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Effect of Plant Growth Regulators on Regeneration Potential of Axenic Nodal Segments of *Dendrobium chrysanthum* Wall. ex Lindl.

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ABSTRACT

Dendrobium chrysanthum **(Orchidaceae) is highly valued in the ornamental industry and has important medicinal properties used in the preparation of herbal medicines in different parts of the world. However, this orchid species is getting depleted from its natural habitats due to unsystematic collections. Therefore, in the present study, an efficient mass propagation protocol through direct shoot bud formation from axenic nodal segments of *D. chrysanthum* has been developed. The synergistic action between thidiazuron (TDZ) and 6-benzyl amino purine (BAP) was applied to enhance proliferation and elongation of shoots from the nodal segments. The explants were inoculated in MS medium fortified with different plant growth regulators *viz.*, 2, 4-dichlorophenoxy acetic acid (2, 4-D), BAP, and TDZ, separately and in combinations. The maximum frequency of explants forming buds (100%), highest number of shoots/explant (14.33±0.14), the bud forming capacity (BFC) index of 14.33, and the maximum length of shoots (1.97±0.04) were obtained in MS medium supplemented with 5 µM each of TDZ and BAP. Hundred percent rooting of regenerated shoots with an average number of 11.26 roots/shoot and having average root length of around 2.45 cm was obtained in MS medium fortified with 10 µM α-naphthaleneacetic acid (NAA). Sixty days after transfer to the greenhouse following *in vitro* acclimatization for 30 days, 79% survival was recorded. The protocol developed will not only help to alleviate the pressure on the natural population under stress, but will also help in meeting its demands in pharmaceutical and ornamental industries, and also form the basis for conservation.**

Keywords: *In vitro* acclimatization, Conservation, Mass propagation, Thidiazuron; 6-benzyl amino purine.

INTRODUCTION

Orchids belonging to the family Orchidaceae, are one of the largest and most evolved flowering plants. Orchidaceae includes about 800 genera and between 25,000 to 30,000 species distributed all over the world (Chowdhery, 2001). Orchids produce one of the most beautiful and enchanting flowers that have fascinated people of all ages. Besides being considered an ornamental treasure in the commercial market, orchids have important medicinal properties used in the preparation of herbal medicines in different parts of the world (Arditti, 1992). According to World Health Organization, 80% of people depend mainly on traditional remedies such as herbs for medicine (Kala, 2005), resulting in increasing demand for medicinal plants.

Dendrobium chrysanthum (Figure 1), commonly known as the "golden orchid", bears beautiful golden yellow flowers at the nodes on pendulous stems. The stems of *D. chrysanthum* possess antipyretic and immunomodulatory properties, because of

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Figure 1. Flowers of *Dendrobium chrysanthum.*

which they are highly valued in traditional Chinese medicines (Pharmacopoeia Commission of PR China, 2005). This species has also been reported to be used by the *Khasi* traditional healers of Meghalaya, India, to treat external injuries and fractured bones (Hynniewta and Kumar, 2008). Many orchids including *D. chrysanthum* have become threatened in nature due to rapid habitat destruction and increased biotic influences. Therefore, to prevent them from becoming extinct, their propagation and conservation need immediate attention (Tandon, 2000; Tandon and Kumaria, 2005).

Micropropagation of orchids, through tissue culture using different explants, has become a significant technique to reproduce/propagate, conserve, and save many species from extinction (Kataki, 1993). Plant growth regulators (PGRs), especially auxin and cytokinin, have been used extensively for large scale propagation of various orchids. Over the years, a number of protocols have been developed through *in vitro* culture of different explants in culture media fortified with auxin and cytokinin, either separately or in combination, to achieve maximum proliferation and elongation of orchid microshoots (Nayak *et al*., 1997; Chen *et al*., 2004; Kosir *et al*., 2004; Luo *et al*., 2008; Cheruvathur *et al*., 2010; Tan *et al*., 2011). However, establishment of protocols for mass propagation of orchids is species-specific and protocol for *D. chrysanthum* is lacking. Besides, using axenic explants for micropropagation of orchids is advantageous over conventional method because of all round availability of the explants and production of uniform and disease-free plants. Therefore, the objective of this study was to identify appropriate concentration and combination of PGRs to develop an efficient propagation protocol for *D. chrysanthum* through direct shoot bud formation from axenic nodal segments for mass production of uniform plants.

