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IN-VITRO CONSERVATION OF SOME COMMERCIALLY IMPORTANT ORCHIDS OF NORTH-EAST INDIA

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Abstract

Using different explants and media composition, the formation of shoot buds and protocorm-like bodies (plbs) were accomplished in *Dendrobium fimbriatum* var. *oculatum*, *Dendrobium wardianum* and *Cymbidium giganteum*. The micropropagated plantlets after hardening in suitable substrata were established. Storage potentialialities of non-encapsulated and encapsulated plbs were studied using various approaches like low temperature, growth retardants, osmotic inhibitors and dehydration treatment. The details of the procedures are described.

Key Words: *Dendrobium Fimbriatum* var. *Oculatum*, *Dendrobium Wardianum*, *Cymbidium Giganteum*, Micropropagation, Protocorm-like Bodies, Establishment, Storage

Abbreviatiations: IAA, indole-3-acetic acid; NAA, ∝-naphthaleneacetic acid; BAP, 6-benzylaminopurine; KN, kinetin; plbs, protocorm-like-bodies; ABA, Abscissic acid; MS, Murashige and Skoog medium

INTRODUCTION

The propagation of orchids through micropropagation has been a common practice ever since Morel (1960, 1964) described the technique for meristem culture for producing virus-free cymbidiums. Because the genotypes of many choice cultivars are highly heterozygous and the traditional methods for propagating orchids by division of off-shoot is slow, the meristem culture technique has been useful in mass propagating a large number of orchid species. Since shoot meristem endangers the mother plant, organ culture is fast emerging as a promising avenue for mass micropropagation of orchids. Reports on the use of leaves (Tanaka, 1987; Vij and Pathak, 1990), axillary buds (Wang, 1988, George and Ravishanker, 1997; Laishram and Devi, 1999), inflorescences (Teng *et al.*, 1997, Payati and Murty, 1999; Sinha and Hedge, 1999), floral stalks (Momose and Yoneda, 1988; Arditti and Ernst, 1993) and roots (Phillip and Nainar, 1988; Vij *et al.*, 1989; Holters and Zimmer, 1990; Chang and Chang, 1998; Zhou, 1998) as explants have appeared.

Different tissue culture techniques have been developed to promote the multiplication of selected clones in orchids (Arditti and Ernst, 1993). But these techniques differ from species to species. The explants used for the tissue culture respond selectively to the nutrient medium depending on their source, physiological state and nutrient environment.

In–vitro conservation refers to maintenance of germplasm in a relatively stable form under more or less defined nutrient conditions in an artificial environment (Withers, 1987). The major a[illegible] in developing *in vitro*

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storage methods is to reduce the frequent demands of subculturing and preserving the unique genetic constitution of the germplasm.

The following paper deals with the micropropagation and conservation of *Dendrobium fimbriatum* var. *oculatum*, *Dendrobium wardianum* and *Cymbidium giganteum*, three commercially important orchids of North-East India.

MATERIALS AND METHODS

Different explant sources namely, offshoots, leaves, young roots, axillary buds and apical meristems were collected from the plants maintained in the Botanical Garden of North-Eastern Hill University, Shillong. About 1-2 cm segments of the explants were cut and cleaned gently scrubbing with a soft brush and mild detergent (0.05%). Explants were then washed in running tap water for about 15-20 min and rinsed with distilled water. The surface sterilization of the explants was done by sodium hypochlorite solution (0.8-1.0% available chlorine) for 15-20 min. Explants were thoroughly rinsed with sterilized distilled water to remove the sterilant. Shoot tips were then stripped off the leaves and the meristems (1-2 mm) excised under aseptic conditions. Leaf bases, leaf tips, root tips and rhizome sections measuring about 2 mm were cut from the larger sections of the explants. The nodal sections containing the axillary buds were also cut into 3-4 mm size after the removal of the leaves, dry sheaths and other external tissues. Five explants/flask were placed in the basal medium with various supplementation of IAA, IBA, NAA, 2,4-D, BAP and KN in a range of 0.0-10.0 µM. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. The cultures were incubated at 25 ± 2 °C under a photoperiod of 14 hr duration using cool-flourescent light of 150 µmoles m^{-2} sec^{-1}. Three replicates were taken for each explant and the experiments were repeated twice.

