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Original Article

EFFECT OF BENZYLAMINO PURINE AND NAPHTHALENE ACETIC ACID ON CALLUS AND PROTOCORM FORMATION OF DENDROBIUM CV. BANYAT PINK

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Abstract: The effect of growth regulators, benzylamino purine (BAP) and naphthalene acetic acid (NAA) on callus and protocorm formation of Dendrobium cv. Banyat Pink were studied. Observations revealed that callus initiation in Banyat Pink was rather slow and only 40% of the tubes showed callus formation on 20th day after inoculation. Best callus spread (1.23 x 0.93cm) was observed in MS medium fortified with 3.0 mg/l BAP and 0.5 mg/l NAA while the smallest callus size was recorded in MS medium with 1.5 mg/l BAP and 1.0 mg/l NAA. The number of protocorms per tube after 30 days of inoculation was higher in MS medium supplemented with BAP at the rate of 1.0 mg/l and 1.5 mg/l. However, after 45 days of inoculation, the numbers of protocorms recorded in the tubes supplemented with 1.5 mg/l of BAP were higher (6.83) than the tubes (5.5) supplemented with 1 mg/l of BAP. The least number of protocorms per tube were observed in MS medium supplemented with 3.0 mg/l of BAP during both the days of observations. The experiment revealed that MS medium supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA was found to be the best for callus formation among all the treatments while maximum number and spread of the protocorm mass was found to be maximum in MS medium supplemented with 1.5 mg/l BAP.

Key words: *Dendrobium*, Banyat Pink, Callus Culture,

INTRODUCTION

Dendrobium is the second largest genus in the family orchidaceae comprising 1600 species distributed in India, Myanmar, Malaysia, Australia, New Zealand, China and Japan [1]. The genus *Dendrobium* exhibits a vast diversity in vegetative and floral characteristics and is of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity. It has become increasingly popular due to its floriferous flower sprays, wide range of colour, size and shape, year round availability and long flowering life of several weeks to months. Banyat Pink (Fig. 1) is an epiphytic, sympodial orchid with deep pink flowers. It is self compatible and responds well to selfing producing true to the type seeds. It is one of the important commercial varieties of *Dendrobium* having very good demand in the domestic as well as international flower market.

Propagation of orchids through seeds is slow in nature. Although the seeds are produced in large numbers (2-3 million per capsule), only 0.2-0.3 percent seeds germinate in nature due to lack of metabolic machinery and functional endosperm. Major advancement in increasing the germination of orchid seeds and reducing flowering time is the development of the green pod culture process.

In vitro culture of immature seeds collected from unripe green capsule in obtaining increased germination as compared to mature seeds has been

successfully accomplished in several orchid genera including *Dendrobium* [2]. However, the technique in terms of use of nutrient media, carbon source, growth regulators and other additives are specific for specific orchids. Hence, there is need of further studies to standardize the technique for other species and varieties for their rapid multiplication that can be employed for commercial production of plants as well as to save the native endangered species which gradually extinct. Based on these points, green pod culture process on *Dendrobium* cv. Banyat Pink was attempted in the present investigation.

MATERIALS AND METHODS

The green pods of *Dendrobium* orchid cv. Banyat Pink, collected from the regional plant resource centre (RPRC), Bhubaneswar, were used for conducting the experiment (Fig. 2). The immature seeds extracted from the green pod (Fig. 3) were used for embryo culture without delay. The seeds were inoculated into the solid nutrient [3] media supplemented with auxin i.e. IAA (Indole-3 acetic acid) 1mg/l and cytokinin i.e. BAP (Benzyl-6-aminopurine) 2.0 mg/l for germination and establishment of culture. Observations on number of tubes showing seed germination and callus initiation were recorded at five days interval, the first one commencing from 10th day of inoculation. The small callus mass initiated were inoculated in MS (Murashige and Skoog) media containing different concentrations of auxin (NAA-0.5 and 1.0 mg/l) and cytokinin (BAP-1.5, 2.0, 2.5 and 3.0 mg/l) for better spread and development of callus. Observations were recorded on spread, nature and colour of callus after 45 days of inoculation. After the formation of callus, the calli mass was transferred to MS media containing seven different concentrations of BAP (cytokinins) for protocorm formation. Observations were recorded on number of protocorm, spread, nature and colour of protocorm mass at 30 and 45 days interval.

