J. Orchid Soc. India, 30: 51-55, 2016 ISSN 0971-5371

MASS PROPAGATION OF *DENDROBIUM AMOENUM* WALL. EX LINDL. THROUGH STEM NODAL EXPLANTS: A STUDY *IN VITRO*

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Abstract

Dendrobium amoenum stem nodal explants (3-6 mm long) procured from *in vitro* raised seedlings, were inoculated on an agar gelled M and MS media alone and in various combinations with IAA, IBA, 2,4-D, NAA, KN (1-5 mgl^{-1} each) and GA_3 (1 mgl^{-1}**).** The regeneration response of the explants varied with their nutrient environment. These failed to respond and perished within 3 wks in basal M medium and most of its combinations with growth hormones. While in MS medium, the growth hormones proved obligatory for regeneration but the frequency and pathway of regeneration varied with the quality and quantity of growth hormones employed. Meristematic loci were initiated along the explants in all the combinations of IAA and each of these subsequently developed into a plantlet via PLB generation. IBA was effective only at $2mgl^{-1}$; it induced PLB generation in only $24.25 \pm 0.95\%$ explants and favoured PLBs proliferations. IBA and KN when used together in combination at 1 mgl^{-1} each acted synergistically to induce accelerated regeneration in $64.25 \pm 0.95\%$ explants; plantlets were obtained in 9.00 ± 0.81 wks.

Introduction

THE METHOD of *in vitro* mass propagation by activation of the axillary buds and further proliferations has been effectively utilized in many orchid taxa [*Arundina* (Mitra, 1971; Arora *et al.*, 2014), *Cymbidium* (Vij *et al.*, 1994), Kanjilal *et al.*, 1999), *Dendrobium longicornu* (Dohling *et al.*, 2012), *Dendrobium Sonia* 'Earsakul' (Priya Kumari *et al.*, 2013) *Phalaenopsis* (Duan *et al.*, 1996), *Vanilla* (George and Ravishankar, 1997), *Vanda* (Decruse *et al.*, 2003; Vij *et al.*, 2000)]. *Dendrobium amoenum* an epiphytic ornamental orchid bears violet coloured and sweetly scented flowers on long pendulous stems which are thickened at the nodes. It is widely distributed along the Himalayas from Garhwal eastwards to Sikkim in more or less lax forests (900-1600 m); the species is also found at comparable elevations in Khasi and Jaintia hills. It blooms during the month of May-June. Its natural populations are, however, getting rare due to depletion of its habitats as a consequence of clearing of forests for agricultural and other developmental purposes. With this aim, an attempt has been presently made to conserve this precious *Dendrobium amoenum* by its mass multiplication using *in vitro* regeneration method by culturing its stem nodal explants.

Materials and Methods

Stem Explant Preparation

Stem nodal explants (3-6 mm long) procured from *in vitro* raised seedlings, were inoculated on an agar gelled M and MS nutrient media either alone or in various combinations with IAA, IBA, 2,4-D, NAA, KN (1-5 mgl^{-1} each) and GA_3 (1 mgl^{-1}).

Culture Media and Culture Conditions

M (Mitra *et al.,* 1976) and MS (Murashige and Skoog, 1962) media alone and in various combinations with IAA, IBA, 2,4-D, NAA, KN (1-5 mgl^{-1} each) and GA_3 (1 mgl^{-1}**)** were used. The pH of nutrient media was adjusted to 5.6 prior to autoclaving at 121^0C at 1 kg cm^{-2} for 20 min. The cultures were maintained under a 12-hr photoperiod of 30 μmol $m^{-2}s^{-1}$ light intensity and a temperature of 25 ± 2^0C, and observed regularly.

Statistical Analysis

One way analysis of variance was performed with respect to each response (average ± standard error against each additive as mention 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.

Results

The regeneration response of 3-6 mm long discs (explants) varied with their nutrient environment. These failed to respond and perished within 3 wks in basal M medium and most of its combinations with growth hormones. While in MS medium, the growth hormones proved obligatory for regeneration but the

