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Protocorm like Bodies and Plantlet Regeneration from *Demdrobium formosum* Leaf Callus

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Abstract: The present study was conducted at the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh during July 2000-July 2002 to investigate the effect of different concentrations of 2,4-D on callusing and PLB formation; BAP (0, 2, 4 and 6 mg l^{-1}) and NAA (0, 0.5 and 1.0 mg l^{-1}) on formation of Protocorm Like Bodies (PLBs) and plantlet regeneration from *Dendrobium formosum* leaf callus. PLB formation, shoot proliferation, root formation, leaf number, shoot length and root length were recorded. The fastest callusing (9.32 days) and the amount (0.73 g $explant^{-1}$) was the greatest with 1 mg l^{-1} 2,4-D at 20 DAE, also the number of PLB was the highest (10.52 $explant^{-1}$) at 60 DAE. Time required for PLB formation was the longest (32.72 days) with 0.50 mg l^{-1} 2,4-D. Plantlet height, root length and number of shoots, leaves and roots per plantlet showed significant variation due to BAP and NAA addition. BAP at 2.5 mg l^{-1} showed the best results in combination with 1 and 2 mg l^{-1} NAA.

Key words: 2,4-D, BAP, NAA, callus, PLB regeneration, orchid

Introduction

Dendrobium is one of the most popular orchids all over the world. The environmental conditions required for the survival and culture of orchid are adequately available throughout the year in Bangladesh. The traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year. Micropropagation is the most frequently used convenient technique for their exploitation as a major trade in developed countries (Goh, 1982; Sagawa and Kunisaki, 1982). There is a lack of reliable protocol to propagate orchid *in vitro*. Moreover, the available protocols has shortfall of low rate of PLB formation, low viability of PL B, consuming long time for obtaining PLB and different responses among PLB and hybrids (Tokuhara and Mii, 1993). The present research was undertaken to observe the effect of 2,4-D on formation of callus and PLBs from leaf explants and their subsequent plantlet regeneration capability with NAA and BAP supplementation.

Materials and Methods

The experiments were carried out in the USDA Biotechnology Laboratory, Department of Biotechnology and Horticulture, Bangladesh Agricultural University, Mymensingh during July 2000-June 2002. Different combinations of 2,4-D, BAP and NAA were supplemented in Ms basal medium (Murashige and Skoog, 1962). The leaf sections were explanted on petri dishes containing MS media supplemented with 0, 0.05, 0.10, 0.50 and 1.00 mg l^{-1}) 2,4-D for callus and PLB formation. The PLBs were then recultured on baby meal jars containing media added with 4 levels of each of BAP (0, 1.25, 2.5 and 5 mg l^{-1}) and NAA (0, 0.5, 1 and 2 mg l^{-1}). The pH of the media was adjusted to 5.8 and then autoclaved at 121°C under 1.16 kg cm^{-2} pressure for 20 min. The culture were allowed to grow at 25+1°C under 16 h photoperiod illuminated with fluorescent tube of 2000-3000 lux. The data for the characters under the present study were statistically analyzed following Completely randomized design (CRD). The analysis of variance was performed and means were compared by least significant difference (LSD) values (Gomez and Gomez, 1984).

Results and Discussion

Statistically significant variation was noticed on callusing and PLB formation with different levels of 2,4-D. The results revealed that callusing was significantly earliest (9.32 days) at 1 mg l^{-1} 2,4-D. It was delayed towards the decrease of 2,4-D amount where the longest period was recorded (20.63 days) in control (Table 1). The trend was almost the same in PLB formation except that the PLBs were not found without 2,4-D. The amount of calli at 20 DAE (0.73 g l^{-1}) and the number of pLBs at 60 DAE (10.52 $explant^{-1}$) were the highest with 1 mg l^{-1} 2,4-D and the least (0.01 g l^{-1} and nil, respectively) without 2,4-D. The enhancement of callusing and PLB formation in the present experiment support the report of Kanjilal *et al*. (1999) who reported that PLB was the greatest with 1 mg l^{-1} 2,4-D in *D. moschatnum*.

