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Shoot Proliferation of *Dendrobium* Orchid with BAP and NAA

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Abstract: The experiment was carried out in the Biotechnology Laboratories Departments of Biotechnology and Genetics & Plant Breeding, Bangladesh Agricultural University, Mymensingh during July-November 2002, to investigate the effect of BAP and NAA on orchid shoot regeneration. In this experiment shoot proliferation, root formation, leaf number, increment of shoot length and days required for shoot proliferation of orchid cultured on MS media containing different concentrations of BAP (0, 1, 2.5 and 5 mg l^{-1}) and NAA (0, 0.1, 0.5 and 1 mg l^{-1}) were observed. The results revealed that the use of different concentrations of BAP and NAA had a significant effect. Among the concentrations, the best shoot proliferation (1.90/explant), root formation (1.93/explant) leaf number (4.25/plantlet), increment of shoot length (0.472 cm) and the least time requirement for regeneration for regeneration (8.8 days) was obtained from 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. At the same time, the lowest shoot proliferation (0.05/explant) was found in control and 0.1 mg l^{-1} NAA, Leaf number (0.4/plantelt) from 5 mg l^{-1} BAP, increment of shoot length (0.252 cm) from control and highest days requirement (23.8 days) from 5 mg l^{-1} BAP +0.1 mg l^{-1} NAA was found.

Key words: Regeneration, BAP, NAA, *Dendrobium*

Introduction

Orchids belong to the largest and most diverse family Orchidaceae, consisting of about 700-800 genera and 25,000 species (Singh and Voleti, 1995). Among them ***Dendrobium*** is an interesting group of orchid known for their intricately fabricated and long lasting colourful flowers. Orchids are propagated both vegetatively and by sexual means but the process 15 kg slow. Moreover, distinct variations in offspring are found. Therefore, to get true to the type plant, clonal propagation is the only means. Many studies on micro-propagation methods have already been conducted to establish a suitable method. Rotor (1949) for the first time tried to propagate ***Phalaenopsis*** clonally using flower stalk buds. Other research reports on the micro-propagation of orchids through tissue culture of leaf (Tanaka, 1987); root tips (Tanaka ***et al.***, 1976) internodal section of flower stalk (Homma and Asahira, 1985; Lin, 1986) and lateral buds from young flower stalks (Ichihashi, 1992) are available, but none of these methods proved effective commercially in producing lot of plantlets in a short period because of low rate of PLB (Protocorm Like Body) formation, low viability of PLB, consuming long time for obtaining PLB and different responses

among PLB and hybrids (Tokuhara and Mii, 1993). To avoid this problem, the multiple shoot proliferation technique using different hormones can be tried. Considering the above idea in mind, the present piece of study was undertaken to standardize and to develop a suitable BAP and NAA combination for shoot regeneration and plantlet establishment.

Materials and Methods

The experiment was carried out in the Biotechnology Laboratories Department of Biotechnology and Genetics & Plant Breeding, Bangladesh Agricultural University, Mymensingh during July-November, 2002 to investigate the effect of BAP and NAA on orchid shoot regeneration. In this experiment ***in vitro*** multiplied shoot buds were cultured on MS medium supplemented with different concentrations of BAP (0, 1, 2.5 and 5 mg l^{-1}) and NAA (0, 0.1, 0.5 and 1 mg l^{-1}). Eighty glass vials were used and arranged under sixteen treatments and five replications. Four ***in vitro*** multiplied one-month-old rootless shoots were placed in each vial. The culture flasks were placed in a growth room and allowed to grow at 25±10°C under 16 hour photoperiod illuminated with fluorescent tube of 2000-3000 lux. The experiment was laid out in Completely Randomized Design (CRD) with 5 replications. The treatment means were compared with LSD values. The data were collected at 10 days interval on number of leaves, shoots and roots per plantlet; increment of shoot length and days required for shoot formation.

Results and Discussion

The main effect of BAP and NAA on shoot proliferation have been presented in Table 1. The highest number of proliferated shoots (1.113/explant) was obtained from 2.5 mg l^{-1} BAP and the least number (0.213/explant) was found in control. In case of NAA, highest (0.91 shoots/explant) and lowest number (0.36 shoots/explant) of proliferated shoots was observed from 0.5 and 1 mg l^{-1} NAA, respectively. Combined use of BAP and NAA showed better shoot proliferation instead of their single use. The highest shoot proliferation (1.90 shoots/explant) was found with 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, whereas the lowest (0.05/explant) was observed at control (Table 3). A few additional shoot like structures were initiated at the basal portion of newly regenerated plantlets. This result is not in full support of Lee ***et al.*** (1999) where they found that shoot number of ***Sarcanthus scolopendrifotius*** was increased in presence of 0.1 mg l^{-1} NAA. This variation might be for the species differences.

