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IN VITRO GROWTH AND DEVELOPMENT OF *DENDROBIUM* HYBRID ORCHID

H. KHATUN[1], M. M. KHATUN[2], M. S. BISWAS[3]
M. R. KABIR[4] AND M. AL-AMIN[5]

Abstract

The experiment was conducted to investigate the combined effect of different plant growth regulators and charcoal supplementation in MS medium on growth and development of plantlets regenerated from protocorm like bodies (PLBs) of hybrid orchid. The combination of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA at different concentrations with charcoal supplementation was studied. The result revealed that the use of different growth regulators had significant effect on different parameters studied. The maximum weight of PLBs (5.123 g) was obtained from the combination of BAP + IBA at 1.0 mg/l each. The highest shoot height (3.239 cm) and maximum number of rooted plantlets (4.473) was obtained from 1.0 mg/l each of BAP + NAA combination. The maximum number of leaves (3.490) and the maximum length of leaves (1.946 cm) were obtained from 1.0 mg/l each of BAP + IBA and the highest leaf width (1.166 cm) was obtained from 0.5 mg/l BAP +1.0 mg/l IBA combination. The highest root length was obtained from 0.5 mg/l each of BAP + IAA and the maximum number of regenerated plantlets (20) was obtained from 0.5 mg/l IAA + 1.0 mg/l IBA combination. However, the maximum fresh weight of single shoot (0.219 g) and the maximum number of roots per plantlet (6.300) was obtained from 1.0 mg/I each of IAA + IBA combination.

Keywords; *Dedrobium*, orchid, hybrid, *In vitro* growth.

Introduction

Orchids belong to the largest and most diverse family Orchidaceae consisting of about 700-800 genera and more than 25000 species (Begum, 2000). They have flowers of wonderful beauty and very good keeping qualities. It has a great value as cut flowers and indoor decoration. Among the orchid genera, *Dendrobium* is one of the most popular orchids all over the world including Bangladesh. Rapid growth, easiness of plantlet regeneration, beauty of flower, year round production in control flowering and long lasting of the flower stalk are the advantages of *Dendrobium* (Talukder *et al.*, 2002). *Dendrobium* hybrid is the most popular orchid for cutflower trade in Asia. Thailand alone exports *Dendrobium* more than $ 12 million to Europe and Germany (Rao, 1977). About 70% of total orchid exports of Singapore were *Dendrobiums* (Singapore Orchid Industry, 2004).

[1]Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh, [2,3,4&5]Biotechnology Division, Bangladesh Agricultural Research Institute, (BARI), Gazipur, Bangladesh.

Generally, orchids are propagated both asexually and sexually. But the traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year (Nasiruddin *et al.,* 2003). Micropropagation of orchids is the most frequently used convenient technique for their exploitation as a major trade in developed countries (Goh and Tan, 1982; Sagawa and Kunisaki, 1982). It is the only method currently available (Goh *et al.,* 1992) which initially starts in early 1960 (Bose *et al.,* 1986). Because commercially mass propagation is possible by producing millions of plantlets using tissue culture techniques (Lim *et al.,* 1985). Orchids can be rapidly propagated through tissue culture techniques by using shoot tips (Saiprasad *et al.,* 2002), leaf (Chen *et al.,* 2001), and stem nodes (Pathania *et al.,* 1998). Due to difficulties in protocorm regeneration and plantlet formation, all procedures mentioned above have become inadequate for meeting commercial need for vegetative propagation. Some of these methods gave a lot of protocorms but they develop slowly or poorly to vital plants. Therefore, the present study was undertaken to investigate which concentration and combination of growth regulators is better for plantlet regeneration and the subsequent growth and development of *Dendrobium* hybrid orchid with charcoal supplementation.

Materials and Method

The experiment was carried out at the laboratory of Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The protocorm like bodies (PLBs) were used as plant material which was obtained from a pod of *Dendrobium* hybrid orchid. Murashige and Skoog (1962) medium was used for culturing the planting materials. The MS medium was supplemented with different plant growth regulators (BAP, NAA, IAA, and IBA) and charcoal. Twenty grams of sucrose, 8.0 g of agar and 1.0 g charcoal were used per litre of the medium. The pH of the medium was adjusted to 5.8 before adding agar. The media were autoclaved at 1.1 kg/cm^2 pressure and 121°C for 20 mn. The inoculated cultures were incubated at 26 ± 1°C under approximately 2000 lux by cool-white fluorescent tubes for 16/8 light/dark cycle. Explants were subcultured at four weeks intervals or when necessary. Experiments were carried out in a completely randomized design (CRD) with three replications.