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MATERIALS AND METHODS

Plant Material

Plants of *D. chrysanthum* were collected from the forests of Nongpyiur, Meghalaya, and maintained in the greenhouse of the Plant Biotechnology Laboratory, Department of Botany, North-Eastern Hill University, Shillong, India. *In vitro* grown seedlings of *D. chrysanthum* were used as the source of material (Hajong *et al*., 2010).

Media and Culture Conditions

Nodal segments measuring about 3-4 mm in size were taken and their leaves, dry sheaths and other external tissues were removed. These segments were inoculated in test tubes containing MS medium (Murashige and Skoog, 1962) fortified with different PGRs *viz.*, 2,4-dichlorophenoxy acetic acid (2,4-D), thidiazuron (TDZ) and 6-benzyl amino purine (BAP), separately and in combinations (in the range of 5-30 µM). The different combinations of growth regulators tested were BAP+2,4-D, TDZ+2,4-D and TDZ+BAP. Nodal explants cultured in hormone-free MS medium served as the control. The pH of the media was adjusted to 5.8 with 0.1N NaOH or HCl prior to autoclaving and the medium was solidified with 0.8% agar. The cultures were maintained at 25±2°C with 12-hour illumination of 60 µmoles m^{-2} sec^{-1} light intensity provided by cool white florescent tubes.

After an initial incubation period of 30 days on hormone-supplemented MS medium, the explants along with the induced shoot buds were transferred to hormone-free MS medium for further proliferation and elongation of the shoot buds. Ten replicates were maintained for each treatment and the experiment was repeated thrice. The regeneration process of the nodal segments was evaluated regularly. Data on percentage of explants forming shoot buds, average number of shoots/explant and average length of shoots were recorded after 30 days of transfer to hormone-free MS medium. The bud forming capacity (BFC) of the nodal explants was calculated based on the average number of shoot buds and percentage response of the explants (Tandon *et al*., 2007).

BFC = (Average number of buds per explant×% of explants forming buds)÷100

Rooting of Shoots

Regenerated shoots were inoculated in MS medium supplemented with different concentrations of NAA (5, 10, 20 and 30 µM) for rooting. Data on frequency of rooting, average number of roots/shoot, and average length of roots were recorded after 30 days of transfer to the rooting medium.

In vitro Acclimatization and Hardening of Plantlets

Plantlets (about 3-4 cm) with well-developed roots were taken out and the agar medium sticking to the roots was removed slowly with a soft brush and washed with sterile water. These plantlets were transferred to 250 ml Erlenmeyer flasks containing 1-1.5 cm^3 brick pieces and charcoal pieces, chopped coconut husks and moss in a ratio of 1:1:1:1. The various substrates were washed thoroughly in tap water, rinsed twice with sterile distilled water and air dried prior to putting in the Erlenmeyer flasks. About 20 ml of distilled water was added to each culture flask in order to maintain the moisture content within the flasks. Three to four plantlets were transferred to each flask and the cultures were maintained at 25±2°C with 12-hour illumination of 60 µmoles m^{-2} sec^{-1} light intensity. After 30 days of *in vitro* acclimatization, the plantlets were transferred to thermocol pots (8 cm diameter) containing the same substrate mix as mentioned above and kept in the greenhouse. Percentage of

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survival of the hardened plants was recorded after 60 days of transfer to the greenhouse.

Analysis of Data

Data were subjected to one-way analysis of variance (ANOVA) using Origin (ver. 8.0), and means were compared using Least Significant Difference (LSD) at $p< 0.01$ significance level.

