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In Vitro Root Formation in *Dendrobium* Orchid Plantlets with IBA

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ABSTRACT

The study was conducted at the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh during May to June 2006 to investigate the effect of different concentrations of IBA (0, 0.5, 1.0, 1.5 and 2.0 mg/l) with Vacin and Went (VW) medium and charcoal on root formation in Dendrobium orchid. Four parameters such as number of roots plantlet^{-1}, length of root, fresh weight of root and days required for root formation were recorded at three DAIs (10, 20 and 30). The use of different concentrations of IBA had significant effects on the parameters examined. The best results were obtained from 1.0 mg/l IBA treatment in which the number of root was 1.81 plantlet^{-1}, length of root 0.35 cm, fresh weight of root 0.16g at 30 DAI and the minimum days to root formation was 10.8.

Key words: In vitro root formation, *Dendrobium* orchid, IBA.

INTRODUCTION

Dendrobium orchids are the most popular orchid genus. They are ornamental plants and have been increasingly grown in many countries, especially in developed temperate countries of the world as pot plants or cut flowers. The environmental conditions required for survival and culture of orchid are adequately available through out the year in Bangladesh. Commercial cultivation of orchids for plant sale as well as for cut flowers production has developed into sizeable industries in many countries and the sale of flower runs in million dollars a year (Singh, 1998). In Bangladesh, the orchid trade is still in its nascent stage. The stems of *Dendrobium* species are used in making baskets in the Philippines, Indonesia and New Guinea, and pseudobulbs of *D. takai* are used as oral contraceptives (Bose and Bhaltacharjee, 1999). The traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year. Orchid is well known for their exploitation as major trade in developed countries (Sagawa and Kunisaki, 1982). *In vitro* culture has proved particularly useful with groups of plants, which are difficult to propagate using conventional techniques (Fay, 1994). When mass propagation of a new hybrid or a variety is needed within a short time, tissue culture is the only method (Goh *et al*., 1992). This is because mass propagation of orchid in commercial exploitation is possible by tissue culture techniques (Lim *et al*., 1985). Comparatively a rapid multiplication of orchid was reported by Intuwong and Sagawa (1974) using shoot tip of a plant, but excision of shoot tip or axillary bud can kill or damage the

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mother plant seriously. To avoid this problem, the multiple shoot proliferation technique using different hormones can be tried. But these shoot proliferating hormones generally do not form roots. Considering the present idea in the mind, the present study was undertaken to standardize and to develop a suitable IBA concentration for root formation and plantlet establishment.

MATERIALS AND METHODS

The experiment was conducted at the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh during May to June 2006 to investigate the effect of different concentrations of indole 3 butaric acid, IBA (0, 0.5, 1.0, 1.5 and 2.0 mg/l) with Vacin and Went (VW) medium and charcoal on root formation in *Dendrobium* orchid. In this experiment *in vitro* multiple shoot buds of *Dendrobium* orchid were cultured on Vacin and Went medium (Vacin and Went, 1949) supplemented with charcoal and different concentrations of IBA (0, 0.5, 1.0, 1.5 and 2.0 mg/l). The vials were arranged under five treatments with three replications. One month old single *in vitro* multiplied rootless shoot was placed in each vial. The cultured vials were placed in a growth room and allowed to grow at 25±1 ^{0} C and less than 16 hour photoperiod illuminated with florescent tube of 2000 – 3000 lux. The data were collected at 10, 20 and 30 days after inoculation on the following parameters except on days to root formation. Number of roots plantlet^{-1} formed was recorded. Root length in centimeter was measured at the distance from the base of a seedling to the tip of the root. Fresh weight of root in milligram was taken using an electrical balance (BDH, DE Series, Model-100A). The inoculated shoot was observed each day after inoculation and days required for new root formation was recorded. The experiment was laid out in Cmpletely Randomized Design (CRD) with five treatments and three replications. The treatment means were compared based on Duncan's Multiple Range Tests - DMRT (Zaman *et al*. 1983).

RESULTS AND DISCUSSION

The comparative effects of different concentrations of IBA with Vacin and Went medium and activated charcoal on *in vitro* root formation of *Dendrobium* orchid are presented in Table 1 and Fig. 1. The results are discussed parameter wise as under:

Fig. 1. Showing *in vitro* root formation in *Dendrobium* orchid on different concentrations of IBA at 30 DAI

