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IN VITRO ROOT FORMATION AND PLANTLET DEVELOPMENT IN DENDROBIUM ORCHID

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Abstract

The experiment was conducted to investigate the combined effect of different plant growth regulators with and without charcoal supplementation for root formation and plantlet development from protocorm like bodies (PLBS) of orchid. The combination of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA at different concentrations were studied. It revealed that the highest number of roots was obtained from 1.0 mg/L each of IAA + IBA combination (6.667) and the highest root length was recorded from 2.0 mg/L BAP + 1.0 mg/L IBA with charcoal supplementation. The treatment combinations, 1.0 mg/L each of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA were found best for producing more rooted plantlets with charcoal supplementation. It revealed that charcoal enhanced the root formation.

Keywords: Orchid, *Dendrobium*, hybrid, *In vitro* rooting.

Introduction

Dendrobium hybrid is the most popular orchid for cut flower trade in Asia. Thailand alone exports *Dendrobium* more than $ 12 million per year to Europe and Germany (Rao, 1977). About 70% of Singapore total orchid exports were *Dendrobiums* (Singapore Orchid Industry, 2004). Commercially orchids are high demanding costly flower. The environmental conditions required for the growth, development and culture of orchids are adequately available throughout the year in Bangladesh. In developed countries, micropropagation of orchids is the most frequently used convenient technique for their exploitation as a major trade (Goh and Tan, 1982; Sagawa and Kunisaki, 1982). When mass propagation of a new hybrid or a variety is needed within a short period of time, tissue culture is the only method to fulfill that objective (Goh *et al.*, 1992). This is because commercially mass propagation of orchid is possible by producing millions of plantlets using tissue culture techniques (Lim *et al.*, 985). Many studies on micropropagation of orchids have been carried out (Fu, 1978; Lin, 1986; Tanaka, 1987; Kobayashi *et al.*, 1991; Ichihashi, 1992). Tokuhara and Mu (1993) reported that the appropriate combination and concentrations of hormones, organic additives and the composition of macro and micro elements in the culture

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medium were of key importance for micropropagation of *Dendrobium* on commercial scale. Sometimes for better root formation, it needs to add some organic additives, such as activated charcoal in the medium. But information on this regard is scarce. Considering the above idea in mind, the present experiment was conducted to observe the combined effect of different growth regulators with charcoal supplementation for root formation and plantlet development.

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) which were collected from a crossed pod of *Dendrobium* orchid were used as plant material. Murashige and Skoog (1962) medium was used for culturing the planting materials. BAP, NAA, IAA, and IBA were used as growth regulators. Twenty grams of sucrose, 8.0 g of agar 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 mm. The inoculated cultures were incubated at 26 ± 1°C under approximately about 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.

Data recorded from each treatment on number of rooted plantlets per sample, number of roots per plantlet and root length were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

In vitro grown PLBs were cultured in MS medium supplemented with different combinations of growth regulators and the concentration is 0.5, 1.0, and 2.0 mg/L each of BAP, NAA, IAA and 1.0 mg/L IBA. Data were recorded at 120 days after inoculation (DAI) and results have been presented in Table 1 and Fig. Ia , Ib, Ic & Id. Root formation and plantlet establishment are shown in Fig.2 (a-e).

Number of roots per plantlet: Among all the treatments, the highest number (6.667) of roots was developed on the hormonal combination of 1 mg/L of IAA + IBA in the presence of charcoal supplement. Without charcoal supplement, the same treatment also showed second highest number of roots (6.557) induction. It revealed that the hormonal combination of IAA and IBA was the best growth regulator for root induction.

BAP + NAA: A significant effect was noticed on number of roots per plantlet at different concentrations and combinations of BAP + NAA and with or without charcoal supplementation. In without charcoal supplementation, the highest

number of root (3.33) was obtained in 1.0 mg/L each of BAP + NAA followed by 0.5 mg/L each of BAP + NAA (Table 1). On the other hand, the highest number of roots (5.183) was observed at 2.0 mg/L each of BAP + NAA followed by 1.0 mg/L each of BAP + NAA (4.167). Results indicated that root development was the best when activated charcoal was added into the medium. Similar findings were also reported by Vij *et al.* (1994).