The main effect of BAP and NAA showed significant variation among their concentrations. Plantlet highest (3.86 cm), number of shoots (2.74 $plantlet^{-1}$) and roots

Table 1: Callus and PLB formation on leaf explants of *Dendrobium formosum* orchid on MS media supplemented with 2,4-D

Treatment 2, 4-D (mg l^{-1})	Days required for callus initiation	Amount of callus at 20 DAE explant^{-1} (g)	Days required for PLB formation	No. of PLBs explant^{-1} at 60 DAE
0	20.63	0.01	-	-
0.05	15.62	0.28	43.27	2.65
0.10	12.48	0.54	36.29	4.76
0.50	9.74	0.68	32.73	5.93
1.0	9.32	0.73	35.84	10.52
$LSD_{(0.05)}$	2.46	0.22	4.08	2.31

Table 2: Main effect of BAP and NAA on growth of plantlets derived from leaf callus and PLB of *Dendrobium formosum* orchid. Data were recorded at 60 DAE

Treatment combinations BAP+NAA (mg l^{-1})		Plantlet height (cm)	Root length (cm)	No. of shoots plantlet^{-1}	No. of roots plantlet^{-1}	No. of leaves plantlet^{-1}
BAP:	0	0.84	0	1.03	0	2.12
	1.25	2.86	0.42	2.13	1.67	2.87
	2.5	3.72	0.86	2.62	2.56	4.42
	5.0	3.86	0.71	2.74	3.50	4.25
$LSD_{(0.05)}$		1.13	0.26	1.03	1.03	1.08
NAA:	0	0.88	0	1.01	0	2.02
	0.5	2.42	0.78	1.89	3.31	2.46
	1.0	3.05	1.26	2.24	4.27	4.01
	2.0	3.14	1.28	2.40	4.42	3.96
$LSD_{(0.05)}$		0.92	0.43	0.81	1.42	0.81

Table 3: Combined effect of BAP and NAA on growth of plantlets derived from leaf callus and PLB of *Dendrobium formosum* orchid. Data were recorded at 60 DAE

Treatment combinations BAP+NAA (mg l^{-1})	Plantlet height (cm)	Root length (cm)	No. of shoots plantlet^{-1}	No. of roots plantlet^{-1}	No. of leaves plantlet^{-1}
0+0	0.86	0	1.02	0	2.04
0+0.5	1.23	0.13	1.08	1.23	2.43
0+1	2.42	0.58	1.32	2.47	2.55
0+2	2.31	0.76	1.21	3.05	2.87
1.25+0	1.53	0.31	1.66	2.83	2.53
1.25+0.5	2.65	0.64	2.05	3.27	2.75
1.25+1	3.16	0.97	2.43	3.69	2.94
1.25+2	2.62	1.13	2.34	3.74	3.22
2.5+0	2.31	0.32	2.01	3.26	2.38
2.5+0.5	3.14	0.58	2.29	3.62	3.83
2.5+1	3.85	0.85	2.68	4.01	4.39
2.5+2	3.66	0.93	2.54	4.14	4.16
5+0	2.63	0.24	1.82	3.02	2.62
5+0.5	2.72	0.43	2.08	3.37	3.21
5+1	3.21	0.62	2.26	3.58	3.77
5+2	2.95	0.73	2.20	3.73	3.68
$LSD_{(0.05)}$	1.02	0.31	0.94	1.21	0.87

(3.50 plantlet^{-1}) were the highest at 5 mg l^{-1} BAP, whereas the root number (0.86 plantlet $^{-1}$) and number of leaves (4.42 explant^{-1}) were recorded maximum at 2.5 mg l^{-1} BAP (Table 2). NAA at 2 mg l^{-1} showed the significantly superior results then other concentrations. It was evident that root was not found without BAP and NAA.

Combined effect of BAP and NAA showed the significant influence on all the parameters studied. Plantlet height (3.85 cm), number of shoots (2.68 plantlet^{-1}) and leaves (4.39 plantlet^{-1}) were found maximum at BAP 2.5 mg l^{-1}+NAA 1 mg l^{-1} (Table 3). Whereas, the minimum was recorded at control (0.86 cm, 1.02 plantlet^{-1} and 2.04 plantlet^{-1}, respectively. The root length (0.93 cm) and number of roots (4.14 plantlet^{-1}) were the highest at BAP 2.5 mg l^{-1} + NAA 2 mg l^{-1} whereas the control plantlets did not produce any root. The highest concentration of BAP and NAA did not show any advantage. The present results are in support of that of Nayak *et al.* (1998), Vij *et al.* (1994), Sheelavantmath *et al.* (2000), Lee *et al.* (1999). Who also found the enhancement of plantlet growth with BAP and NAA application.

It may me suggested that 1 mg l^{-1} 2,4-D is suitable for callusing and PLB formation of *Dendrobium formosum* orchid. For plantlet growth 2.5 mg l^{-1} BAP in the best with 1 mg l^{-1} NAA.

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