The concentrations of growth regulators were used 0.5, 1.0, 2.0 mg/l in case of BAP, NAA, and IAA, while 1.0 mg/l in IBA and 1.0 g/l. charcoal. Data were collected at 60 days after inoculation (DAI) for weight of PLBs, height of shoot, number of leaves per plantlet, length of leaves, width of leaves, fresh weight of single shoot and number of regenerated plantlets per sample. Data collected were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The protocorm like bodies (PLBs) were cultured in MS medium supplemented with different combinations and concentrations of growth regulators (0.5, 1.0, and 2.0 mg/l each of BAP, NAA, IAA, and 1.0 mg/l IBA) and also 1.0 g/l charcoal. Results have been presented in Table I and proliferation of PLBs and plantlet formation are shown in Fig. 2.

Table 1. Combined effect of different growth regulators with charcoal supplementation on *in vitro* growth and development of PLBs of hybrid orchid at 60 days after inoculation.

Concentration (mg/l)		Wt of PLBs (g)	Height of shoot (cm)	No. of leaves	Length of leaves (cm)	Width of leaves (cm)	Fresh wt of single shoot (g)	No. of rooted plantlets	No. of roots/ plantlet	Length of root (cm)
BAP	NAA									
0	0	2.083d	0.840d	1.083c	0.846b	0.116c	0.024b	0.000d	0.000d	0.000c
0.5	0.5	3.330b	1.083c	2.120a	0.626c	0.226b	0.133a	3.287b	1.810c	0.0l0c
1.0	1.0	4.690a	3.239a	1.187bc	1.710a	0.356a	0.213a	4.473a	2.477b	1.080a
2.0	2.0	2.217c	2.150b	1.283b	0.800bc	0.290ab	0.029b	1.441c	3.117a	0.493b
BAP	IAA									
0	0	2.183d	0.846d	1.082c	0.817b	0.126b	0.025b	0.000d	0.000d	0.000d
0.5	0.5	2.637c	1.494c	2.120a	0.745b	0.166b	0.015b	1.183b	2.533c	2.722a
1.0	1.0	4.816a	2.527a	1.66Th	1.850a	0.259a	0.136a	3.183a	3.296b	1.423c
2.0	2.0	3.623b	2.083b	1.173c	0.833b	0.155b	0.028b	1.140c	4.080a	1.767b
BAP	IBA									
0	0	2.082d	0.817d	1.075d	0.786d	0.124c	0.026d	0.000d	0.000d	0.000d
0.5	1.0	4.450b	1.530c	2.157c	1.583b	1.166a	0.143a	1.100c	1.000c	0.569b
1.0	1.0	5.123a	2.800a	3.490a	1.946a	0.206b	0.079b	2.417a	2.320a	1.000a
2.0	1.0	3.640c	1.762b	2.467b	1.267c	0.107c	0.057c	1.407d	1.300b	0.405c
IAA	IBA									
0	0	2.0806	0.824d	l.081c	0.826b	0.115c	0.024b	0.000d	0.000c	0.000d
0.5	1.0	2.167c	1.870b	1.178c	0.529c	0.4623a	0.040b	3.467b	3.167b	1.250
1.0	1.0	4.514a	2.500a	2.680a	1.757a	0.225b	0.219a	4.417a	6.300a	2.347a
2.0	1.0	3.490b	1.243c	1.49Th	0.799b	0.102c	0.015b	2.917c	3.267b	2.064b

Means followed by a common letter are not significantly different at *5%* level

Weight of PLBs: Significant variation in weight of PLBs was found with various combinations of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA. In case of BAP + NAA, the highest weight of PLBs was obtained from 1.0 mg/l each of BAP + NAA and the lowest from control treatment (Table 1). In case of BAP + IAA, the highest weight of PLBs was obtained from 1.0 mg/I each of BAP + IAA

and the lowest was also obtained from control treatment. Similar results were obtained from BAP + IBA and IAA + IBA treatment combination. Among all treatment combinations, the highest weight of PLBs was obtained from 1.0 mg/l each of BAP + IBA. Results indicated that in all treatment combinations, 1.0 mg/l each of growth regulators is found suitable for increment of PLBs

Height of shoot: The combined effect of charcoal and different combinations of growth regulators on shoot height showed significant differences at 60 DAI. The highest shoot height was obtained from 1.0 mg/l each of BAP + NAA followed by BAP + IBA at the same concentration (Table 1). The results are in agreement with the findings of Prasad *et al.* (2001). They found that the highest mean shoot height was obtained from 1.0 mg/l each of BAP + NAA on MS medium. On the other hand, in case of IAA + IBA combination, the maximum height was obtained from l.0 mg/l each of IAA + IBA. Results indicated that combination of cytokinin and auxin gave good result for shoot height. Results are in agreement with the findings of Khatun *et al.* (2008) where they reported that lower concentration of auxin and cytokinin favoured the enhancement of plantlet growth.