Table 1. Combined effect of growth regulators on *in vitro* rooting of orchid at 120 days after inoculation in MS medium.

Growth regulators	Concentrations (mg/L)	Number of roots/plantlet		Length of root (cm)	
		Without charcoal	With charcoal	Without charcoal	With charcoal
BAP+NAA	0+0	0.000d	0.000d	0.000d	0.000d
	0.5 + 0.5	2.000b	3.233c	l.l10b	0.833b
	1.0 + 1.0	3.330a	4.16Th	2.442a	l.203a
	2.0 + 2.0	l.517c	5.183a	0.842c	0.383c
BAP+IAA	0 +0	1.282d	0.000d	0.282d	0.000d
	0.5 + 0.5	3.125c	4.210c	3.576a	2.%7a
	1.0+ 1.0	4.21Th	5.283a	l.583c	2.033b
	2.0 + 2.0	5.220a	4.34Th	l.700b	0.002c
BAP+IBA	0+0	l.283d	0.000d	0.313d	0.000d
	0.5 + 1.0	2.000b	2.038c	0.916a	l.480c
	1.0 + 1.0	l.817c	3.333a	0.716b	2.003b
	2.0+ 1.0	2.583a	2.333b	0.520c	5.367a
IAA+IBA	0+0	0.000d	l.167c	0.000d	0.293c
	0.5 + 1.0	4.800c	4.66Th	1.367c	2.140b
	1.0 + 1.0	6.557a	6.667a	2.823a	2.667a
	2.0+ 1.0	5.365b	4.500b	l.560b	2.130b

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

BAP + IAA: In case of BAP + IAA combination, the maximum number of roots per plantlet (5.22) was obtained from 2.0 mg/L each of BAP + IAA without charcoal supplementation and the lowest number (1.282) was recorded from control treatment. On the other hand, 1.0 mg/L each of BAP + IAA combination gave the highest number of roots (5.283) with charcoal supplementation.

BAP + IBA: In BAP + IBA combination, the highest number of roots (3.333/plantlet) was obtained in 1.0 mg/L each of BAP + IBA with 1.0 g/L charcoal supplementation followed by 2.0 mg/L BAP + 1.0 mg/L + IBA (2.333). On the other hand, in without charcoal, the highest number of roots (2.583) was observed is 2.0 mg/L BAP + 1.0 mg/L + IBA.

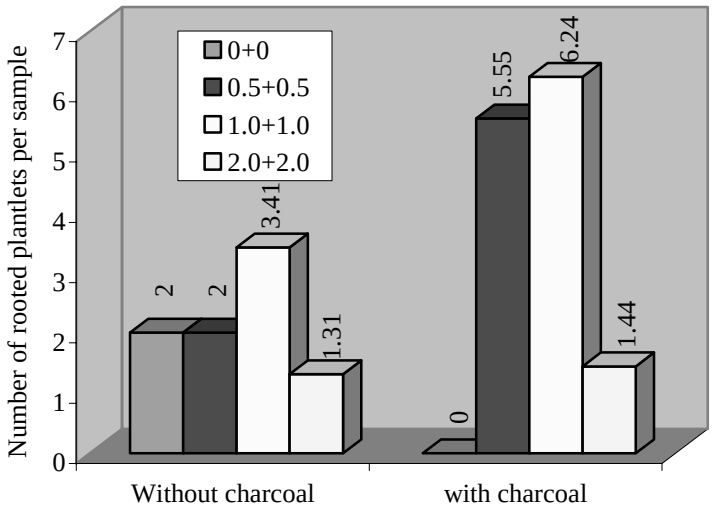

Fig. 1a. Combined effect of BAP+NAA on *in vitro* grown rooted plantlets with and without charcoal supplementation at 120 days after inoculation

