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Studies on *in vitro* regeneration of orchids (*Dendrobium nobile)* using shoot explant

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Abstract
Shoot tips of *Dendrobium* sp. were used as explants and inoculated on MS medium at different concentrations. The maximum callus formation (83%) and the maximum number of callus initiated (12.00) were observed in combination of NAA and BAP at 1mgL^{-1} into MS medium after 80 days of culture. Subcultured callus were inoculated on MS medium supplemented with different combinations of NAA (0.5, 1.0, 1.5, 2.0 mgL^{-1}) and BAP (0.5, 1.0, 1.5, 2.0 mgL^{-1}) for shoot regeneration. The maximum number of shoot (12.00), the highest shoot length (3.613 cm) and the plantlet regeneration were observed in 0.5 mgL^{-1} NAA + 1.0 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 regeneration percentage (91.44%) were observed in 0.5 mgL^{-1} NAA + 1.0 mgL^{-1} BAP after 60 days of culture. Finally, regenerated plantlets were transferred into half strength MS medium to obtain plants.

Keywords: Callus, MS media, subculture, shoot

Introduction
Orchids are the most beautiful flowers in God's creation, comprise a unique group of plants. 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). In Dendrobium, due to absence or restricted seed setting in hybrids, the propagation via seeds is difficult. Conventional propagation of hybrids by the division of offshoots, the plants are very slow and consume years to develop. Now-a-days *In vitro* culture has proved particularly useful for plants, which is difficult to propagate with conventional techniques. Large numbers of genetically identical plants can be produced rapidly. Genetic modifications can be introduced into thousands of plant quickly, after modifying only a few plants. Species that are difficult to grow from seeds or from cuttings can be produced. So, we have conducted the experiment by taking the following objectives**.** To study the effect of different concentrations of hormones on callus initiation**.** To identify the effective concentration of plant growth regulators for shoot and root formation.

Materials and methods
Shoot 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 HCl prior to autoclaving for 15 min at 121 ^{0}C. Full strength MS medium was used for the development of callus from shoots. Besides, MS medium was also used for the subculture of callus and development of roots. Five different concentrations namely NAA (0.5, 1.0, 1.5, 2.0, 2.5 mgL^{-1}) were added to MS medium for the development of callus from shoot tips. For the development of shoot, we used BAP (0.5, 1.0, 1.5, 2.0, 2.5 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/cm2 of pressure at 121^{0}c for 20 minutes.

The application of the vortex-assisted MSPD method to the analysis of real samples showed TCS in some fish liver and fish gill samples at trace levels. 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.

Results and Discussion

Effect of NAA and BAP on growth and development of callus initiation from shoot tips

Mean Number of callus initiated

Concentration of NAA and BAP significantly influenced the plant development (Riva, 2013). The maximum number of callus (12.0) was observed in combination of NAA, BAP at 1.0 mgL^{-1} (Fig.1.a,) and minimum number of callus (2.0) was found at 2.5 mgL^{-1} NAA after 80 days of culture using shoot tips (Fig. 1-a.). The higher concentration of NAA the less amount of callus induction took place. This results partially supports the findings of S.S. Riva (2013) who showed the highest number of callus induction took place at combination of NAA and BAP.