RESULTS

Micropropagation

The nodal segments showed varied responses on MS medium supplemented with different plant growth regulators either separately or in combinations (Table 1). With PGRs incorporated singly in medium, 100% of the explants formed buds in MS+5 µM BAP, MS+10 µM BAP and MS+5 µM TDZ. Highest number of shoots/explant (9.46±0.13) and BFC index of 9.46 were obtained in MS+5 µM TDZ, closely followed by 6.84±0.69 number of shoots/explant and BFC index of 6.84 in MS+10 µM BAP. However, maximum length of shoots (1.52±0.02) (cm) was obtained in MS medium incorporated with 5 µM BAP. The BFC index was further enhanced with the combination of PGRs at different concentrations in the medium; 100% explants forming buds, highest number of shoots/explant (14.33±0.14), BFC index of 14.33 and the maximum length of shoots (1.97±0.04) (cm) were obtained in MS medium containing 5 µM each of TDZ and BAP (Figures 2-A, -B and -C). It was observed that with increase in concentration of PGRs in MS medium, there was a decrease in percentage response of the explants forming buds (Table 1). Decrease in the number of shoots/explant, BFC index, and average length of shoots were also recorded at higher concentration of PGRs. It was also observed that addition of certain combination of PGRs in MS medium resulted in increase of the parameters studied till the second higher level of concentration and, thereafter, a steady decrease was noted from the third higher level of concentration onwards. The explants exhibited a peculiar response at higher concentration of TDZ in the medium. Stunted shoots and callus induction from the base of the explants were observed. Prolonged culture in the same medium did not improve the rate of shoot bud proliferation. However, transfer of explants to hormone-free MS medium after an incubation period of 30 days enhanced further proliferation and elongation of the shoot buds.

Rooting of Shoots

Regenerated shoots cultured in MS medium supplemented with NAA resulted in 100% shoots forming roots in MS+10 µM NAA (Table 2). Maximum root number/shoot (11.26±0.32) with 2.45±0.043 cm average length of roots were obtained in the same medium (Figure 2-D). At the lowest level of NAA (5 µM), the percentage of shoots forming roots, number of roots/shoot, and the average length of roots declined. At 20 µM and 30 µM of NAA, the frequency of shoots forming roots and the other growth parameters studied decreased further.

Acclimatization and Hardening

After 30 days of *in vitro* acclimatization (Figure 2-E), the plantlets were transferred to the greenhouse and were hardened in about 60 days after transfer, wherein 79% survival was recorded (Figure 2-F). It was observed that during acclimatization, the plantlets shed the *in vitro* leaves, which were replaced by new leaves. In some cases, new shoots were seen to grow out from the base of the *in vitro* shoots.

DISCUSSION

The use of PGRs for efficient micropropagation of orchids by stimulating induction and proliferation of shoot buds

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Table 1. Morphogenetic responses and BFC index of nodal segments of *Dendrobium chrysanthum* in MS medium supplemented with different plant growth regulators after 60 days of culture[a].