For storage at low temperature, Plbs obtained were cultured at –10, 0 and 4 °C (in the dark). Cultures stored at low temperatures were transferred to room temperature (24±2 °C) every 7 days for 3 weeks and the data was collected on the plb survival, growth and development. For encapsulation, artificial seeds were produced using sodium alginate (2 - 5%) and calcium chloride (50-150 mM) solutions in growth regulator free-MS liquid medium. Both uncapsulated and encapsulated plbs were also plated out in MS medium after desiccation, hourly, to determine their survival rate. To study the potentialities for slow growth storage, the plbs were inoculated in MS medium supplemented with mannitol (0-15%), sucrose (2-8%) and abscissic acid (0 – 5 mg/l). Data were collected every 30 days for 3 months.

Around 6-8 months old plantlets of about 6-8 cm in height were used for the transfer to the pots. These plantlets were taken out gently from the flask with the help of the forceps and separated from each other. The adhering agar was gently removed. The plantlets were then rinsed in sterile water to remove the last bit of adhering agar, blot dried and transferred to pots containing various potting mixtures for hardening and establishment.

RESULTS AND DISCUSSION

The morphogenetic responses of various explants to different growth regulator treatments varied in the orchid species studied (Fig 1). The apical meristems and axillary buds were found to be suitable for micropropagation of the dendrobes. The apical meristem enlarged and produced the protocorm mass in about 3-4 weeks in the medium (devised by Kumaria and Tandon, 1994) supplemented with IAA and BAP, both separately and in combination. The maximum number of plbs was formed on apical meristem in the medium

supplemented with 10.0 μM IAA separately and 10.0 μM IAA in combination with 0.5 μM BAP. A balanced supply of auxins and cytokinins (2.5 μM each) was found to be effective. The axillary buds responded better to the medium containing NAA. Plb-formation from axillary buds occurred in the medium containing 5.0 μM NAA. Formation of plbs as well as shoot buds was stimulated in the medium supplemented with 10.0 μM NAA and 0.5 μM BAP. The plbs were multiplied by further cutting and subculturing in fresh medium. The use of apical meristems and axillary buds for clonal propagation of orchid species has been successfully accomplished (Holters and Zimmer, 1990; Arditti and Ernst, 1993). The other explants of *D. fimbriatum var. oculatum* failed to respond in culture. The use of growth regulators was found to be beneficial and resulted in different morphogenetic responses. In case of *D. wardianum*, the nodal buds started developing in about 3 weeks of culture in MS medium containing BAP alone or BAP with IAA/NAA. Buds cultured in the medium containing 10.0 μM BAP alone developed multiple shoots (7-10 shoots/culture) in 50% cultures in 8 weeks time. With the increase in the concentration, however, a decline in the response was observed. Multiple shoots were also observed in buds cultured in the medium containing combinations of IAA+BAP and NAA+BAP. However, the number of shoots developed and the percentage response were less compared to the response obtained by using BAP alone. Leaf bases containing NAA (2.5-5.0 μM) + BAP (5.0 –10.0 μM), showed no response in the form of plb initiation after 6 weeks of culture. The optimum plb formation resulted in the medium containing 2.5 μM NAA and 10.0 μM BAP. Other growth regulators tried failed to induce plb-formation or multiple shoot development. Restriction of morphogenetic potential to the leaf bases only from among the leaf tissues in *D. wardianum* is in accord with the earlier suggestion of Zimmer and Peiper (1975) that the leaf base is, in general meristematic in monocots and upon isolation and culture it differentiates into plants. Also, Abdul Karim and Hairani (1990) have reported a greater proliferative potential of leaf base than the leaf tip in the dendrobes. Shoot tips, root tips and rhizome segments were found suitable explants for mass propagation of the cymbidium. The shoot tips enlarged and assumed a ball shaped structure resembling protocorm in MS medium containing 1.0 μM NAA and 2.5 μM BAP. A period of 8-12 weeks was required to develop into a spherical plb. At times the plb showed proliferation into many plbs. Root tips exhibited various responses depending upon the size and the growth regulators used in the medium, the best being in IAA and KN combination of 10.0 and 2.5 μM each, when the root tips showed enlargement at the tip and finally differentiated into 3-4 plbs after 4-6 weeks. Rhizome sections of size 2-3 mm showed elongation producing plbs at regular intervals. The use of shoot tips, root tips and rhizome segments as explants for successful propagation of orchids has been reported in certain cases (Vij *et al.*, 1989; Latha and Seeni, 1991; Chang and Chang, 1998; Zhou, 1998). In IAA and KN combination of 10.0 μM and 2.5μM respectively, the rhizome pieces showed light greenish callusing at the cut ends with a few plbs arising out of the callus. In 1.0 μM each of NAA and BAP in the medium, the rhizomes elongated producing roots and shoots at regular intervals and about 25% of the rhizomes produced clusters of plbs at the axil of the root and the rhizome. In NAA and BAP combination of 2.5 μM each of the growth regulators, the rhizome segments branched out like ginger, producing plbs at the tips. On leaving the plbs undisturbed, the formation of complete plantlets took

