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Original Article

In Vitro Propagation of *Dendrobium nanum* through rhizome bud culture

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Abstract

A simple and dependable procedure for *in vitro* propagation of an orchid *Dendrobium nanum* Hook. F has been developed. Explants of rhizome buds were introduced on MS basal medium supplemented with using hormones such as NAA, BAP and Kinetin. Maximum percentage of callus induction was obtained from the rhizome bud explants of *D. nanum* cultured on MS basal medium with 2.0μM/l^{-1}NAA and 1.2 μM /l^{-1} kinetin. The maximum effect of shoot induction (15.78 ± 0.37mm) was observed on basal medium supplemented with 0.5μM /l^{-1} BAP. The micropropagated orchid from this study was successfully reintroduced into their natural habitat (85% of survival after 3 months).
***Key words*:** Orchidaceae; *Dendrobium nanum;* rhizome bud; Micropropagation
Abbreviations: NAA – 2-naphthalene acetic acid; MS – Murashige and Skoog medium; Kin – kinetin; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; BAP-benzyl-amino purine

Introduction

Orchidaceae is the most species-rich plant family in the world, with estimate 17500 to 35000 species (Maridass, 2006). Orchids can be found from the tropics to the arctic regions, as epiphytes (typically tropical), terrestrials (typically temperate), and even include subterranean forms (Cribb *et al*., 2003). Orchids are experiencing a steady decline in tropical countries due to the destruction of natural forest areas. A large proportion of orchid species are rare and endangered as a direct or indirect result of human activities, including collection, habitat destruction and degradation, and loss of pollinators and fungal partners (Batty *et al*., 2002; Cribb *et al*., 2003; Zettler *et al*., 2003). The greatest potential for loss of orchid species is in the tropics, where species diversity is the highest, and this is the area in which orchids have been studied least (Batty et al., 2002; Cribb et al., 2003). However, orchids also exhibit considerable diversity and many species are threatened. Although there has been extensive research on these species (Batty et al., 2002; Cribb et al., 2003), much more research work is needed before they can be effectively conserved. It is essential to take measures for the conservation and propagation of these endangered orchid species. Hence, *In vitro* propagation of endangered plants can offer considerable benefits for the rapid cultivation of species that are at risk, that have limited reproductive capacity and exist in threatened habitats (Fay, 1992). *In vitro* propagation methods are essential components of plant genetic resource management and they are becoming increasingly important for conservation of rare and endangered plant species (Sudha *et al*.,1998; Benson *et al*., 2000; Iankova *et al*., 2001; Bhatia *et al*., 2002).

The *Dendrobium* is the second largest genus of Orchidaceae, which is composed of approximately 1500 species scattered in the world, and there is the most popular and highly valued orchids in the market (Chen and Ji, 1998). *In vitro* propagation of *Dendrobium* sp has been investigated with variable results depending on the tissue culture techniques, the species or cultivar and explants as a source. In micropropagation, various parts of the plant such as shoot tip, flower stalk, root, and leaf have been utilized as a explants (Yasugi and Shinto, 1994). A tissue culture procedure for clonal propagation of *Dendrobium sp*. was developed by Morel in 1960. There is no information about regarding micropropagation of wild Dendrobium

sp. Moreover, for mass propagation of Orchid, protocorm propagation from seed and/or nodal explants of adult plants was an effective method, and it was superior to shoot tip on account of an exponential propagation rate (Wang *et al*., 1995). The present study has resulted in high-frequency of shoot multiplication and rooting, starting from explants obtained from wild population of *D. nanum* in the South India and the micropropaged explants were successfully reintroducted into their natural habitat.

Materials and Methods

Collection of Explants

D. nanum was collected in KMTR region, South India and was used as source of explants. Which was maintained under standard green house conditions.

Sterilization

The young rhizomes were disinfected with 70% ethanol for 30 sec followed by surface sterilization with 3% sodium hypochlorite (supplemented 2–3 drops of Tween 80 each 500 ml) for 20 min and then washed for 4–5 times in sterile distilled water. The rhizome was cut into small pieces (5mm), and the pieces were washed with sterile water.

Culture

The explants were placed onto cultured on the basal media of MS medium, (Murashige and Skoog,1962), supplemented with various concentrations of auxins, NAA (naphthalene acetic acid), IAA (indole acetic acid), IBA (indole butyric acid) and cytokinins BAP (benzyl-amino purine) and kinetin. The auxins concentrations were 0.5, 1.0,2.0,5.0,10.0 and 2.0μM/L, while the cytokinins were 0.5, 1.0,2.0,5.0,10.0 and 2.0μM/L. The pH of all media were adjusted to 5.8 with 0.1*M* KOH before sterilization. All media with 0.7% agar and 2% sucrose were autoclaved at 121°C for 20 min. The explants were cultured in 220 ml glass jars containing 30ml medium, which were closed with semipermeable plastic caps. All the cultures were kept in the culture room at 26 °C and 10/14h photoperiod under 1000-2000 lux light intensity.