RESULTS AND DISCUSSION

Data in Table 1 showed that germination process of seeds initiated after 25 days of culture and within 30 days, 15 tubes out of 20 showed light green colouration on the surface of agar medium inside the culture tube and within 35 days of culture all the tubes showed light green coloration indicating the completion of germination process and initiation of callus. Observations recorded after 45 days of inoculation

Table 1: Number of culture tubes showing germination and callus initiation (mean ± S. Em; n=3). Figures in parenthesis indicate corresponding percentage.

Tubes	Days after inoculation (DAI)					
	10	15	20	25	30	35
Showing germination and callus initiation	-	-	-	8±0.45 (40%)	15±0.32 (75%)	20±0.55 (100%)

on the effect of plant growth regulators i.e. BAP and NAA on callus development and spread have been presented in Table 2. The maximum spread of callus (1.23x 0.93 cm) was obtained in MS medium fortified with 3.0 mg/l BAP in combination with 0.5 mg/l NAA (T_4) which produced a compact dark greenish callus mass followed by the combination of 2.5 mg/l BAP and 1.0 mg/l NAA (1.1 cm x 0.66 cm) and 2.0 mg/l BAP with 1.0 mg/l NAA (1.2 cm x 0.53 cm). On the other hand, MS medium fortified with 1.5 mg/l BAP and 1.0 mg/l NAA produced the minimum spread of callus (1.2 cm x 0.4 cm). The other combinations of growth regulators which produced less spread of callus were 1.5 mg/l BAP and 0.5 mg/l NAA as well as 3.0 mg/l BAP along with 1.0 mg/l NAA (1 cm x 0.5 cm each).

The data presented in Table 3 showed the effect of various concentrations of BAP on protocorm formation and multiplication after 30 and 45 days of inoculation. It was observed that the number of protocorms per tube after 30 days of inoculation were higher (5 in each case) in MS medium fortified with BAP 1.0 mg/l (T_3) and 1.5 mg/l (T_4). However, after 45 days of inoculation the protocorm number was significantly higher in T_4 (6.83) followed by T_3 (5.50). On the other hand, the least number of protocorms per tube were observed (2.94 and 3.33) in T_7 i.e. MS medium supplemented with 3.0 mg/l of BAP which was at par with T_1 i.e. MS medium alone (2.94 and 3.50). As indicated in Table 3, the spread of protocorm mass was maximum in T_4 (i.e. BAP 1.5 mg/l) both at 30 and 45 days of inoculation followed by T_5 which was fortified with 2.0 mg/l BAP. The size of protocorm under T_4 and T_5 were 1.2 cm x 0.7 cm and 1.25 cm x 0.63 cm, respectively after 45 days of inoculation. The spread was observed to be minimum in T_7 (0.73cm x 0.34cm and 0.92cm x 0.46cm) during both the observations.

In the present investigation, MS medium fortified with 1.0 mg/l IAA and 2.0 mg/l BAP stimulated to germination process and initiated callus formation. All the culture tubes showed completion of

Table 2: Effect of Plant Growth Regulators on spread (size), nature and colour of callus after 45 days on MS as basal medium (mean ± S. Em; n=3). MS= Murashige and Skoog

Treatment No.	Treatments (mg/l)		Size of callus (cm)		Nature of the callus	Colour of the callus
	BAP	NAA	Length	Breadth		
T_1	1.5	0.5	1.00 ± 0.04	0.50 ± 0.04	Compact	Dark green
T_2	2.0	0.5	1.06 ± 0.05	0.50 ± 0.03	Compact	Green
T_3	2.5	0.5	1.10 ± 0.03	0.56 ± 0.01	Compact	Green
T_4	3.0	0.5	1.24 ± 0.02	0.93 ± 0.02	Compact	Dark green
T_5	1.5	1.0	1.20 ± 0.05	0.40 ± 0.01	Compact	Dark green
T_6	2.0	1.0	1.20± 0.07	0.53 ± 0.01	Compact	Dark green
T_7	2.5	1.0	1.10 ± 0.04	0.66 ± 0.01	Friable	Dark green
T_8	3.0	1.0	1.00 ± 0.05	0.50 ± 0.01	Friable	Dark green
S. Ed±	-	-	0.06	0.03		
CD (5%)	-	-	0.13	0.06		
CD (1%)	-	-	0.18	0.08		