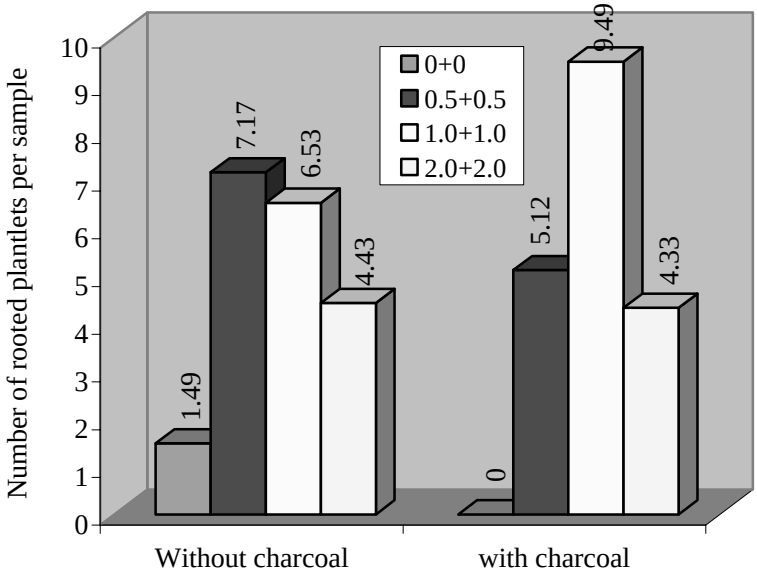

Fig. 1b. Combined effect of BAP+IAA on *in vitro* grown rooted plantlets with and without charcoal supplementation at 120 days after inoculation

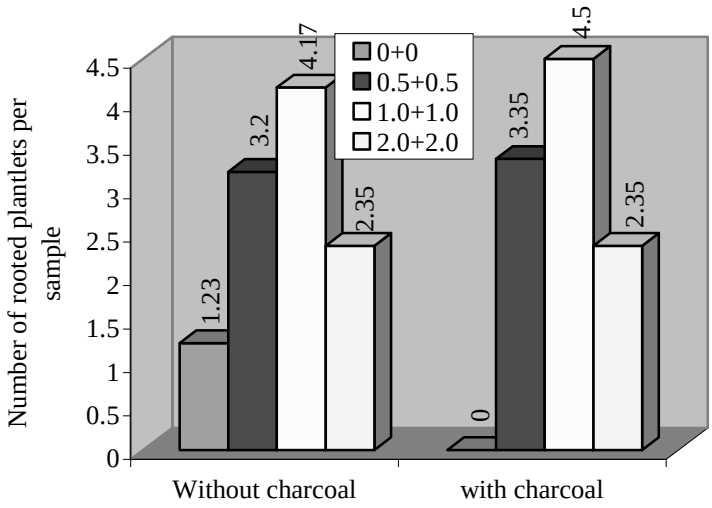

Fig. 1c. Combined effect of BAP+IBA on *in vitro* grown rooted plantlets with and without charcoal supplementation at 120 days after inoculation

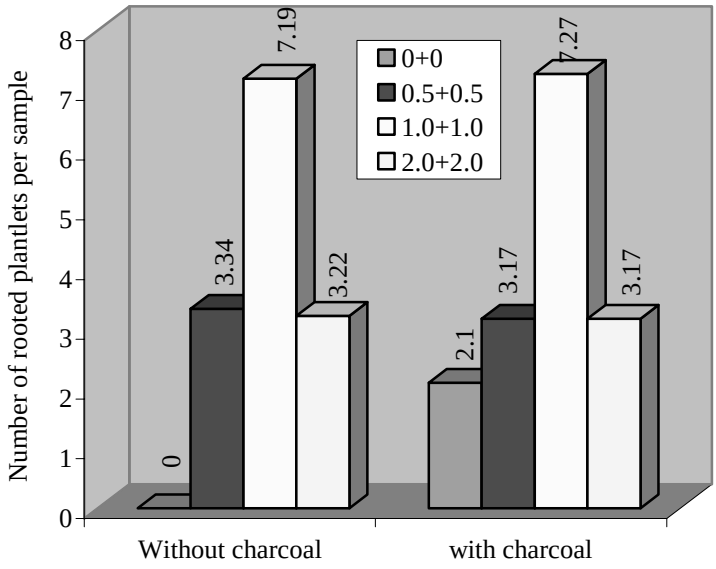

Fig. 1d. Combined effect of IAA+IBA on *in vitro* grown rooted plantlets with and without charcoal supplementation at 120 days after inoculation