Regenerating shoots of 2–2.5cm in height were separated from calli and cultured onto solid rooting medium for vigorous growth and rooting. After 30 days of culture, these were well rooted and shoots were transferred to pots and shifted green house of 25°C with low natural light, and with 95% relative humidity. These plantlets were acclimatized in outside conditions for 15days. The rooted plantlets (3-5cm in height) were washed with water to remove residual media, and dipped into 50% 800× carbendazim for 15min, then they were directly transplanted to substrate viz. vermiculite, sawdust and humus: sawdust (1:1) under high relative humidity. Survival rate was recorded after 30 days of transplantation.

Statistical analysis

Experiments design was thoroughly randomized and they were repeated three times. Every treatment had three replications. Observations on the number and height of shoots were recorded after 30 days of culture. All the growth of roots and shoots length values are statistically (mean ± error) calculated by SPSS-11.0 version.

Results and Discussion

The basal media with the supplementation of NAA or Kinetin was effective in initiating callus on *D. nanum* rhizome bud (Table-1). Optimum concentrations were 2.0μM/l^{-1}NAA and 1.2 μM /l^{-1} kinetin for callus formation, while percentage of callus induction was decreased at 2.5μM /l^{-1}NAA and 1.5mg/l^{-1} kinetin (Table-1).

As shown in Table 1, After 3 to 4 weeks, rhizome was induced to callus on each explant. The green and healthy rhizome buds developed into shoots of 5.30-15.78mm length after 12 weeks of culture. These cultures were statistically significant in differences in average number of shoots and shoot regeneration frequency with the length of explants represented in Table-1. The maximum regeneration % frequency of shoot induction was observed on the rhizome with 15.78 ± 0.37mm length. The maximum shoot induction (15.78 ± 0.37mm) was observed on basal medium supplemented with BAP (0.5μM/l^{-1}). The induced shoots developed very well after 12week. At this stage, they were transferred to the rooting medium. Two to three healthy roots developed from shoots after 4 weeks of subculture on MS basal medium (Fig.1). Of the MS medium supplemented with three auxin such as IAA, IBA and NAA tested for inducing

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roots, Ms medium combination with IAA and IBA did not show root initiation. At 4-day intervals half-strength MS liquid medium was added to the pots with plantlets. The plants were transferred to the greenhouse after 4 weeks, where they were acclimatized