Table 3: Effect of BAP on number, spread, nature and colour of protocorms on MS medium (mean± S. Em; n=3)

Treatment No.	Treatments (mg/l)	Number ofprotocorms per tube		Spread of protocorm mass (cm)				Nature of protocorm mass		Colour of protocorm mass	
	BAP	30 DAI	45 DAI	30 DAI		45 DAI		30 DAI	45 DAI	30 DAI	45 DAI
				Length	Breadth	Length	Breadth				
T_1	0.0	2.94±0.13	3.50±0.12	0.86±0.02	0.42±0.03	0.98±0.01	0.47±0.03	Compact	Compact	Green	Green
T_2	0.5	4.83±0.04	5.17±0.15	0.86±0.02	0.46±0.02	1.06±0.07	0.56±0.02	Friable	Friable	Green	Green
T_3	1.0	5.00±0.12	5.50±0.15	0.94±0.01	0.54±0.02	1.22±0.06	0.55±0.02	Friable	Friable	Green	Green
T_4	1.5	5.00±0.20	6.83±0.09	0.93±0.01	0.56±0.02	1.20±0.05	0.70±0.04	Compact	Compact	Green	Green
T_5	2.0	4.67±0.17	5.05±0.10	0.98±0.01	0.52±0.02	1.25±0.05	0.63±0.02	Friable	Friable	Green	Green
T_6	2.5	4.00±0.12	4.67±0.18	0.86±0.02	0.53±0.03	0.94±0.05	0.62±0.02	Friable	Friable	Green	Green
T_7	3.0	2.94±0.07	3.33±0.17	0.73±0.01	0.34±0.02	0.92±0.02	0.46±0.01	Compact	Friable	Green	Green
S. Ed ±	-	0.099	0.057	0.006	0.026	0.064	0.018	-	-	-	-
CD (5%)	-	0.21	0.12	0.013	0.055	0.14	0.038	-	-	-	-
CD (1%)	-	0.29	0.17	0.018	0.076	0.19	0.052	-	-	-	-

germination process within 35 days (Table 1). This is in agreement with other published works on callus formation in orchid species such as *Oncidium, Paphiopedilum* orchid, *Dendrobium fimbriatum* Lindl. [4-6]. Similar findings have also been reported where cytokinins as such or in combination with auxin (IAA) stimulated germination in orchid seeds [7]. Table 2 indicated the spread of callus in different treatments. The maximum spread of callus was obtained in MS medium fortified with 3.0 mg/l BAP in combination with 0.5 mg/l NAA which produced a compact, dark green colour callus mass followed by the combination of 2.5 mg/l BAP and 1.0 mg/l NAA and 2.0 mg/l BAP with 1.0 mg/l NAA. It may be noted that at 45 days after inoculation (DAI), the spread (size), nature and colour of callus mass were found most satisfactory when higher concentration of BAP in combination of lower concentration of NAA was used. On the other hand, the spread was lesser with lower concentration of BAP in combination with higher concentration of NAA (Table 2). The frequency of callus formation was varied with different types and concentration of plant growth regulators [8]. The effect of cytokinins on orchid seed germination, callus formation and seedling growth was reported to be different on different species [9]. Meesawat and Kanchanapoom [10] reported the vigourus proliferation of Dendrobium crumenatum in a medium supplemented with suitable concentration of plant growth regulators and peptone. In some species it promoted callus formation and increased the fresh weight, while in others induced the formation of numerous shoots without affecting fresh weight. Different plant species and different plant parts may react differently in different types, concentration and combinations of hormone. The frequencies of callus induction may be varied due to the endogenous hormone contents in plants, their uptake, type of