IAA + IBA: In IAA and IBA combination, 1.0 mg/L each of IAA + IBA combination gave the second highest number of roots (6.557/plantlet), while with

charcoal supplementation, the highest number of roots (6.667/plantlet) was observed at the same concentration and combination (1.0 mg/L each of IAA + IBA). Results indicated that in all the treatment combinations, charcoal enhanced the root formation (Table 1. The results are in agreement with the findings of Vij *et al.* (1994) where they examined the regeneration potential of *Cymbidium pendulum in vitro* using IAA, IBA, 2, 4-D, NAA and GA_3. Activated charcoal has not only good adsorption properties but also creates partial darkness. Probably the activated charcoal fortified media enhanced induction of roots (Bhadra and Hossain, 2003). Results also indicated that root formation was found best at 1.0 mg/L each of IAA + IBA combination with charcoal supplementation. The result is partially supported by Talukder *et al.* (2002) where they reported that in combined effect, root formation was found best with 1.0 mg/L IBA. This result is also partially agreed with Pathania *et al.* (1998) where they found that both Vacin and Went (VW) and Knudson C (KC) media favoured rooting when supplemented with 1 mg/L IBA.

Length of root: Root length was significantly influenced by different levels of growth regulators and also with and without charcoal supplementation. Among all the treatments, the highest root length (5.367 cm) was obtained from the hormonal combination of 2.0 mg/L BAP + 1.0 mg/L IBA in the presence of charcoal supplementation. In without charcoal supplementation, 0.5 mg/L each of BAP + IAA gave the second highest root length (3.576 cm) followed by with charcoal supplementation at the same concentration (2.967 cm). It revealed that the hormonal combination of BAP and IAA was the best growth regulators for root length.

BAP + NAA: In BAP and NAA combination, the highest root length (2.442 cm) was found with 1.0 mg/L both of BAP + NAA without charcoal supplementation, while in charcoal supplementation, the highest root length (1.203 cm) was obtained at the same concentration (1.0 mg/L both of BAP + NAA) (Table I).

BAP + IAA: In without charcoal supplementation, the highest root length (3.576 cm) was obtained in 0.5 mg/L each of BAP + IAA and the lowest root length was obtained from control treatment (0.282 cm). On the other hand, in charcoal supplemented media, the highest root length (2.967 cm) was also obtained from 0.5 mg/L each of BAP + IAA. Result indicated that root length was found better in the hormonal combination of BAP + IAA.

BAP + IBA: In case of BAP + IBA combination, the root length was significantly highest (5.367 cm) at 2.0 mg/L BAP + 1.0 mg/L IBA with charcoal supplementation than without charcoal (Table 1) where highest root length (0.916 cm) was obtained from 0.5 mg/L BAP + 1.0 mg/L IBA.