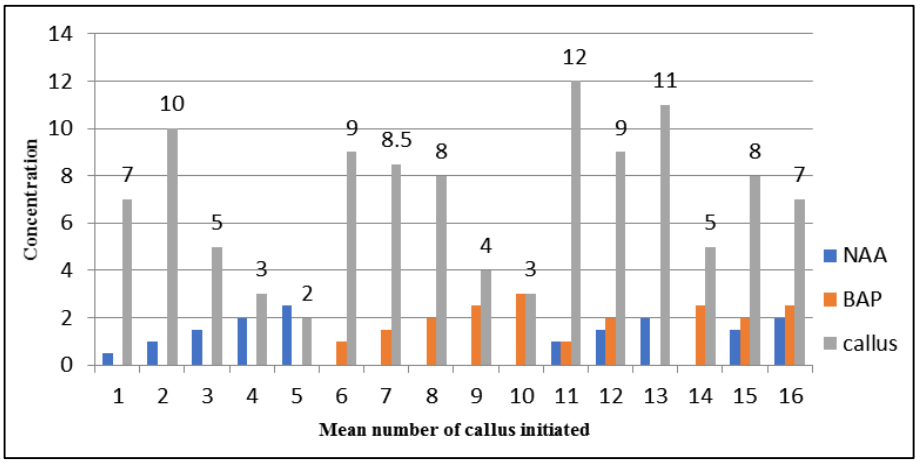

Fig 1: Effect of different concentrations of NAA and BAP on callus initiation

Combined effect of NAA and BAP on plant regeneration

Effect of shoot initiation

MS medium supplemented with different concentrations and combination of NAA, IBA and BAP significantly influenced the number, weight and height of shoots. The maximum number of shoots (12.00) was observed in 0.5 mgL^{-1} NAA + 1.0 mgL^{-1} BAP (Fig.2.). Again minimum number of shoots (0.66) per vial was found at 2.0 mgL−1 NAA + 1.5 mgL−1 BAP 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.

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 (mgL^{-1})		Number of shoots	Weight of shoots (g)	Length of shoot (cm)	Number of roots	Length of root (cm)	Plantlet regeneration (%)
BAP	NAA						
0.5	0.5	1.66	0.667	1.26	2.33	0.256	63.33
	1.0	2.33	0.326	2.25	1.93	0.325	52.63
	1.5	1.76	0.210	2.10	2.06	1.256	44.32
	2.0	0.76	0.166	1.69	1.96	0.630	33.33
1.0	0.5	12.33	0.625	3.61	3.50	2.325	91.44
	1.0	5.66	0.456	3.06	2.23	0.636	84.33
	1.5	4.33	0.266	2.76	2.56	1.565	76.66
	2.0	0.69	0.336	2.06	2.75	0.761	65.33
1.5	0.5	7.33	0.544	2.86	2.33	0.565	65.44
	1.0	3.00	0.336	2.53	1.66	0.825	34.24
	1.5	2.66	0.226	2.23	2.06	1.236	55.33
	2.0	1.33	0.132	1.86	1.77	1.069	42.44
2.0	0.5	3.66	0.407	2.86	3.56	0.568	54.33
	1.0	2.00	0.266	2.73	2.66	0.869	45.22
	1.5	0.66	0.132	1.93	1.23	0.635	35.23
	2.0	1.00	0.262	2.23	2.56	0.325	26.67

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 + 1.0 mgL−1 BAP and minimum shoot length (1.33 cm) was found at 2.0 mgL^{-1} NAA + 1.5 mgL^{-1}BAP (Table-1). This result partially supported by Khatun (2005) who showed that 1.0 mg each of BAP and NAA performed better growth and development of orchid.

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 (3.50) was observed in 0.5 mgL−1 NAA + 1.0 mgL−1 BAP (Fig.3.b) and minimum number of roots (0.33) per vial was found at 2mgL−1 NAA + 2 mgL−1 BAP (Table1). These results 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 (1.32cm) was observed in 1.0 mgL−1 NAA + 1.0 mgL−1 BAP and minimum root length (0.32 cm) was found at 2 mgL−1 NAA + 2mgL−1 BAP. This result partially supported by Khatun (2005) who showed that 1.0 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 cannot work properly in high concentration.

A. **B.**

Fig 1: Effects of NAA and BAP at different concentrations for callus induction

Fig 2: Sub culturing of callus and shoot formation and root initiation

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 (91.44%) was observed in 0.5 mgL−1 NAA + 1.0 mgL−1 BAP and the lowest plantlets regeneration (26.67%) was found at 2.0 mgL−1 NAA + 2.0 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

Conclusion

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 NAA and BAP. It can be concluded from the obtained result that MS medium supplemented with could be used for the production of callus from leaf tips and MS medium supplemented with 0.5 mgL−1 NAA + 1.0 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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