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In Vitro Regeneration of *Dendrobium* sp. of Orchid Using Leaf Tip as Explant

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Abstract

Plant growth regulators (PGRs) namely, 2,4-D, NAA and BAP were added into Murashige and Skoog (MS) medium to observe the effect of PGRs on the growth and development of *Dendrobium* sp. orchid. Leaf tips of *Dendrobium* sp. were used as explants and inoculated on MS medium supplemented with 2, 4 D (0, 0.5, 2.5, 5, 10 mgL^{-1}) for development of PLBs. The maximum PLBs formation (90%) and the maximum number of PLBs (16.00) were observed in 10 mgL^{-1} 2, 4-D into MS medium after 60 days of culture. Subcultured PLBs were inoculated on MS medium supplemented with different combinations of NAA (0, 0.5, 2.5, 5 mgL^{-1}) and BAP (0, 0.5, 2.5, 5 mgL^{-1}) for shoot regeneration. The maximum number of shoot (11.00), the highest fresh weight (0.6233g) and the highest shoot length (3.613 cm) were observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP after 60 days of culture. Even, the maximum number of root (4.00), the maximum root length (1.627cm) and the maximum plantlet regeneration percentage (93.33%) were observed with the combined effect of 0.5 mg NAA and 0.5 mg BAP after 60 days of culture. Finally, regenerated plantlets were transferred into half strength MS medium to obtain plants.

Key words: Growth regulator, Plantlets, PLBs (Protocorm like bodies), Subculture

Introduction

Orchids are costly for their marvelous long lasting flowers. There are many varities of orchid like *Bulbophyllum, Epidendrum, Dendrobium, Pleurothallis, Vanilla Muchara, Vanda* etc found in different parts of the world. In the world of them *Dendrobium* has more than 1100 species . They are widely distributed throughout the world ranging from southern Asia to New Guinea and Australia (Puchooa, 2004). They exhibit incredible range of diversity in shape, size and color of their flowers. Several orchid species are cultivated for their various economic uses especially in floriculture. Though orchids are grown primarily as ornamentals, many are used as herbal medicines, food, and other cultural value by many different cultures and tribes in the different parts of the world (Khasim *et al*., 1999). Naturally, orchids have a long juvenile period and needs symbiotic association with mycorrhiza for seed germination and further development (Ovando *et al*., 2005). On the other hand, many orchid species are threatened due to their habitat destruction and indiscriminate collection. Plant tissue culture techniques will circumvent these limitation by providing necessary inorganic and organic nutrients for growth and development. Moreover, tissue culture techniques have been widely used for the *in vitro* mass multiplication of several commercially important orchids (Kanjilal *et al*., 1999; Malabadi *et al*., 2004).

Several orchid species had been successfully propagated *in vitro* using different explants such as leaves, flower inflorescence, roots, and pseudobulb (Martin K. P., Madassery, 2006). Though the available literature reveals the accomplishment of many investigators to propagate orchids *in vitro*, there are fewer reports on *in vitro* culture of *Dendrobium* using leaf tip specially in Bangladesh. The present study was carried out with the following objectives to establish an efficient *in vitro* regeneration protocol for large scale propagation and effective conservation of this highly prized orchid species.

- To establish a suitable protocol for the proliferation of protocorm like bodies.
- To find out effective concentrations of plant growth regulators for proliferation of shoots and formation of roots.

Materials and Methods

Leaf tips of *Dendrobium* sp. of orchid were used as explants of this experiment. Explants were placed on the MS (Murashige and Skoog, 1962) medium supplemented with plant growth regulatrors. The pH of the medium was adjusted to 5.2 with 1N KOH or HCl prior to autoclaving for 15 min at 121^0C. Full strength MS medium was used for the development of PLBs (Protocorm Like Bodies) from leaf tips and shoots regeneration from PLBs. Besides, half strength MS medium was also used for the subculture of PLBs and development of roots. Five different concentrations namely 2, 4-D (0, 0.5, 2.5, 5, 10 mgL^{-1}) were added to MS medium for the development of PLBs from leaf tips. For the development of shoot, we used BAP (0, 0.5, 2.5, 5 mgL^{-1}) and NAA (0, 0.5, 2.5, 5 mgL^{-1}) in different combinations. The culture vials containing the media were autoclaved with 1.16 kg/cm^2 of pressure at 121^0c for 20 minutes. All the culture vials were placed in a culture room and allowed to grow at 25±1°C under 16 hour photoperiod illuminated with fluorescent tube of 2000-3000 lux. After 60 days of culture, data (number and length of PLBs, root, shoot) were recorded. MSTATC software was used to analyze the data by ANOVA1 function .