place in about 4 weeks. The leaf tips and leaf bases dried in course of time. Complete plantlets with well- developed green, healthy roots were formed within 12-16 weeks. Plbs of *D. wardianum* were successfully encapsulated in calcium alginate beads to synthetic seeds which were then stored and the conversion frequencies studied (Sharma and Tandon, 1992). Optimum storage of the plbs for 90 days in 44% cultures was achieved using 10% mannitol. ABA at 2 mg/l concentration could suppress the growth in 35% cases. These plbs, when transferred to re–growth medium, showed 100% conversion to plants. Poor performance was observed when plbs were subjected to low temperature storage in the dark. 30% plbs could survive a temperature of 4° C for a week. All the 'synthetic seeds' developed into plantlets *in–vitro*. Those stored at 4° C could germinate even after 180 days, though poorly. High germination percentage in case of encapsulated plbs than non-encapsulated plbs could be due to the matrix that not only facilitates regular nutrient supply but also protects the dispersed, delicate tissues from mechanical injury during handling and from desiccation. Reduced growth rate, delayed proliferation and growth of the plbs of *Cymbidium giganteum* resulted in the medium supplemented with sucrose (8%) and ABA (1 mg/l) both individually and in combination (Corrie and Tandon, 1993). Reduction in the number of plb by ABA treatment is in agreement with the work of Fujimura and Komamine (1975), Kamada and Harada (1981). Three hours of desiccation was found to be optimum for plb survival.

The clonally propagated plantlets were transferred to clear clay pots of 10 cm diam containing different mixtures of compost. For the dendrobes the compost consisted of charcoal, brick pieces, coconut husk, vermiculite and moss in varying ratios. The compost comprising charcoal chunks, brick pieces, vermiculite (1:1:1/2) and a layer of moss was found to be the most suitable for the healthy growth of the plantlets. The cymbidium being terrestrial orchid grew well on the compost containing soil, sand, cowdung (1:1:1), brick pieces, charcoal, decayed leaves, wood pieces and bark. The potted plants were hardened in the glasshouse. The minimum and maximum temperatures of the glasshouse at the time of hardening were 16° C and 22° C respectively, and the relative humidity was 70-80%. The plants were fed with 1/10 of the nutrient solution. Feeding the plantlets with nutrient salt solutions has been reported to be beneficial for the promotion of orchid plant growth (Mukherjee, 1983). Plants were hardened and established in about 6-7 weeks. This paper gives the complete protocol for the mass multiplication and conservation of *Dendrobium fimbriatum* var. *oculatum* Hk. f., *Dendrobium wardianum* Warner and *Cymbidium giganteum* Wall., three commercially important orchids of North-East India.

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