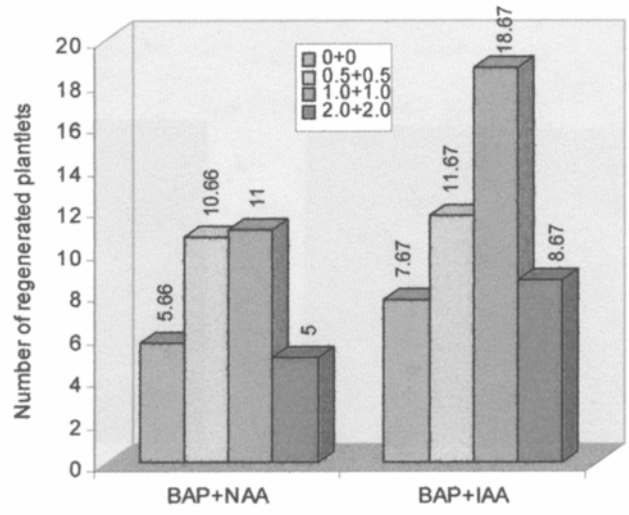
Number of leaves: The number of leaves produced per plantlet showed significant variation at various combinations of growth regulators. The highest number of leaves was obtained from 1.0 mg/l each of BAP + IBA followed by IAA + IBA at the same concentration. Whereas, the minimum was recorded at control treatment of all combinations of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA.

Length of leaves: Significant variation was observed in leaf length among different combinations and concentrations of growth regulators. In case of BAP + NAA, the highest leaf length was obtained from 1.0 mg/l of each BAP + NAA and the lowest from 0.5 mg/l each of BAP + NAA (Table 1). In BAP + IAA combination, similar results were obtained where 1.0 mg/l each of BAP + IAA produced highest leaf length. Among all combinations, highest leaf length was obtained from 1.0 mg/l each of BAP + IBA, while the lowest was observed from 0.5 mg/l IAA + 1.0 mg/l IBA treatment combination.

Width of leaves: The combined effect of different growth regulators showed significant variation on width of leaves. In case of BAP + NAA, BAP + IAA, the highest width of leaves was observed in 1.0 mg/l each of BAP + NAA and BAP + IAA and the lowest was obtained from control treatment (Table 1). Among all the treatment combinations, the highest width of leaf was obtained from 0.5 mg/l BAP + 1.0 mg/l IBA and the lowest from 2.0 mg/l JAA +1.0 mg/l IBA.

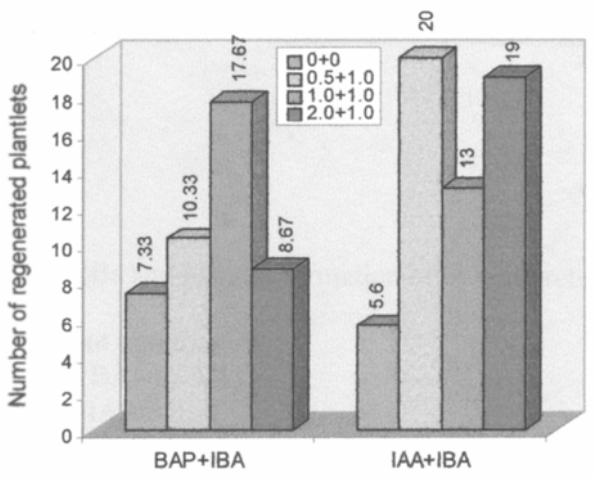
Fresh weight of single shoot: Significant variation was observed in fresh weight of single shoot in all the treatment combinations. The maximum fresh weight of shoot was obtained from 1.0 mg/l each of IAA +IBA followed by BAP + NAA at

the same concentration (Table 1). On the other hand, the minimum was recorded from 0.5 mg/l each of BAP + IAA and 2.0 mg/l IAA + 1.0 mg/I IBA treatment combination.