Treatments [Growth regulators (μM)]		Percentage of explants forming buds (%)	Average no. of shoot buds formed/explant	Bud forming capacity (BFC)	Average length of shoots (cm)
Control		96.66 ± 3.33^{a}	1.00 ± 0^{n}	0.97	0.90 ± 0.01^{d}
2,4-D					
5		83.33 ± 3.33^{abcde}	2.00 ± 0^{i}	1.66	0.70 ± 0.01^{ef}
10		60.00 ± 0^{ghij}	1.52 ± 0.17^{jkl}	0.91	0.57 ± 0.02^{ghi}
20		50.00 ± 0^{jklm}	1.00 ± 0^{n}	0.50	0.32 ± 0.02^{no}
30		40.00 ± 0^{mno}	1.00 ± 0^{n}	0.40	0.30 ± 0.02^{o}
BAP					
5		100 ± 0^{a}	6.23 ± 0.16^{e}	6.23	1.52 ± 0.02^{b}
10		100 ± 0^{a}	6.84 ± 0.69^{e}	6.84	0.97 ± 0.02^{d}
20		86.66 ± 3.33^{abcd}	1.62 ± 0.09^{ijkl}	1.40	0.75 ± 0.01^{e}
30		83.33 ± 3.33^{abcde}	1.52 ± 0.10^{jkl}	1.26	0.75 ± 0.02^{e}
TDZ					
5		100 ± 0^{a}	9.46 ± 0.13^{c}	9.46	0.95 ± 0.01^{d}
10		63.33 ± 3.33^{fghi}	1.68 ± 0.18^{ijk}	1.06	0.59 ± 0.02^{gh}
20		56.66 ± 3.33^{hijk}	1.52 ± 0.17^{jkl}	0.86	0.35 ± 0.01^{mno}
30		53.33 ± 3.33^{ijkl}	1.50 ± 0.20^{jklm}	0.80	0.31 ± 0.01^{o}
BAP	2,4-D				
5	5	86.66 ± 3.33^{abcd}	1.46 ± 0.09^{klm}	1.27	0.97 ± 0.02^{d}
5	10	90.00 ± 0^{abc}	1.93 ± 0.15^{ij}	1.73	0.75 ± 0.02^{e}
5	20	76.66 ± 3.33^{bcdef}	1.26 ± 0.09^{klmn}	0.97	0.55 ± 0.02^{hij}
5	30	70.00 ± 5.77^{defgh}	1.14 ± 0.07^{mn}	0.80	0.42 ± 0.01^{lm}
10	5	83.33 ± 3.33^{abcde}	1.44 ± 0.10^{klm}	1.20	0.63 ± 0.02^{fg}
10	10	90.00 ± 5.77^{abc}	12.15 ± 0.14^{b}	10.93	1.16 ± 0.03^{c}
10	20	76.66 ± 6.66^{bcdef}	1.26 ± 0.09^{klmn}	0.96	0.46 ± 0.01^{kl}
10	30	63.33 ± 3.33^{fghi}	1.00 ± 0^{n}	0.63	0.35 ± 0.01^{mno}
20	5	76.66 ± 3.33^{bcdef}	1.52 ± 0.15^{jkl}	1.17	0.75 ± 0.05^{e}
20	10	76.66 ± 3.33^{bcdef}	5.48 ± 0.22^{f}	4.20	0.48 ± 0.01^{jkl}
20	20	66.66 ± 3.33^{efghi}	1.00 ± 0^{n}	0.66	0.44 ± 0.02^{lm}
20	30	66.66 ± 6.66^{efghi}	1.00 ± 0^{n}	0.66	0.36 ± 0.01^{mno}
30	5	63.33 ± 3.33^{fghi}	1.26 ± 0.10^{klmn}	0.80	0.44 ± 0.02^{lm}
30	10	73.33 ± 3.33^{cdefg}	1.68 ± 0.10^{ijk}	1.23	0.46 ± 0.01^{kl}
30	20	56.33 ± 3.33^{hijk}	1.00 ± 0^{n}	0.56	0.34 ± 0.02^{mno}
30	30	46.33 ± 6.66^{klmn}	1.00 ± 0^{n}	0.46	0.32 ± 0.02^{no}
TDZ	2,4-D				
5	5	40.00 ± 0^{mno}	1.25 ± 0.13^{klmn}	0.50	0.53 ± 0.03^{hijk}
5	10	63.33 ± 3.33^{fghi}	2.00 ± 0.18^{i}	1.27	0.56 ± 0.04^{ghi}
5	20	-	-	-	-
5	30	-	-	-	-
10	5	43.33 ± 3.33^{lmn}	1.00 ± 0^{n}	0.43	0.50 ± 0.03^{ijkl}
10	10	56.66 ± 3.33^{hijk}	1.00 ± 0^{n}	0.57	0.44 ± 0.03^{lm}
10	20	40.00 ± 0^{mno}	1.00 ± 0^{n}	0.40	0.42 ± 0.02^{lm}
10	30	30.00 ± 0^{o}	1.00 ± 0^{n}	0.30	0.40 ± 0.03^{lmn}
20	5	43.33 ± 3.33^{lmn}	1.00 ± 0^{n}	0.43	0.43 ± 0.03^{lm}
20	10	43.33 ± 3.33^{lmn}	2.00 ± 0.28^{i}	0.87	0.45 ± 0.02^{ijk}
20	20	40.00 ± 0^{mno}	1.00 ± 0^{n}	0.40	0.41 ± 0.01^{lmn}
20	30	36.66 ± 3.33^{no}	1.00 ± 0^{n}	0.37	0.30 ± 0.02^{o}
30	5	36.66 ± 3.33^{no}	1.00 ± 0^{n}	0.37	0.47 ± 0.03^{jkl}
30	10	43.33 ± 3.33^{lmn}	1.15 ± 0.10^{lmn}	0.50	0.46 ± 0.02^{kl}
30	20	30.00 ± 0^{o}	1.00 ± 0^{n}	0.30	0.46 ± 0.03^{kl}
30	30	-	-	-	-