Results and Discussion

Effect of 2, 4-D on growth and development of PLBs from leaf tips

Number of PLBs per vial

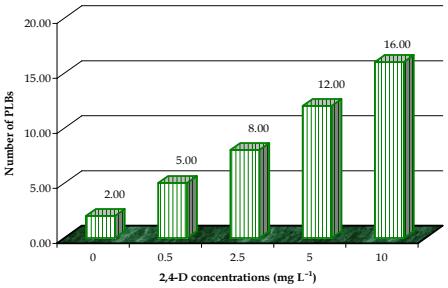
Concentration of 2, 4–D significantly influenced the plant development (Samad, 2011). 2, 4-D is a synthetic auxin, which is a class of plant hormones. It is absorbed through the leaves and is translocated to the meristems of the plant. The maximum number of PLBs (16.0) was observed in 10 mgL^{-1} 2, 4-D (Fig.1.a,) and minimum number of PLBs (2.0) was found at 0 mgL^{-1}2, 4-D after 60 days of culture using leaf tips (Fig. 1-a.). High concentration of 2, 4-D has the ability to proliferate plant within very short time (Fig.2.a). This result partially supports the findings of Jaime and Teixeira (2014) who showed the fresh weight of plantlets and PLBs production increased in the presence of high levels ($8mg^{-l}$) of 2, 4-D.

PLBs formation (%)

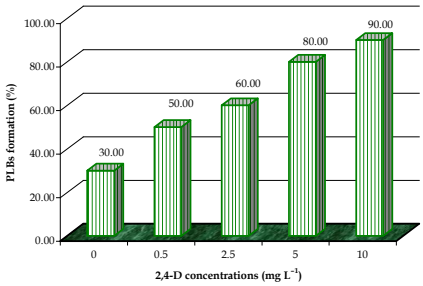
High concentration of 2, 4-D influenced the response of plant regeneration (Gaj ,2004). The maximum PLBs formation response (90%) was observed in 10 mgL^{-1} 2, 4-D and minimum response (30%) was found at 0 mgL^{-1} 2, 4-D after 60 days of culture (Fig.1.b). This result partially supports the observation of Lee (1999) who treated *Cymbidium* orchid with 2, 4-D and PLBs formation response was accelerated with the 2, 4-D concentration.

Subculture of PLBs

Proliferated PLBs were subcultured onto half strength MS medium. Huge number of PLBs (Fig. 2.b) was found by the subculture within few days (Fig.2.c).



a



b

Fig. 1. a. Effects of 2, 4-D on the number of PLBs formation from leaf tips and b. Effects of different concentrations of 2, 4-D on PLBs formation (%) after 60 days of culture

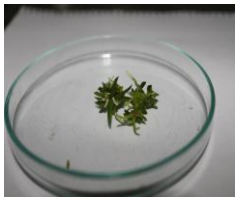
a

b

c

Fig. 2. a. Initiation of PLBs from single leaf tip of *Dendrobium* sp. orchid on MS medium supplemented with 10 mgL^{-l} 2, 4–D after 22 days of culture b. Proliferation of PLBs in MS medium supplemented with 10 mgL^{-l} 2,4-D after 60 days of culture c. Subculture of PLBs into half strength MS media.

a



b

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Fig. 3. a. Initiation of shoot in MS medium supplemented with 0.5mgL^{-l} NAA+ 0.5 mgL^{-l} BAP , b. Proliferation of roots of *Dendrobium* sp. orchid on half strength MS medium after 60 days of culture and c. Hardening of rooted plantlets

Combined effect of NAA and BAP on plant regeneration

Effect on shoot initiation

MS medium supplemented with different concentrations and combination of NAA and BAP significantly influenced the number, weight and height of shoots. The maximum number of shoots (11.00) was

observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP (Fig.3.a). Again minimum number of shoots (0.66) per vial was found at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP followed by 5 mgL^{-1} BAP + 0 mgL^{-1} NAA after 60 days of culture (Table-1). BAP is a first-generation synthetic cytokinin that elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division, whereas, auxin like NAA participate in local and long-distance signalling, with the same transport mechanism as purines and nucleosides. Although high concentration of auxin and cytokinin sometimes cause toxicity. The shoots length affected significantly due to supplement of NAA and BAP into the medium. After 60 days of culture, the maximum shoot length (3.61cm) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and minimum shoot length (1.33 cm) was found at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP (Table-1). This result partially supported by Khatun (2005) who showed that 0.5 mg each of BAP and NAA performed better growth and development of orchid.

Fresh weight of shoot was also significantly affected by combination of NAA and BAP. The maximum fresh weight (0.62g) of was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and minimum fresh weight (0.06g) of shoots was found (Prasad *et al*. 2001) at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP (Table-1).