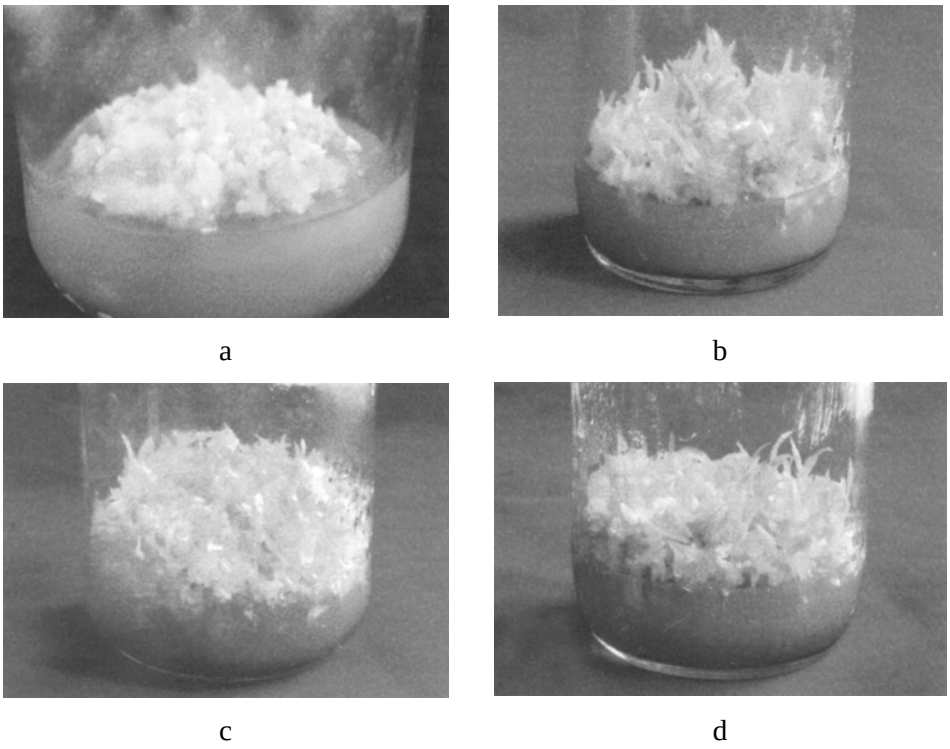
Plant growth regulartors

Fig. 1a. Combined effect of different concentrations of BAP+NAA and BAP+IAA on regenerated plantlets at 60 DAI.



Plant growth regulartors

Fig. 1b. Combined effect of different concentrations of BAP+IBA and IAA+IBA on regenerated plantlets at 60 DAI.



a b

c d

Fig.2 (a-d). Proliferation of PLBS and plantlet formation of hybrid orchid in MS medium at 60 DAI.

a) Control (without hormone).
b) 1 mg/l each of BAP+NAA.
c) 1 mg/l each IAA+IBA.
d) 1 mg/l each of BAP+IBA.

Number of rooted plantlets: A significant difference was found on number of rooted plantlets among the combined effects of charcoal and different combinations and concentrations of growth regulators. The maximum number of rooted plantlets was observed from 1.0 mg/l each of BAP + NAA followed by IAA + IBA at the same concentration (Table 1). On the other hand, control treatment did not produce any root.

Number of roots per plantlet: The number of roots produced per plantlet showed significant variation at various concentrations and combinations of growth regulators. In case of BAP + NAA, the highest number of roots was obtained from 2.0 mg/l each of BAP + NAA and control treatment did not produce any root. Similar results were also obtained from BAP + IAA treatment combination, where maximum number of roots was obtained at 2.0 mg/l each of BAP + IAA. On the other hand, the maximum number of roots was obtained

from 1.0 mg/l each of BAP + IBA and LAA + IBA. In overall treatment combinations, the highest number of roots (6.3) was obtained from 1.0 mg/l each of IAA + IBA (Table 1). Result indicated that auxin treatment combination gave the highest number of roots than auxin and cytokinin combination. Similar observations were also reported by Khatun and Al-Amin (2006).

Length of root: A significant difference was found on root length at different concentrations and combinations of growth regulators. In case of BAP + NAA treatment combination, the maximum root length was obtained from 1.0 mg/l each of BAP + NAA and no root was observed in control treatment as a result root length was not measured. On the other hand, among all the treatment combinations, the maximum root length was obtained from 0.5 mg/l each of BAP + IAA followed by 1.0 mg/l each of IAA + IBA.

