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Micropropagation of Orchid *Dendrobium barbatum* Lindl. (Orchidaceae) from Fleshy Stem Segments

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An efficient protocol for micropropagation of orchid *Dendrobium barbatum* Lindl. (Orchidaceae) using the fleshy stem segments was developed. Murashige Skoog (MS) basal medium *supplemented* with 1.0 mg L of Naphthalene Acetic Acid (NAA) + 2 .0mg L of Benzyl Amino Purine (BAP) for multiple shoot induction. The multiplied shoots were then transferred to half-strength MS medium supplemented with 1 mg L of Indole-3 acetic acid (IAA) or Indole-3 butyric acid (IBA) or NAA. Best response for shoot multiplication was achieved when the medium was supplemented with 1 mg L^{-1} NAA and 2mg L^{-1} BAP. While that for rooting with 1 mg l IBA. The plantlets were hardened off and survived plants were released to the shade house then to their epiphytic environment.

Key words: Benzyl Amino Purine (BAP),*Dendrobium barbatum* fleshy stem segment, *In vitro*.

The genus *Dendrobium* (Family: Orchidaceae) exhibits a vast diversity in vegetative and floral characteristics and is of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity (Jones *et al* .1998). *D. barbatum* Lindl. is a mildly fragrant, sympodial epiphytic orchid which has elongated stem, pluri-nodul, leaves lanceolate arising from the top of pseudo bulb, having a geographical distribution ranging from Western Ghat of South India, North east India, Nepal and Myanmar to a few countries of South – east Asia. In these regions, *D. barbatum* ranks among the important ornamental orchid. It has graceful, pendulous racemes of medium sized flowers, usually of pristine white, except that of a pink blotch at the tip of sepals, petals and the throat of the labellum. The plant is generally propagated through Pseudobulb, but the rate of multiplication is slow, normally giving rise to 2-4 plants per year. The orchid resources of the world in general, North east region of India and in Western Ghat in particular is depleting day by day due to habitat loss. The collection of wild *Dendrobium* countries at levels ranging from hobbyist to large scale illegal trade. Endemic orchids of the North east India and Western Ghat are facing the grim possibility of extinction under intense biotic pressures. Hence, conservation and sustainable utilization assume greater importance to save the dwindling orchids (Kishor *et al* . 2006).

The available literature reveals that micropropagation has been achieved using immature or mature embryos, protocorms and shoot tip explants in *Dendrobium* barbatum Zhang *et al*. 1993, Liu *et al* 1988, Shiau *et al* .2005). *In vitro* generation of *D. barbatum* seeds has been reported by Alam *et al* (2002). However, there is no report on Micro propagation of *D. barbatum* using fleshy stem as explants sources. Hence the present study was aimed at *in vitro* Micropropagation and conservation approach of this Orchid plant.

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MATERIALS AND METHODS

Plant Material

Plants of *D. barbatum* were collected from their natural habitat in Pallini Hills Western Ghats,Tamil Nadu, India (Fig-1a) and grown in shade net house environment at the Herbal garden, Department of Botany, National College, Tieuchirappalli, Tamil nadu. The nodal portions of succulent stem were tested for multiple shoot induction. The young succulent stems were excised from one year old explant and kept under tap water with Tween20 (4 -5drops) for 20 min. Nodal segments (1-2 cm long) was cut from these stems and was treated with fungicide Bavistin (0.05-0.1 %w/v) and antibiotic Streptomycin (0.5-1.0 %w/v) for 10 min each. Every time the nodal segments were rinsed thrice in distilled water and then they were surface sterilized with aqueous solution of $HgCl_2$ (0.1% w/v) for 2-3 min and washed thrice with sterilized distilled water. The nodal segments were cut gently with sterilized blade and used as explants

Multiple shoot regeneration from fleshy stem segment cultures

The experiment was performed with 1.0-2.0 cm excised nodal segment explants. Each segment was cultured in test tubes each bottle, containing 15 ml MS medium supplemented with different concentrations of Benzyl Amino Purine (BAP) (0.5,1.0, 2.0 and 4.0 mg L) individually, and in combination with Naphthalene Acetic Acid (NAA) at 1 mg L . The medium was solidified with 4 g L. Difco Bacto agar (Himedia, India) at pH 5.8. The cultures were incubated for 60 days at 25±2°C under cool white fluorescent light with 16 h photoperiod. The multiple shoot regeneration was achieved by sub cultured on MS medium for about 2-3 times to get desired size.