Number of roots plantlet^{-1}

At 10 DAI, significant influence on number of roots plantlet^{-1} was found with IBA. IBA at 1 mg/l showed the highest number (0.20 plantlet^{-1}) of new roots and other treatments did not produce any new root. This result is partially supported by Talukder *et al*. (2002) who did not find any root at the concentrations of 0.0 mg/l, 1.0 mg/l and 5.0 mg/l BAP with MS medium. At 20 DAI, the number of roots plantlet^{-1} with 1 mg/l IBA was found to be the highest (1.2 plantlet^{-1}) and that with 0.0 mg/l IBA

was found to be the lowest (0.20 plantlet^{-1}) among the five treatments. No significant difference was observed in the number of roots plantlet^{-1} between 0.0 mg/l and 2 mg/l IBA. At 30 DAI, no significant difference was observed in the number of roots explant^{-1} among five treatments. The highest (1.81plantlet^{-1}) number of root explant was obtained from 1 mg/l IBA treatment (Table 1). These results were partially supported by Talukder *et al*. (2002), where they found 1.62 roots plantlet^{-1} from 2 mg/l IBA with MS media at 30 DAI. Rovindra *et al*. (2004) observed that the shoots of *Vanda coerulae* produced roots cultured on ½VW medium supplemented with 11.42 μM IAA. Talukder *et al*. (2003) showed that the 1.93 /explant root of the orchid at 2.5 mg/l BAP + 0.5 mg/l NAA. Doods (1991) found that shoots of *Dendrobium* hybrids rooted on VW medium supplemented with 2 mg/l IBA and IAA. Sheelavantmath *et al*. (2000) found that the regenerated shoots of *G. densiflorum* rooted on MS with NAA at 1.0 μM. Vij *et al* (1994) found that root of *Cymbidium pendulum* was the best when activated charcoal was used in to the basal medium.

Table 1. Effect of different concentrations of IBA on *in vitro* root formation of regenerated plantlets in *Dendrobium* orchid

Treatment (mg/l IBA)	Number of roots plantlet^{-1} at different DAIs			Length or root (cm) at different DAIs			Fresh weight of roots (g) at different DAIs			Days to root formation
	10 DAI	20 DAI	30 DAI	10 DAI	20 DAI	30 DAI	10 DAI	20 DAI	30 DAI	
(0.0)	0.00b	0.20b	0.60d	0.00b	0.18c	0.25	0.00b	0.06d	0.08d	16.0c
(0.5)	0.00b	0.30b	0.50d	0.00b	0.12d	0.20	0.00b	0.04c	0.06d	16.2c
(1.0)	0.20a	1.20a	1.81a	0.18a	0.24a	0.35	0.03a	0.12a	0.16b	10.8d
(1.5)	0.00b	0.60b	1.00c	0.00b	0.20b	0.28	0.00b	0.08b	0.10c	17.6b
(2.0)	0.00b	0.40b	1.40b	0.00b	0.19c	0.26	0.00b	0.07c	0.15a	19.4a
CV (%)	11.53	2.99	23.82	23.58	13.60	19.98	19.07	27.03	23.86	17.94

In a column, figures followed by same letter(s) don't differ significantly at P<0.01 as per DMRT

Note: DAI = Days after inoculation
IBA = Indole 3 butatric acid
CV = Coefficient of variation

Length of root

At 10 DAI, the length of single root of the treatment 1 mg/l IBA was significantly higher (0.18 cm) than those of other treatments. At 20 DAI, the length of single root of treatment 1 mg/l IBA was found to be the highest (0.24 cm) and that of treatment of 0.5 mg/l IBA was the shortest (0.12 cm) among the five treatments. No significant difference was observed in length of root of the concentrations between 0.0 mg/l and 2.0 mg/l IBA. At 30 DAI, no significant difference was observed in length of single root among the concentrations. The highest (0.35 cm) and lowest (0.20 cm) lengths of root was obtained from the concentrations 1.0 mg/l and 0.5 mg/l IBA, respectively (Table 1). The result of the present study was supported by Talukder *et al*. (2002), where they obtained 0.51 cm root with 1.0 mg/l IBA in MS medium. Nasiruddin *et al*. (2003) found the highest length of root of *Dendrobium formosum* at 0.5 mg/l 2, 4-D. Lim *et al*. (1985) observed that IBA at 0.1 mg/l was the best for producing tall roots in *D. moniliforms.*

Fresh weight of root plantlet $^{-1}$

At 10 DAI, significant variation of fresh weight of single root was found with different concentrations of IBA. The treatment 1 mg/l IBA showed the highest (0.03 g) fresh weight of root. At 20 DAI, the concentration 1 mg/l IBA gave significantly higher fresh weight of root (0.12 g) than those of other treatments. At 30 DAI, the fresh weight of single root of the treatment 1 mg/l IBA was the highest (0.16 g) and that of treatment 0.5 mg/l IBA was the lowest (0.06 g) among the five treatments (Table 1). This result is in agreement with Pathania *et al*. (1998) who reported that Vacin and Went medium favored rooting when supplemented with 1 mg/l IBA.

Days to root formation

The concentration effect of IBA was found significant for days to root formation. For root initiation, the longest time (19.4 days) was required by 2 mg/l IBA and least time (10.8 days) was required by 1 mg/l IBA (Table 1). Similar results were reported by Talukder *et al*. (2002), where they observed that days to root formation were the minimum (12.2 days) at 1 mg/l IBA while the maximum days (18.5 days) were required at 2.5 mg/l IBA in *Dendrobium* orchid.

CONCLUSION

It can be concluded from the present findings that 1 mg/l IBA might be recommended for root formation in *Dendrobium* orchid in comparison with other concentrations.

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