Number of regenerated plantlets: Statistically significant variation was noticed on number of regenerated plantlets with different combinations and concentrations of growth regulators. The highest number of regenerated plantlets (20.00) was obtained from 0.5 mg/l IAA + 1.0 mg/l IBA followed by BAP + IAA (18.67) at 1.0 mg/I (Fig. 1a & 1b). The results are in agreement with the findings of Khatun *et al.* (2007) where they reported the highest number of regenerated plantlets at 1.0 mg/l of each BAP + IAA. On the other hand, the lowest number of plantlets (5.00) was obtained from 2.0 mg/l each of BAP + NAA.

The results from the present study suggest that the hormonal combination of BAP + IBA is found suitable for shoot regeneration and for root development; IAA + IBA combination is more suitable. On the other hand, the combined effect of growth regulators, 1.0 mg/l each of growth regulators is found suitable.

Conclusion

Considering the above results, the combination of BAP + IBA is the best for regeneration of *Dendrobium* hybrid orchid and the best concentration of each growth regulator was 1.0 mg/l.

References

Begum, F. 2000. Training Courses on Orchid Production in Bangladesh organized by Hortex Foundation, 5th June, 2000. BARI, Joydebpur, Bangladesh. pp: 4-5.

Bose, T.K., S.K. Mitra and M.K. Sadhu. 1986. Propagation of Tropical and Sub-tropical Horticultural Crops. Naya Prokash, Calcutta, India. pp.481-510.

Chen, J. T., W.C. Chang, J. K. Chan and W.C. Chang. 2001. Effect of auxins and cytokinins on direct somatic embryogenesis on leaf explants of *Oncidium* "Gower Ramsey." Plant Growth Regulation **34** (2): 229-232.

Goh, C.J. and H. Tan. 1982. CIonal propagation from leaf explants in *Renantanda* orchid hybrid. *Orchid Rev.* **90:** 295-296.

Gob, C.J. A.A. Sim and G. Lim. 1992. Mycorrhizal associations in some tropical orchids. *Lindleyana* **7** (1): 13-17.

Khatun, M. M. and M. Al-Amin. 2006. *In vitro* germination and micropropagation from F_1 seeds of *Dendrobium* orchid. *Bangladesh J. P1. Breed. Genet.* **19**(2): 37-42.

Khatun, H., M. M. Khatun, D. Khanam, M. A. Malek and M. Al-Amin. 2007. Effect of different plant growth regulators on plantlet regeneration in *Dendrobium* hybrid orchid. *Bangladesh J. Agril. Res.* **32**(4): 629-635.

Khatun, M. M., H. Khatun, D. Khanam, M. A. Malek and M. Al-Amin. 2008. *In vitro* culture of orchid. *Bangladesh J. Agril. Res.* **33**(2): 319-325.

Lim-Ho, C.L., G.C. Lee and L.K. Phua. 1985. Clonal propagation of orchids from flower buds. Proc. 50 th Asian Orchid Cong. A.N. Rao (ed.). 1984, Singapore. p. 90-1 10.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15:** 473-497.

Nasiruddin, K.M., R. Begum and S. Yasmin. 2003. Protocorm like bodies and plantlet regeneration from *Dendrobium formosum* leaf callus. *Asian Journal of Plant Sciences* **2**(13): 955-957.

Pathania, N. S., O.P. Sehgal, P. Debojit, B.S. Dilta and D. Paul. 1998. Studies on micropropagation in *Dendrobium* cv. Sonia. *J. Orchid Soc. India.* **12** (1-2): 35-38.

Prasad, G. V. S. S., I. V. S. Rao and P. V. Reddy. 2001. *In vitro* propagation of orchid. *Dendrobium* cv. Sonia. *Indian J. Plant Physiol.* **6**(3): 284-288.

Rao, A.N. 1977. Tissue culture in the orchid industry. In: Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. J. Reinert and Y.P.S. Bajaj (eds.). McGraw- Hill, New York. pp. 44-69.

Sagawa, Y. and J.T. Kunisaki. 1982. Clonal propagation of orchids by tissue culture. In: A Fujiwara, Plant Tissue Culture, Maruzen, Tokyo. pp. 683-684.

Saiprasad, G. V. S., P. Raghuveer and R. Polisetty. 2002. Effect of various nutrient media on the production of protocorm like bodies (PLBs) and multiple shoots.

Singapore Orchid Industry. 2004. Assignment. Radio Singapore International. Talukder, S. K., K.M. Nasiruddin, S. Yasmin, R. Begum and S. Sarkar. 2002. *In vitro* root formation on orchid plantlets with IBA and NAA. *Progress. Agric.* **13**(1&2): 25-28.