For days requirement to proliferate new shoots the main effect of BAP and NAA have been presented in Table 1. The longest time (20.4 days) was required from 5 mg l^{-1} BAP followed by control (16.9 days) and the shortest time (13.6 days) was recorded at 2.5 mg l^{-1} BAP. Effect of NAA on time requirement for shoot proliferation was found significant, where the longest time (22.77 days) was required with 0.1 mg l^{-1} NAA and the shortest time (13.80 days) was required with 0.5 mg l^{-1} NAA.

It was found that the days required for new shoot proliferation varied significantly with different combinations of BAP and NAA. Among the treatments, the best treatment was 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA which required only 8.8 days for new shoot proliferation. On the other hand the longest period (23.6 days) was required for control to regenerate a new shoot

Table 1: Main effect of BAP and NAA on shoot proliferation and shoot length of *Dendrobium* orchid

	Number of proliferated shoots				Shoot length (cm)				
Hormones	10 DAI	20 DAI	30 DAI	40 DAI	10 DAI	20 DAI	30 DAI	40 DAI	Days to shoot
BAP (mg l^{-1})									
0	0.000	0.125	0.200	0.213	0.07	0.10	0.22	0.26	16.90
1	0.000	0.363	0.588	0.600	0.08	0.19	0.26	0.33	16.05
2.5	0.100	0.663	0.910	1.113	0.08	0.20	0.27	0.32	13.60
5	0.000	0.150	0.325	0.325	0.05	0.11	0.21	0.26	20.40
LSD (0.05)	0.019	0.074	0.129	0.069	0.006	0.006	0.008	0.006	1.630
NAA (mg l^{-1})									
0	0.000	0.188	0.363	0.400	0.07	0.15	0.26	0.31	18.26
0.1	0.000	0.138	0.363	0.363	0.05	0.12	0.21	0.27	22.77
0.5	0.100	0.625	0.798	0.913	0.09	0.20	0.29	0.34	13.80
1	0.000	0.350	0.500	0.575	0.07	0.13	0.21	0.26	17.05
LSD (0.05)	0.019	0.074	0.129	0.069	0.006	0.006	0.008	0.006	1.630

Table 2: Main effect of BAP and NAA on number of leaves and roots per plantlet of *Dendrobium* orchid

	Number of leaves per plantlet				Number of roots per plantlet			
Hormones	10 DAI	20 DAI	30 DAI	40 DAI	10 DAI	20 DAI	30 DAI	40 DAI
BAP (mg l^{-1})								
0	0.05	0.388	0.638	0.838	0	0.225	0.375	0.413
1	0.275	0.963	1.4	1.475	0	0	0	0
2.5	0.488	1.588	2.338	2.55	0.063	0.325	0.563	0.645
5	0	0.3	0.65	0.713	0	0.163	0.288	0.288
LSD (0.05)	0.052	0.09581	0.1038	0.1165	0.00631	0.05285	0.04893	0.05285
NAA (mg l^{-1})								
0	0.113	0.488	0.863	0.975	0	0	0	0
0.1	0.113	0.55	0.963	1.138	0	0	0	0
0.5	0.413	1.425	1.963	2.113	0.063	0.488	0.8	0.895
1	0.175	0.775	1.238	1.35	0	0.225	0.425	0.45
LSD (0.05)	0.05285	0.09581	0.1038	0.1165	0.00631	0.05285	0.04893	0.05285

(Table 3). Shoot length increased significantly with the use of BAP and NAA where the highest (0.33 cm) and lowest value (0.26 cm) was obtained from 2.5 mg l^{-1} and control. On the other hand, 0.5 and 1 mg l^{-1} NAA showed the highest (0.34 cm) and the lowest (0.26 cm) shoot length, respectively (Table 1). The combined effect of BAP and NAA showed the highest value (0.47 cm) from 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and the lowest value (0.25 cm) was obtained from control at 40 DAI (Table 3).

The main effect of BAP for the highest leaf number (2.55/explant) was found with 2.5 mg l^{-1} BAP and lowest value (0.71/explant) was obtained with 5 mg l^{-1} BAP. In case of NAA, the highest number of leaves (2.11/explant) was found from 0.5 mg l^{-1} NAA followed by 1 mg l^{-1} NAA (1.35/explant) and the lowest value (0.975/explant) was obtained from 0 mg l^{-1} NAA (Table 2).

In combined effect, the highest value (4.25/explant at 40 DAI) was obtained with 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and the lowest value was found (0.40/explant) with 5 mg l^{-1} BAP (Table 4).