[a] Values are mean±SE, n= 10. Means followed by different letters within the same column are significantly different at $p< 0.01$.

Table 1. continued

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Table 1. continued

Treatments [Growth regulators (µM)]		Percentage of explants forming buds (%)	Average no. of shoot buds formed/explant	Bud forming capacity (BFC)	Average length of shoots (cm)
TDZ	BAP				
5	5	100 ± 0^{a}	14.33 ± 0.14^{a}	14.33	1.97 ± 0.04^{a}
5	10	96.66 ± 3.33^{a}	9.89 ± 0.20^{c}	9.55	0.93 ± 0.02^{d}
5	20	60.00 ± 5.77^{ghij}	3.50 ± 0.12^{g}	2.10	0.54 ± 0.01^{hijk}
5	30	36.66 ± 6.66^{no}	1.63 ± 0.15^{ijkl}	0.60	0.52 ± 0.02^{hijk}
10	5	$93.33 \pm 6.66_{ab}$	6.85 ± 0.16^{e}	6.40	1.17 ± 0.05^{c}
10	10	86.66 ± 3.33^{abcd}	8.75 ± 0.15^{d}	7.58	0.93 ± 0.03^{d}
10	20	63.33 ± 3.33^{fghi}	2.95 ± 0.19^{gh}	1.87	0.65 ± 0.02^{fg}
10	30	60.00 ± 0^{ghij}	1.44 ± 0.12^{klm}	0.86	0.57 ± 0.02^{ghi}
20	5	46.66 ± 6.66^{klmn}	1.00 ± 0^{n}	0.47	0.70 ± 0.05^{ef}
20	10	63.33 ± 3.33^{fghi}	8.07 ± 0.39^{d}	5.11	0.60 ± 0.02^{gh}
20	20	56.66 ± 3.33^{hijk}	1.00 ± 0^{n}	0.57	0.52 ± 0.03^{hijk}
20	30	43.33 ± 3.33^{lmn}	1.00 ± 0^{n}	0.43	0.50 ± 0.02^{ijkl}
30	5	70.00 ± 5.77^{defgh}	1.57 ± 0.11^{ijkl}	1.10	0.52 ± 0.02^{hijk}
30	10	83.33 ± 3.33^{abcde}	2.64 ± 0.09^{h}	2.20	0.50 ± 0.01^{ijkl}
30	20	63.33 ± 3.33^{fghi}	1.00 ± 0^{n}	0.63	0.51 ± 0.01^{hijk}
30	30	36.66 ± 3.33^{no}	1.00 ± 0^{n}	0.37	0.47 ± 0.01^{jkl}

[a] Values are mean±SE, n= 10. Means followed by different letters within the same column are significantly different at $p< 0.01$.

Table 2. Rooting of in *vitro* regenerated shoots of *Dendrobium chrysanthum* in MS medium supplemented with different concentrations of NAA.