Received: April 6, 2016 ; Accepted: June 23, 2016

frequency and pathway of regeneration varied with the quality and quantity of growth hormones employed (Figs. 1-5). IAA at 1-3 mgl^{-1} induced regeneration response in nearly 50% explants whereas at its higher concentration (4-5 mgl^{-1}), 75% explants responded. The explants regenerated via PLBs within 3-4 wks (Figs. 1-3) which subsequently differentiated into plantlets (Fig. 5). Meristematic loci were initiated along the explants in all the combinations of IAA and each of these subsequently developed into a plantlet via PLB generation, the plantlets rooted profusely when IAA was used at 1 and 3mgl^{-1}. IBA was effective only at 2mgl^{-1}; it induced PLB generation in only 24.25 ± 0.95% explants and favoured PLBs proliferations (Fig.4). NAA favoured proliferations via PLBs formation in the explants when used at 1mgl^{-1} and plantlets were obtained in 11.00 ± 0.81 wks. NAA when used at its higher concentrations (2-5mgl^{-1}) and KN at all the concentrations used in the medium proved inhibitory. The explants responded via PLBs formation in 3.00 ± 0.81 wks and plantlets were obtained in 11.00 ± 0.81 wks in medium containing MS + 2,4-D (5 mgl^{-1}). When KN and IAA were used together, the frequency of regeneration and onset of morphogenetic processes were variously affected depending upon their concentration in the medium. At 1 mgl^{-1} each of these growth hormones, 75.75 ± 0.95% explants responded and generated plantlets within 13.25 ± 0.95 wks. Increased concentration of either of these hormones detrimentally affected the regeneration frequency. Replacement of IAA with NAA in the above combination though enhanced the frequency of regeneration (NAA:KN, 2:5), it was however reduced (NAA:KN, 5:1/2). Plantlet formation was delayed in combinations containing NAA and KN at 1:5 and vice versa. IBA and KN in the combination at 1 mgl^{-1} each acted synergistically to induce accelerated regeneration in 64.25 ± 0.95% explants, plantlets were obtained in 9.00 ± 0.81 wks. Enhanced concentration of IBA and KN and 2,4-D and KN in the nutrient pool, however, proved lethal (Table 1).

Discussion

The stem segments have earlier been used for regeneration purposes in *Arundina* (Mitra, 1971; Arora

Table 1. *In vitro* regeneration response in *Dendrobium amoenum* through stem nodal culture on MS medium.

Growth regulators	Explants responded	Percentage response	Time taken in wks for the development of PLB	Root	Plantlet
MS + IAA_1	4.00 ± 0.81^{abc}	50.75 ± 0.95^{d}	3.25 ± 0.50^{ab}	7.75 ± 0.95^{bcd}	11.00 ± 0.81^{bcd}
MS + IAA_2	4.00 ± 0.81^{abc}	50.81 ± 0.81^{d}	4.00 ± 0.81^{bc}	10.00 ± 0.81^{f}	12.75 ± 1.89^{defg}
MS + IAA_3	4.00 ± 0.81^{abc}	50.00 ± 0.81^{d}	3.50 ± 0.57^{abc}	7.75 ± 0.50^{bcd}	12.75 ± 0.95^{defg}
MS + IAA_4	5.75 ± 0.50^{d}	74.25 ± 0.95^{f}	4.00 ± 0.81^{bc}	7.00 ± 0.81^{abc}	13.00 ± 0.81^{efg}
MS + IAA_5	5.50 ± 0.57^{cd}	75.75 ± 0.95^{f}	4.00 ± 0.81^{bc}	6.75 ± 0.50^{abc}	13.25 ± 0.95^{efg}
MS + IBA_2	2.75 ± 0.95^{a}	24.25 ± 0.95^{a}	4.00 ± 0.81^{bc}	11.75 ± 0.95^{g}	14.00 ± 0.81^{g}
MS + 2,4-D_5	4.00 ± 0.81^{abc}	50.00 ± 0.81^{d}	3.00 ± 0.81^{ab}	6.50 ± 0.57^{ab}	11.00 ± 0.81^{bcd}
MS + NAA_1	6.00 ± 0.81^{d}	64.50 ± 1.29^{e}	3.25 ± 0.50^{ab}	6.75 ± 0.50^{abc}	11.00 ± 0.81^{bcd}
$IAA_1 + KN_1$	6.00 ± 0.81^{d}	75.75 ± 0.95^{f}	4.00 ± 0.81^{bc}	7.00 ± 0.81^{abc}	13.25 ± 0.95^{efg}
$IAA_1 + KN_5$	3.75 ± 0.95^{ab}	36.50 ± 1.29^{b}	3.75 ± 0.95^{abc}	8.25 ± 0.50^{cde}	11.75 ± 0.95^{cde}
$IAA_2 + KN_5$	2.75 ± 0.50^{a}	36.50 ± 1.29^{b}	4.75 ± 0.50^{cd}	10.50 ± 1.29^{fg}	11.75 ± 1.25^{cde}
$IAA_5 + KN_1$	4.00 ± 0.81^{abc}	50.50 ± 1.29^{d}	3.25 ± 0.50^{ab}	9.75 ± 0.95^{ef}	10.25 ± 0.95^{abc}
$IAA_5 + KN_2$	4.50 ± 1.29^{bcd}	50.75 ± 0.95^{d}	3.25 ± 0.50^{ab}	7.00 ± 0.81^{abc}	9.25 ± 0.95^{ab}
$IBA_1 + KN_1$	5.75 ± 0.95^{d}	64.25 ± 0.95^{e}	3.50 ± 0.57^{abc}	6.00 ± 0.81^{a}	9.00 ± 0.81^{a}
$NAA_1 + KN_1$	5.75 ± 0.50^{d}	75.00 ± 0.81^{f}	6.00 ± 0.81^{d}	9.75 ± 0.95^{ef}	13.75 ± 0.50^{fg}
$NAA_1 + KN_5$	2.50 ± 0.57^{a}	25.25 ± 0.5^{a}	6.00 ± 0.81^{d}	10.75 ± 1.25^{fg}	14.00 ± 1.41^{g}
$NAA_2 + KN_5$	3.00 ± 0.81^{ab}	38.50 ± 1.29^{c}	2.50 ± 0.57^{a}	10.00 ± 1.41^{f}	12.00 ± 1.41^{cdef}
$NAA_5 + KN_1$	3.00 ± 0.81^{ab}	36.50 ± 1.29^{b}	4.00 ± 0.81^{bc}	7.25 ± 0.95^{abc}	12.75 ± 0.95^{defg}
$NAA_5 + KN_2$	3.00 ± 0.81^{ab}	36.50 ± 1.29^{b}	4.00 ± 0.81^{bc}	8.00 ± 0.81^{bcd}	10.75 ± 1.25^{abc}