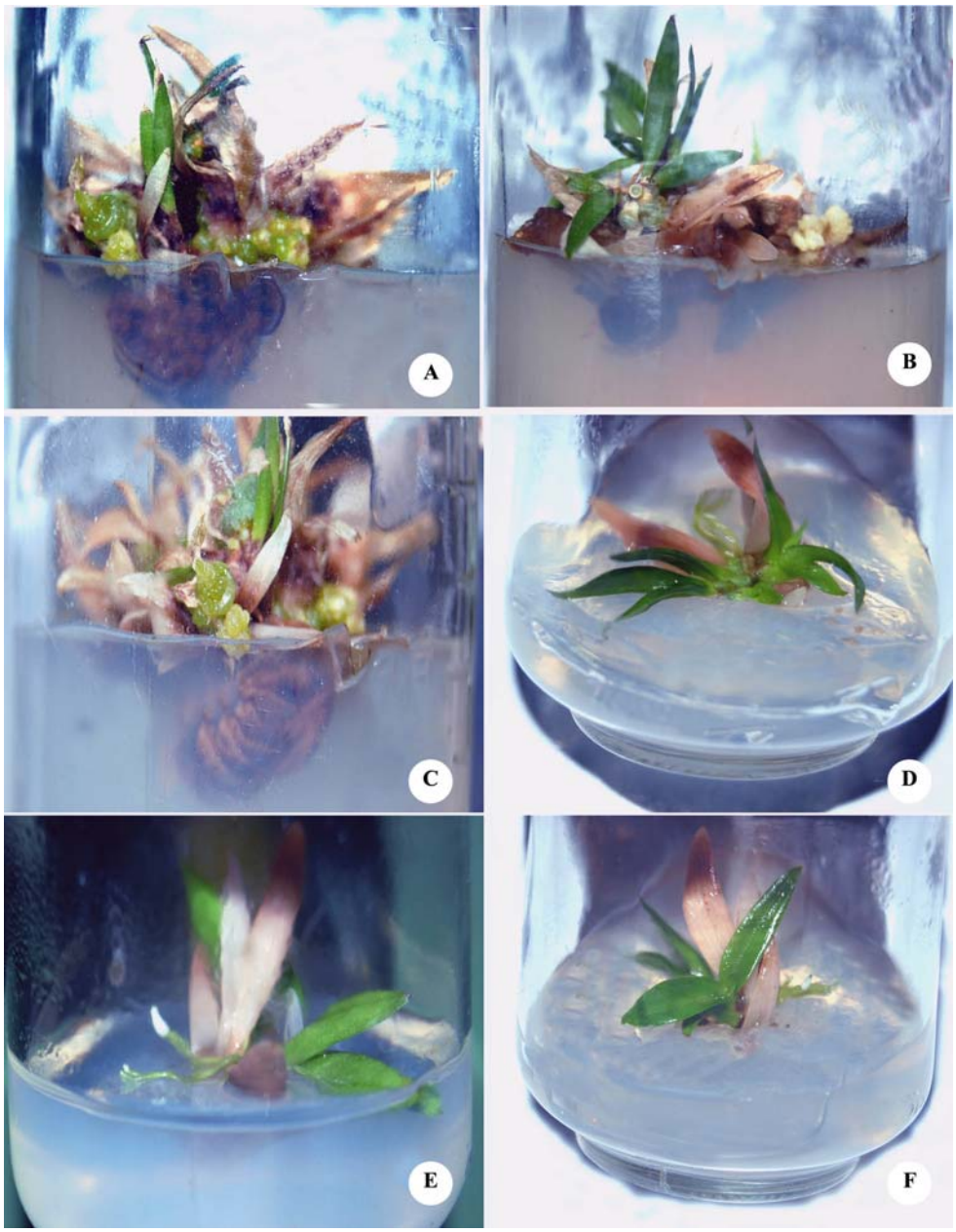

Fig. 1: A-C Callus responses shoot formation; D-F shoots formation on MS medium

Table-1: Callus formation on basal medium supplemented with NAA and Kinetin concentrations of *Dendrobium nanum* Hook f

MS medium combination with		No. of explant inoculated	No. of explants proliferated in to callus	% of Callus response
NAA(μM)	Kinetin(μM)			
0.9	0.3	15	3	20.00
1.0	0.6	15	5	33.33
1.5	0.9	15	9	60.00
2.0	1.2	15	15	100.00
2.5	1.5	15	7	46.66

Table – 2: Effect of MS medium with different concentrations of NAA and BAP on rhizome section culture of *Dendrobium nanum* Hook f.

Medium	No. of explants inoculated	No. of explants culture responses	% of cultures responses	No. of shoots/ explant	Shoot length (mm)
MS + NAA-concentrations					
MS + 0.5 μM	15	3	20.00	–	–
MS + 1.0 μM	15	8	53.33	–	–
MS + 2.0 μM	15	11	73.33	–	–
MS + 5.0 μM	15	12	80.00	–	–
MS + 10.0 μM	15	7	46.66	–	–
MS + 20.0 μM	15	9	60.00	–	–
MS + BAP-concentrations					
MS + 0.5 μM	15	2	13.33	2.50 ± 0.05	5.30 ± 0.75
MS + 1.0 μM	15	4	26.66	4.00 ± 0.57	7.75 ± 1.55
MS + 2.0 μM	15	14	93.33	4.75 ± 0.47	8.05 ± 1.67
MS + 5.0 μM	15	15	100.00	5.00 ± 0.91	15.78 ± 0.37
MS + 10.0μM	15	12	80.00	4.00 ± 0.54	7.30 ± 1.24
MS + 20.0 μM	15	11	73.33	4.40 ± 0.51	7.97 ± 1.23

Values are means ± standard error

Discussion

The results presented in this work shows that rhizome buds of *Dendrobium nanum* was growing in the field of cultured *in vitro* in a MS basal medium supplemented with BAP - enriched media to induce shoot and root formation (Fig-1). In previous reports different basal media supplemented with natural organic compounds have been used to enhance *in vitro* shoot and root development of several orchid species *Cymbidium kanran* (Paek *et al*.,1990; Chung *et al*., 1985), *Cymbidium faberi* (Hasegawa *et al*., 1985), *Cymbidium naveomagenatam, C.goeringii* (Paek and Kazoi,1998) and *Cymbidium forrestii* (Paek and Yeung,1991), *Dendrobium candium,D. loddigesi, D. waggi* and *D. moniliforme* (*Zeng et al*.,1998; *Shiau et al*.,2005. The conclusion of the present study, we have developed a simple and efficient protocol for rapid micropropagation to get a large number of plantlets from rhizome bud cultures of *Dendrobium nanum* could be used for large-scale propagation and *ex situ* conservation of this orchid species.

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