Treatments (µM)	Rooting (%)	Average number of roots/explant	Average length of roots (cm)
5	90.00 ± 0.00^{ab}	6.90 ± 0.35^{b}	1.67 ± 0.087^{b}
10	100.00 ± 0.00^{a}	11.26 ± 0.32^{a}	2.45 ± 0.043^{a}
20	86.66 ± 3.33^{b}	5.53 ± 0.27^{c}	1.21 ± 0.042^{c}
30	83.33 ± 3.33^{b}	5.40 ± 0.33^{c}	0.95 ± 0.019^{d}

Values are mean ± SE, n = 10. Data was recorded after 30 days of transfer to the rooting medium. Means followed by different letters within the same column are significantly different at $p<0.01$.

from various explants is well established. The type, concentration, and combination of PGRs exert differential influence on various explants (Arditti and Ernst, 1993). In the present study, highest frequency of explant response was observed in MS medium supplemented singly either with 5 or 10 µM BAP or 5 µM TDZ. However, the highest number of shoots/explant and BFC index was observed in MS medium with 5 µM TDZ. TDZ, a substituted phenyl urea (N-phenyl-1,2,3-thidiazol-5-ylurea) derivative exhibits strong cytokinin-like activity (Mok *et al*., 1982; Thomas and Katterman, 1986) and has been reported to be effective in high rates of regeneration and axillary shoot proliferation in a number of plants including several species of orchids (Huetteman and Preece, 1993; Nayak *et al*., 1997; Chang and Chang, 1998; Chen *et al*., 2004; Cocu *et al*., 2004; Faisal *et al*., 2005; Ferreira *et al*., 2006). On the other hand, BAP was found to be more effective in boosting the elongation of shoots. The importance of BAP in stimulating shoot elongation has been highlighted in *Vanilla planifolia* (Geetha and Shetty, 2000), *Dendrobium formosum* (Nasiruddin *et al*., 2003) and *Achillea millefolium* (Shatnawi, 2013). Combination of TDZ and BAP (5 µM each) in the

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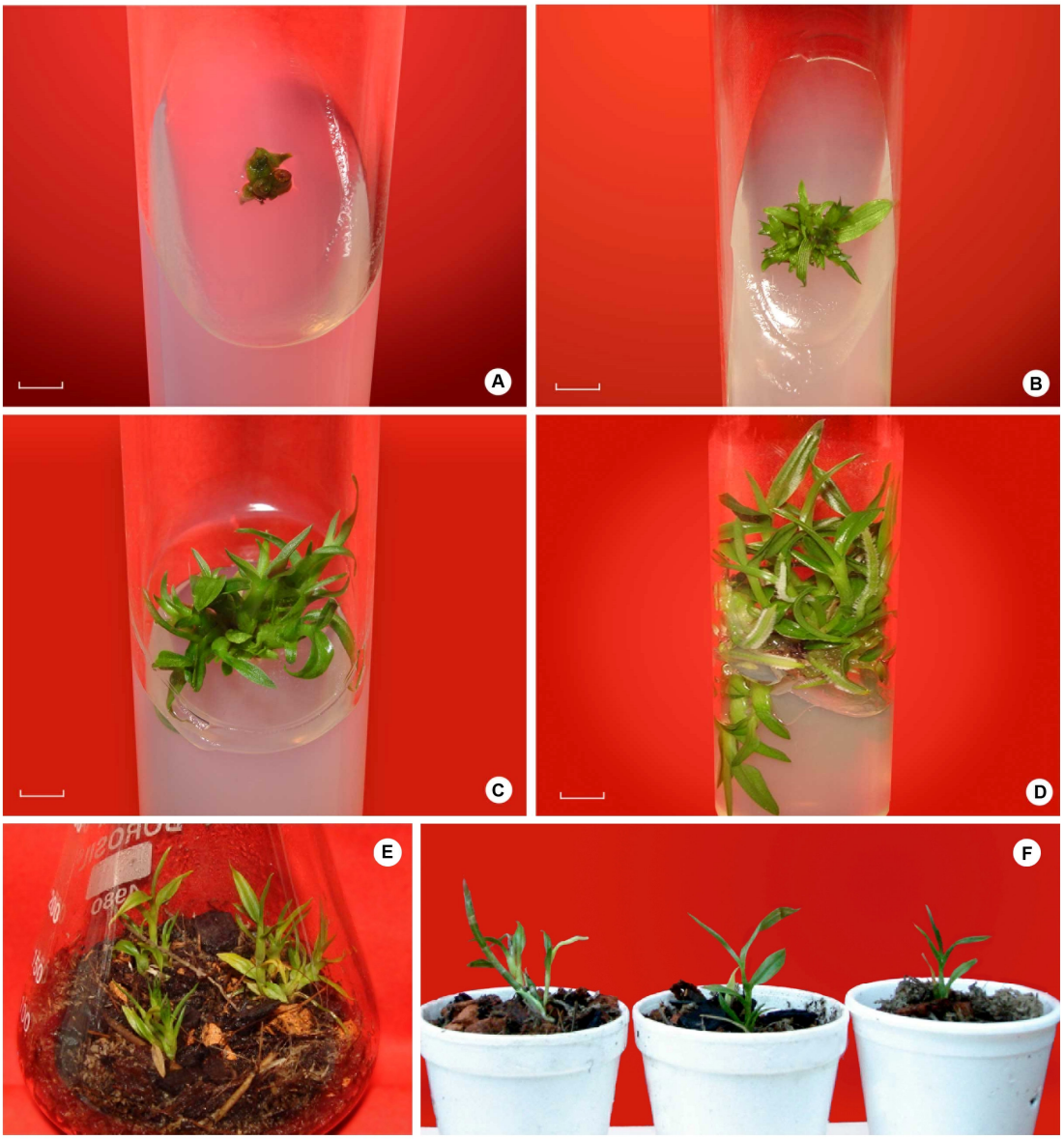