Effect on root initiation

High concentration of cytokinin induces growth of shoot buds, while high concentration of auxin induces root formation (Khatun, 2005). The maximum number of roots (4.00) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP (Fig.3.b) and minimum number of roots (0.33) per vial was found at 5 mgL^{-1} NAA + 5 mgL^{-1} BAP (Table1). These result were supported by Nayak *et al.* (1998) who observed that a NAA and BAP combination induced rooting in regenerated shoots thereby producing complete plantlets. Significant effect was observed on the effect of NAA and BAP which influenced the roots length after 60 days of culture. From table 1 the maximum root length (1.62cm) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and minimum root length (0.41cm) was found at 5 mgL^{-1} NAA + 5 mgL^{-1} BAP. This result partially supported by Khatun (2005) who showed that 0.5 mgL^{-1} each of BAP and NAA performed better growth and development of orchid. However, high concentrations of auxin inhibit root elongation instead of enhance adventitious root formation. Similarly, cytokinin also can not work properly in high concentration.

Table 1. Combined effect of different concentrations of NAA and BAP on development of shoot initiation, root formation and plantlet regeneration at 60 days after culture

Treatment combinations (mg L^{-1})		Number of shoots	Weight of shoots (g)	Length of shoot (cm)	Number of roots	Length of root (cm)	Planlet regeneration (%)
BAP	NAA						
0	0	0.667 j	0.0667 h	1.333 f	1.000 efg	0.817 ef	26.67 h
	0.5	3.000 ef	0.3200 cde	2.610 bcde	3.333 c	1.393 abc	53.33 de
	2.5	1.667 hi	0.2100 efgh	2.000 def	2.000 d	1.227 bcd	53.33 de
	5.0	1.333 hij	0.1300 fgh	1.827 ef	0.667fg	0.630 fg	33.33 gh
0.5	0	2.000 gh	0.2200 efg	2.333 cde	2.000 d	1.217 bcd	53.33 de
	0.5	11.00 a	0.6233 a	3.613 a	4.000 a	1.627 a	93.33 a
	2.5	5.667 c	0.3767 cd	3.000 abc	3.333 c	1.517 a	80.00 b
	5.0	3.667 de	0.3433 cde	2.720 abcde	1.000efg	1.183 bcd	60.00 cd
2.5	0	1.333 hij	0.2100 efgh	1.813 ef	1.333fg	0.953 de	40.00 fg
	0.5	6.667 b	0.5400 ab	3.377 ab	3.667 b	1.557 a	80.00 b
	2.5	4.000 d	0.4233 bc	2.827 abcd	2.667 c	1.443 ab	66.67 c
	5.0	3.000 ef	0.3333 cde	2.277 cde	0.667fg	0.730 ef	53.33 de
5.0	0	0.667 j	0.1200 gh	1.337 f	0.667fg	0.723 ef	33.33 gh
	0.5	5.667 c	0.4067 bcd	2.810 abcd	1.667de	1.133 cd	66.67 c
	2.5	2.667 fg	0.2733 def	2.523 bcde	1.000efg	0.947 de	46.67 ef
	5.0	1.000 ij	0.2000 efgh	2.000 def	0.333g	0.413 g	46.67 ef

Each treatment had three replications and 5 PLBs were cultured per vial.
** = Significant at 1% level of probability

Plantlet regeneration (%)

NAA and BAP had influenced significantly on the percentage of plantlet regeneration. Although individually BAP and NAA can able to regenerate plant but BAP along with NAA was very effective for plant regeneration. The highest percentage of plantlets

regeneration (93.33%) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and the lowest plantlets regeneration (26.67%) was found at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP (Table-1).

Hardening of in vitro grown plantlets

The plantlets were taken out from the vials carefully and washed under tap water to remove medium from the basal part of plantlets. Then a plastic pot was taken and a small piece of aluminium foil was wrapped inside the plot. After that, wooden powder and charcoal mixture was set into the small plastic pot. Then basal part of the plant was planted into the pot. The plantlets were kept in the hardening room under shade and supplied water two times a day as fog and given gradual exposure to light (Fig.3.c). The hardening room temperature was 25 ± 1^0 C. The light was provided by the fluorescent tube for 16 hours per day in the culture room.

Conclusions

The present study was undertaken with a view to investigate the effect of different plant growth regulators (PGRs) on growth and development of plantlets derived from PLBs using leaf tips. Leaf tips and PLBs were cultured on MS medium supplemented with different concentrations of 2, 4-D, NAA and BAP. It can be concluded from the obtained result that MS medium supplemented with 10 mgL^{-1} 2, 4-D could be used for the production of PLBs from leaf tips and MS medium supplemented with 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP could be recommended for the large scale production of shoots from PLBs as well as for the highest development of root.

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