Fig. 1

Fig. 2

Fig. 3

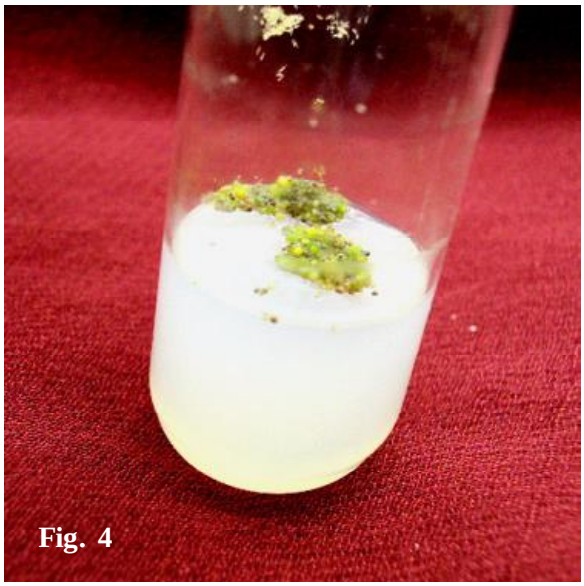

Fig. 4

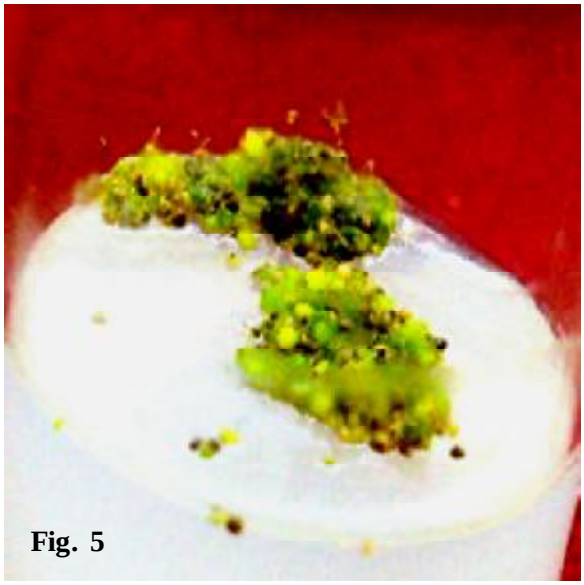

Fig. 5

Fig. 1: Fully grown Banayat Pink plant with flowers. **Figs. 2,3:** Mature pods and seeds of Banyat Pink. Fig. 2 Green mature pods, Fig. 3 Powder like seeds taken out of the pod, which is used as explants. **Fig. 4,5:** Protocorm proliferation and development of Banyat Pink. Fig. 4 Protocorm proliferation in MS medium + BAP 1.5 mg/l. Fig. 5 Magnified view of the protocorm proliferation showing development of protocorms (yellow rounded bodies).

auxins and cytokinins supplemented and also their mode of action [11].

The observations on number, spread, nature and colour of the protocorm formation is presented in Table 3. Data recorded after 30 and 45 days of inoculation indicated that MS medium supplemented with 1 mg/l BAP or 1.5 mg/l BAP produced maximum number of protocorms (5 in each case). At 30 DAI, significantly higher number (6.83) of protocorms per culture tube were formed with 1.5 mg/l BAP (Fig. 4) followed by 1.0 mg/l BAP after 45 days of inoculation. The least number of protocorms were formed with 3 mg/l BAP, which is at par with the control i.e. MS medium without BAP after 45 days of inoculation. The same trend was also noticed with respect to spread of protocorm. It was found that the size of protocorm mass after 45 days of inoculation was maximum (1.2 cm x 0.7 cm) with 1.5 mg/l BAP, while it decreased to a minimum size of 0.92 cm x 0.46 cm when BAP concentration was increased to 3.0 mg/l. Positive effects of adding cytokinins (e.g. benzyladenine or kinetin) to the media on protocorm development is also reported by Rasmussen [12] and Roy et al. [13]. It might be due to enhancement of cell division, normally observed by the action of cytokinins. However, in the present investigation, a concentration of 1.5 mg/l BAP was most effective while higher or lower concentrations of BAP were not much effective for protocorm development. BAP at 1.5 mg/l not only improved the number and size of protocorm but also produced a green compact mass of protocorms having high potential for development of new plantlets in Banyat Pink (Fig. 5).

CONCLUSION

The results obtained in the experiment indicated that the exogenous plant growth regulators are important for callus induction in orchids. The concentration of 1.5 mg/l BAP is the optimum concentration for effective protocorm development in Dendrobium cv. Banayat Pink.

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