiple shoots in *Dendrobium oculatum.*

Present results indicate that pseudobulb explants in *Dendrobium nobile* have been successfully used for regeneration purposes and these data indicate that utility of such explants may be explored in many other related orchid taxa for *in vitro* propagation purposes.

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