Table 3: Combined effect of BAP and NAA on shoot proliferation and shoot length of *Dendrobium* orchid at different days after culture initiation

	Number of proliferated shoots				Shoot length (cm)				
BAP + NAA (mg l^{-1})	10 DAI	20 DAI	30 DAI	40 DAI	10 DAI	20 DAI	30 DAI	40 DAI	Days to shoot
0 + 0	0	0.0	0.05	0.05	0.06	0.068	0.222	0.252	23.6
0 + 0.1	0	0.05	0.05	0.05	0.06	0.116	0.214	0.26	18.0
0 + 0.5	0	0.25	0.50	0.5	0.09	0.12	0.236	0.28	15.0
0 + 1	0	0.20	0.25	0.50	0.06	0.104	0.204	0.254	18.6
1 + 0	0	0.60	0.90	0.95	0.12	0.31	0.408	0.462	12.0
1 + 0.1	0	0.20	0.55	0.50	0.051	0.116	0.194	0.28	18.8
1 + 0.5	0	0.40	0.65	0.70	0.1	0.208	0.226	0.32	14.8
1 + 1	0	0.20	0.25	0.25	0.046	0.110	0.206	0.264	18.6
2.5 + 0	0	0.10	0.30	0.35	0.046	0.108	0.198	0.256	21.4
2.5 + 0.1	0	0.30	0.60	0.65	0.052	0.114	0.212	0.264	13.0
2.5 + 0.5	0.4	1.40	1.49	1.90	0.11	0.366	0.464	0.472	8.80
2.5 + 1	0	0.85	1.25	1.55	0.11	0.206	0.222	0.274	11.2
5 + 0	0	0.05	0.25	0.25	0.046	0.106	0.202	0.256	21.4
5 + 0.1	0	0.00	0.25	0.25	0.048	0.114	0.21	0.266	23.8
5 + 0.5	0	0.40	0.55	0.55	0.052	0.116	0.228	0.274	16.6
5 + 1	0	0.15	0.25	0.25	0.048	0.116	0.206	0.26	19.8
LSD (0.05)	0.039	0.149	0.258	0.138	0.017	0.012	0.017	0.012	1.712

Table 4: Combined effect of BAP and NAA on number of leaves and roots of *Dendrobium* orchid at different days after culture initiation

	Number of leaves per plantlet				Number of roots per plantlet			
BAP + NAA (mg l^{-1})	10 DAI	20 DAI	30 DAI	40 DAI	10 DAI	20 DAI	30 DAI	40 DAI
0 + 0	0.0	0.30	0.5	0.7	0	0.0	0.0	0.0
0 + 0.1	0.0	0.35	0.4	0.65	0	0.0	0.0	0.0
0 + 0.5	0.2	0.8	1.05	1.20	0	0.6	0.95	1.05
0 + 1	0.0	0.1	0.6	0.8	0	0.3	0.55	0.6
1 + 0	0.45	1.25	1.75	1.9	0	0.0	0.0	0.0
1 + 0.1	0.05	0.5	1.1	1.15	0	0.0	0.0	0.0
1 + 0.5	0.55	1.55	2.1	2.15	0	0.0	0.0	0.0
1 + 1	0.05	0.55	0.65	0.7	0	0.0	0.0	0.0
2.5 + 0	0.0	0.3	0.85	0.9	0	0.0	0.0	0.0
2.5 + 0.1	0.4	1.25	1.75	2.05	0	0.0	0.0	0.0
2.5 + 0.5	0.9	3.0	3.95	4.25	0.25	1.0	1.65	1.93
2.5 + 1	0.65	1.8	2.8	3.0	0	0.3	0.6	0.65
5 + 0	0.0	0.1	0.35	0.4	0	0.0	0.0	0.0
5 + 0.1	0.0	0.1	0.6	0.7	0	0.0	0.0	0.0
5 + 0.5	0.0	0.35	0.75	0.85	0	0.35	0.6	0.6
5 + 1	0.0	0.65	0.9	0.8	0	0.3	0.550	0.55
LSD (0.05)	0.140	0.191	0.207	0.229	0.021	0.105	0.097	0.105

The main effect of BAP and NAA on root initiation have been presented in Table. 2 where the highest number of roots (0.645/explant) at 40 DAI was obtained from 2.5 mg l^{-1} BAP and no root

was found from 1 mg l^{-1} BAP. For NAA, the highest number of roots (0.90/explant) was found from 0.5 mg l^{-1} NAA and the control and 0.1 mg l^{-1} NAA were found rootless.

From the combined effect the highest number (1.93/explant) of root was obtained with 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA followed by 0.5 mg l^{-1} NAA (1.05 roots/explant) at 40 DAI (Table 4).

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