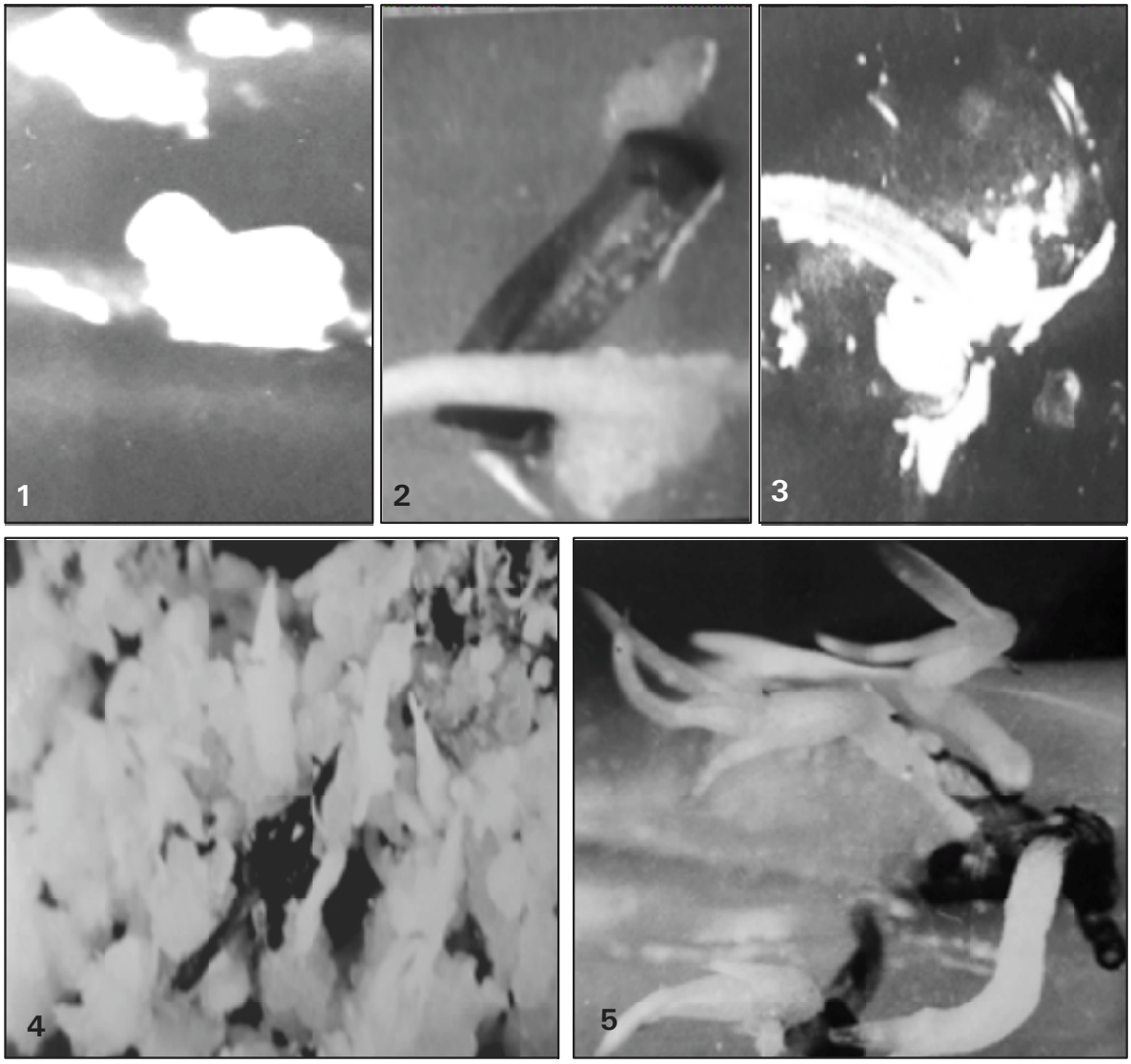

Fig. 1-5. *In vitro* mass propagation of *Dendrobium amoenum* via stem nodal explant culture: 1-3, Explants regenerated via PLBs [MS + IAA (2 mgl^{-1}), initiation of meristematic loci along the explants [MS + IAA (4 mgl^{-1})] and subsequent development into plantlets via PLB generation [MS + IAA (3 mgl^{-1})]; 4, PLBs generation and proliferations [MS + IBA (2 mgl^{-1}); 5, Complete plantlets [MS + IAA (1mgl^{-1})].

et al., 2014); *Cymbidium* (Vij *et al.*, 1994), Kanjilal *et al.*, 1999), *Dendrobium* (Kim *et al.*, 1970; Mosaich *et al.*, 1974); *D. longicornu* (Dohling *et al.*, 2012); *Phalaenopsis* (Sagawa, 1961; Scully, 1965; 1966; Wang, 1989); *Vanda* Miss Joaquim (Sagawa and Sehgal, 1967); and *Vanilla* (Philip and Nainar, 1986; Duan and Hong, 1989). Philip and Nainar (1986) correlated the inability of the nodal explants with their size and medium composition in *Vanilla*, since 2 cm long explants failed to proliferate in Gamborg's (1966) liquid medium whereas 3 cm ones generated PLBs in Knudson 'C' (1946) liquid medium. In the present studies, 0.3-0.6 cm long explants were used. Their inability to proliferate on agar gelled basal M medium and most of its combinations with growth hormones and ability to proliferate on solidified MS medium hint at the importance of nutritional recipe. However, the ability of the explants to proliferate along the cut ends in the present species may be associated with the wound stimulus. Earlier, Teo (1978) considered a solid medium as obligatory for differentiation in *Haemeria discolor.*

During the present study, meristematic loci were initiated along the explants in all the combinations of IAA and each of these subsequently developed into a plantlet via PLB generation. Earlier, Arora *et al.* (2014) successfully tested the regeneration competence of stem discs of *Arundina graminifolia* and reported that IAA was effective when used at 2-4 mgl^{-1}. IBA was effective only at 2mgl^{-1}; it induced PLB generation in