Rooting of Regenerated multiple shoots

For the root induction experiments, the multiple shoots obtained from the above experiment were used. Small clumps of shoots were cultured in glass test tubes 100 ml each containing 15 ml MS basal medium supplemented with 1.0 mg L Indole-3 acetic acid (IAA) or Indole -3 butyric acid (IBA) or NAA. The cultures were incubated for 30 days under the conditions as described for the previous experiment. For all the media mentioned above, the pH was adjusted to 5.8 before autoclaving at 121ÚC, 15 Ib for 15 min. The culture bottles were capped with two layered aluminium foil before autoclaving and scaled with Parafilm M^R (Himedia,India) after inoculation. The best result was achieved at 1.0 mg L IBA for rooting.

RESULTS AND DISCUSSION

The efficacy of multiple shoot formation differed with different concentrations and combinations of BAP and NAA. First the axillary buds enlarged and they underwent (Fig 1 b) direct regeneration of shoots. A low concentration of BAP (0.5 mg) (Fig 1 c) favoured direct shoot development and regeneration. Higher concentrations of BAP (2-4 mg L) favoured regeneration through callus formation. (Fig 1 d)

During the present study, it was found that the direct shoot development from the axillary buds was significally promoted by different concentrations of BAP (0.5- 4.0 mg L) in combination with NAA (1.0 mg L). After, 60 days of culture, the combination of BAP (2.0 mg L) and NAA (1.0 mg L) showed development of highest number of shoots. Further increase in concentration of BAP (4.0 mg L) in combination with NAA (1.0 mg L), however did not produce higher number of shoots. According to George (1993) exogenous application of cytokinins and auxins has been known to be important for shoot induction and elongation of many plant species *In vitro*, while BAP and NAA, respectively are the two most commonly used cytokinin and auxin for shoot induction (Nasiruddin *et al*.2003). In the present study, relatively higher number of regenerated micro shoot were produced when compared to the report of Vij *et al 1989)*. The synergistic effect of BAP and NAA towards multiple shoot formation from axillary buds without intermediate callusing is evident from the present study and it is in conformity with the findings of (Herrera *et al* 1990) who purported that exogenous auxins could be effectively employed along with cytokinin for better response of multiple shoot development of *Digitalis thapsi*.

Other studies reported the requirement of the nutrient media with organic growth supplements for multiple shoot induction in a few *Dendrobium* orchids. Peptone was supplemented in the medium to induce multiple shoots in

a) *Dendrobium barbatulum* Lindl. Habit
b) Shoot regeneration in 20 days
c) Shoot regeneration in 30 days
d) Shoot regeneration in 45 days
e) Young clone in rooting medium
f) Young rooted clone

Fig. 1. In vitro micropropagation of orchid *Dendrobium barbatulum* Lindl

*D.*antennathum (Kukulezanka & Wojerechowska. 1983), while yeast extract or urea for *D.chrysanthum* (Vji and Pathak .1989) . Present results have shown that organic supplements were not required to induce multiple shoots in the micropropagation of *D. transparens*.Root induction was favoured by MS + IBA (1.0 mg L). The auxin, IBA also exerted better response on longer root length. These results were supported by (Aktar *et al* 2007) where they found highest length of root of *Dendrobium* sp. At 1.0 mg L IBA in MS medium. These responses can be explained by the promotive effect of auxins on root initials as observed by (De Klerk *et al* 1997).

Plantlets were transferred into potting mixture (brick and charcoal in 2:1 ratio) and successfully acclimatized in a growth chamber. More than 60% of the transplanted plants survived and they were shifted under the shade house, acclimatized and then in natural epiphytic condition.

In conclusion, a simple and efficient protocol for mass clonal propagation of *D.barbatum* from fleshy stem explants has been established. Using this protocol, it is possible to produce viable, uniform and healthy plants with maximum survival rate that can be used for large scale cultivation. Furthermore, the protocol may facilitate conservation of this potential orchid from extinction in the natural population.

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