Figure 2. Complete plant regeneration from nodal segments, i*n vitro* acclimatization and hardening of *Dendrobium chrysanthum* plantlets: **(A)** Shoot bud initiation; **(B)** Shoot proliferation - in MS medium supplemented with 5 µM each of TDZ and BAP; **(C)** Shoot proliferation and elongation in 60 days in basal MS medium; **(D)** Multiple shoots with roots in MS medium with 10 µM NAA 30 days after transfer to rooting medium; **(E)** *In vitro* acclimatization of plantlets, **(F)** Hardened plantlets 60 days after transfer to greenhouse. Scale bars= 5mm.

medium was found to further enhance the number of shoots in *D. chrysanthum*. Similar synergistic effect between a cytokinin and a cytokinin-like substance has been observed in *Rhynchostylis gigantean*, *Miscanthus ogiformis* and *Jatropha curcas* (Nielsen *et al*., 1995; Le *et al*., 1999; Kumar *et al*., 2010). In the present study, it was also observed that continuous culture in the same medium did not improve the rate of shoot bud proliferation and elongation. These problems were, however, overcome by transferring the regenerated shoots to hormone-free MS medium 30 days after

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inoculation in PGR supplemented medium. Such peculiar effect of TDZ may be attributed to its ability to induce cytokinin accumulation (Victor *et al*., 1999) or to enhance the accumulation and translocation of auxin (Murch and Saxena, 2001).

Application of auxin to microshoots is stated to intensify the number of adventitious roots. The regenerated shoots of *D. chrysanthum* responded best in terms of rooting, number of roots/shoot, and average length of roots in MS medium fortified with 10 µM NAA. The role of NAA in stimulating root formation has been illustrated in *Dactylorhiza* species, *Dendrobium candidum*, *Geidorum densiflorum*, *Vinca minor* and *Vanilla planifolia* (Sheelavantmath *et al*., 2000; Shiau *et al*., 2005; Wotavova-Novotna *et al*., 2007; Raouf Fard *et al*., 2008; Tan *et al*., 2011). However, rooting of *D. chrysanthum* shoots was suppressed at higher concentrations of NAA.