only 24.25 ± 0.95% explants and favoured PLBs proliferations. IBA and KN when used together in combination at 1 mgl^{-1} each acted synergistically to induce accelerated regeneration in 64.25 ± 0.95% explants; plantlets were obtained in 9.00 ± 0.81 wks. The role of PGRs, especially combination containing cytokinins and auxins, in initiating the regeneration response in the nodal explants has been greatly emphasized in a number of orchid species (Arora *et al.,* 2014; George and Ravishankar, 1997; Kanjilal *et al.*, 1999; Laishram and Devi, 1999; Nayak *et al.*, 1997; Vij *et al.*, 1994). Dohling *et al.* (2012) studied multiple shoot induction from nodal explants (1-2 cm) procured from *in vivo* grown plants of *Dendrobium longicornu* using MS medium supplemented with NAA, 2,4-D and BAP. The maximum explant response (86.6%) was obtained in medium supplemented with NAA at 30 μM while maximum number of shoots (4.42) and maximum bud forming capacity (3.51) were observed in medium containing BAP (15 μM) and NAA (15μM) in combination. Recently, Priya Kumari *et al.* (2013) successfully tested the regeneration potential of *Dendrobium* Sonia Earsakul using stem nodal explants (1-2 cm) on MS medium supplemented with auxins and cytokinins. Early bud break was observed in MS medium supplemented with BAP (4 mgl^{-1}) and for shoot multiplication, treatment combination of KN (2 mgl^{-1}) and NAA (0.01 mgl^{-1}) proved best for shoot multiplication and maximum numbers of healthy shoots. Presently, stem nodal explants were successfully used for regeneration in *Dendrobium amoenum* and the present study hints at their utility for mass propagation and conservation in the presently investigated species and other related taxa.

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