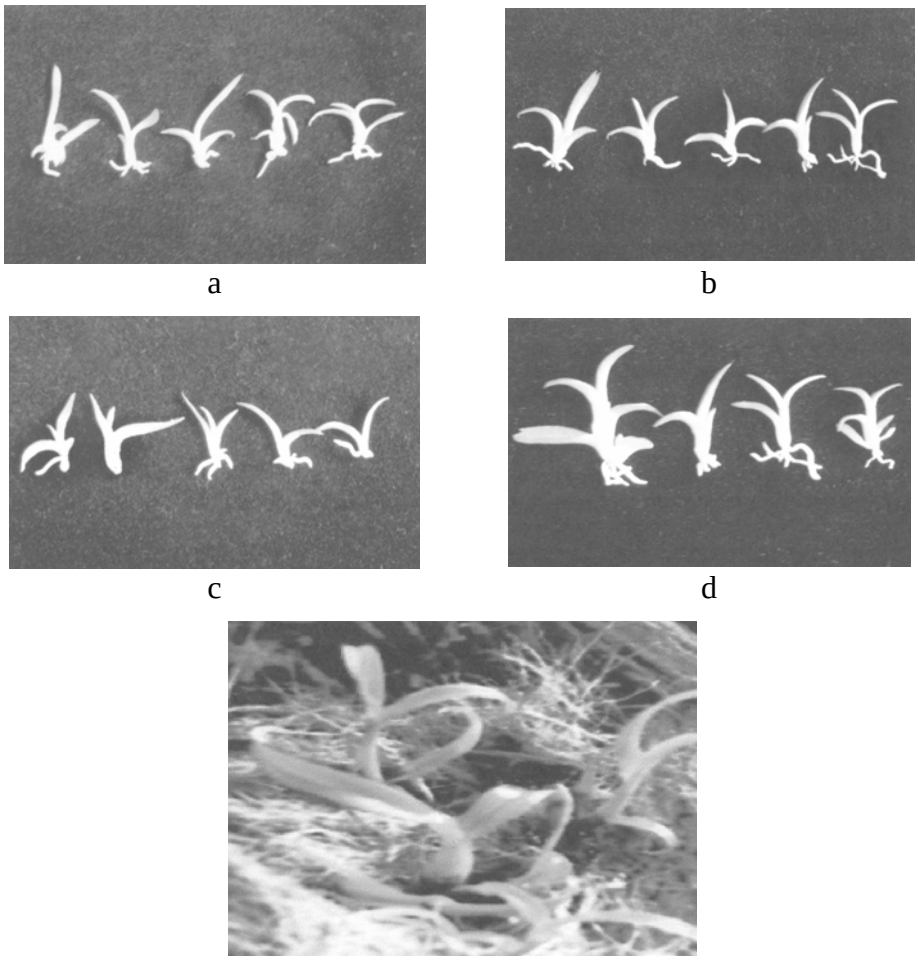

Fig. 2. (a-e). Root formation and establishment of plantlets in Dendrobium orchid

a) 1 gm/1 each of BAP + NAA
b) 1 gm/1 each of IAA+IBA
c) 1 gm/1 each of BAP + IBA
d) 1 gm/1 each of BAP + IAA
e) Established plantlets in coconut husk

IAA + IBA: The highest root length (2.823 cm) was obtained from without charcoal supplementation at 1.0 mg/L each of IAA + IBA. At the same concentration (1.0 mg/L each of IAA + IBA) in charcoal supplementation, the highest root length (2.667 cm) was observed which was lower than that of without charcoal supplementation (Table 1). Result indicated that in charcoal supplemented media, root length was not always the highest but root number was always higher.

Number of rooted plantlets: Significant variation was observed in number of rooted plantlets at different concentrations and combinations of growth regulators and also with and without charcoal supplementation. Results indicated that in all the treatment combinations, 1.0 mg/L each of BAP + NAA, BAP + IAA, BAP + IBA, and IAA + IBA were found best for producing more rooted plantlets in charcoal supplementation. Among all the treatment combinations, the highest number of rooted plantlets (9.49) was observed in the hormonal combination of BAP + IAA.

BAP + NAA: In BAP + NAA combination, the highest number of rooted plantlets was obtained from 1.0 mg/L each of BAP + NAA without charcoal treatment (3.41), while at the same concentration, the highest number (6.24) was observed in charcoal supplemented media (Fig. I a).

BAP + IAA: In BAP and IAA combination, 0.5 mg/L each of BAP and IAA gave the maximum rooted plantlets (7.17) followed by 1.0 mg/L each of BAP + IAA (6.53) in without charcoal treatment. On the other hand, the highest number of rooted plantlets (9.49) was observed from 1.0 mg/L each of BAP + IAA in charcoal supplemented media and control treatment produced rootless plantlets (Fig. 1b). Result indicated that charcoal enhanced root formation.

BAP + IBA: In BAP + IBA combination, both charcoal and without charcoal supplemented media, the highest number of rooted plantlets was obtained from 1.0 mg/L each BAP + IBA where charcoal treated media produced more rooted plantlets (4.5) than without charcoal (4.17) (Fig. 1 c).

IAA + IBA: In case of IAA +IBA combination, both charcoal and without charcoal supplemented media, the highest number of rooted plantlets was obtained from 1.0 mg/L each IAA + IBA where charcoal treated media produced more rooted plantlets (7.27) than without charcoal (7.19) (Fig.ld).

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