A micropropagation system can be deemed beneficial only by the successful transfer of plantlets from tissue-culture vessels to the ambient conditions found *ex vitro* (Hazarika, 2003). A significant number of micropropagated plants do not survive when transferred from *in vitro* conditions to greenhouse or field environment which have substantially lower relative humidity, higher light and septic environment compared to the *in vitro* conditions. However, Pospisilova *et al*. (1999), Hazarika (2003), and Deb and Imchen (2010) reported that *in-vitro* acclimatization of plantlets prior to their *ex vitro* transplantation is important in producing healthy plantlets. Their findings are similar to the results obtained in our study wherein a high survival percentage was achieved when the plantlets were transferred to the greenhouse following *in vitro* acclimatization. During *in vitro* acclimatization, leaves of *D. chrysanthum* plantlets were replaced by new ones and fresh shoots emerged from the base of some of the *in vitro* raised shoots. Some workers reported that in many plant species, the *in vitro* formed leaves were unable to develop further under *ex vitro* conditions and were replaced by newly formed leaves (Preece and Sutter, 1991; Diettrich *et al*., 1992). Formation of new shoots during acclimatization of *Calopogon tuberosus* and *Bletia purpurea* has also been reported (Kauth *et al*., 2006; Dutra *et al*., 2008). The change in morphological, anatomical, and physiological features of newly developed leaves and shoots helped in improved hardening and survival of the orchid plants.

The protocol developed in the present study is highly efficient and reproducible for mass propagation of this highly valued epiphytic orchid, *D. chrysanthum*. The protocol developed will not only help alleviate the pressure on the natural population under stress due to over exploitation, it will also help meet its demands in both pharmaceutical and ornamental industry, and also form the basis for conservation.

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تاثیر تنظیم کننده های رشد گیاه روی پتانسیل باز زایی قطعات گره ناآلوده ارکیده*Dendrobium chrysanthum* Wall. ex Lindl

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چکیده

گونه *Dendrobium chrysanthum* ارکیده از ارزش زیادی در صنعت گل های زینتی برخوردار است و دارای خواص دارویی مهمی است و در تهیه دارو های گیاهی در نقاط مختلف جهان به کار می رود. با این همه، به علت برداشت های نا موزون، این گونه ارکیده در زیستگاه طبیعی اش درحال کم شدن است.بنا بر این، هدف پژوهش حاضر این بود که روشی کار آمد برای تکثیر توده ای از طریق ایجاد مستقیم جوانه شاخه از گره نا آلوده *D. chrysanthum* به دست آورد.برای این هدف از کنش هم افزایی بین thidiazuron (TDZ) و6-benzyl amino purine (BAP) برای ارتقای پرآوری و رشد ساقه (شاخه) از محل گره استفاده شد. ریز نمونه های گیاه در محیط MS که با تنظیم کننده های گوناگون شامل 2,4-dichlorophenoxy acetic acid (2, 4-D)، BAP و TDZبه صورت مخلوط یا جدا جدا غنی شده بود تلقیح شد. بیشترین بسآمد ریز نمونه ها که جوانه زدند(۱۰۰٪) و بیشترین تعداد شاخه در ریز نمونه(14.33 ± 0.14) با شاخص ظرفیت تشکیل جوانه (BFC) برابر ۱۴.۳۳ و بیشترین طول شاخه (1.97 ± 0.04 cm) در محیط MSکه با 5 میکرومول از موادTDZ وBAP غنی شده بود به دست آمد. در محیط MSکه با ۱۰ میکرومول آلفا نفتالین استیک اسید (NAA) غنی شده بود ریشه زنی ساقه (شاخسار)های باز زایی شده ۱۰۰ در صد و میانگین تعداد ریشه در هرساقه ۱۱.۲۶ با میانگین طول ۲.۴۵ سانتی متر بود. شصت روز بعد از انتقال به گلخانه و در پی ۳۰ روز سازگاری درون شیشه ای، در صد بقا ۷۹درصد ثبت شد. روشی که در این پژوهش ابداع شد نه تنها در کاهش فشار از روی جامعه طبیعی که تحت تنش است کمک خواهد کرد، بلکه در تامین تقاضای صنایع دارویی و گل تزئینی و بر پایی مبنایی برای حفاظت از این گونه نیز موثر است.

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