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IN VITRO SEED CULTURE STUDIES IN *DENDROBIUM* ORCHID CV. BANYAT PINK

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Abstract

An experiment entitled *in vitro* seed culture studies in *Dendrobium* orchid cv. Banyat Pink was carried out to investigate the effect of growth regulators like IAA, BAP and NAA on seed germination, callus and protocorm formation and seedling development. The results revealed that the seeds obtained from green capsules ("pods") inoculated on MS medium supplemented with 1.0 mgl^{-1} IAA and 2.0 mgl^{-1} BAP recorded cent per cent germination within 35 days after inoculation (DAI). The best callus spread was observed in MS medium fortified with 3.0 mgl^{-1} BAP in combination with 0.5 mgl^{-1} NAA. Maximum number (6.83/culture) and spread of protocorm mass was found on MS medium supplemented with 1.5 mgl^{-1} BAP at 45 DAI.

Introduction

DENDROBIUM IS the second largest genus in the family Orchidaceae which comprises of about 1600 species distributed in Australia, Burma, China, Japan, India, Malaysia, and New Zealand (Bose and Bhattacharjee, 1980). This genus 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. The cultivar Banyat Pink 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 high demand in the domestic and international market. Although symbiotic seed germination studies have been conducted in other orchid species, very little information is available on *in vitro* germination of seeds obtained from the green pods of this variety of *Dendrobium*.

Further, though an attempt has been made to propagate orchid species *in vitro* using various explants (seeds, stem, root *etc.*) (Anuprabha and Pathak, 2012; Arora *et al*., 2014, 2016; Bhattacharjee and Hossain, 2015; Borah *et al*., 2015; Chauhan *et al*., 2010, 2015; Hegde, 2012; Hoque *et al*., 2016; Kaur and Pathak, 2014; Laqishram and Devi, 1999; Pathak *et al*., 1992, 2011, 2012, 2016; Sibin and Gangaprasad, 2016; Sibin *et al*., 2014; Verma *et al*., 2013; Vij *et al*., 1989, 1994, 1995) so as to develop protocols for their *in vitro* propagation, the data is meager in terms size of the orchid family. Hence, the present investigation assumes a greater significance so far as the orchid propagation is concerned.

Materials and Methods

The present study was conducted in the Plant Tissue Culture Laboratory, Department of Floriculture, Orissa University of Agriculture and Technology, Bhubaneswar.

Plant Material

Green pod of *Dendrobium* orchid cv. Banyat pink was used for conducting the experiment. The immature seeds obtained from the green pod were used for embryo culture.

Maintenance and Establishment of Culture

The seeds were inoculated on solid MS nutrient media (Murashige and Skoog, 1962) supplemented with 2 concentrations of auxin *i.e.,* IAA (0.5 mgl^{-1} and 1.0 mgl^{-1}) in combination with four different concentrations of cytokinin *i.e*., BAP (1.5 mgl^{-1}, 2.0 mgl^{-1}, 2.5 mgl^{-1}, and 3.0 mgl^{-1}) for germination study and establishment of culture. Observations on number of cultures showing seed germination and callus initiation were recorded.

Callus Formation

The small calli mass that were initiated, were inoculated in MS media containing different concentrations of NAA (0.5 $mgl^{-1}$and 1.0 mgl^{-1}) and BAP (1.5 mgl^{-1}, 2.0 mgl^{-1}, 2.5 mgl^{-1}, and 3.0 mgl^{-1}) to assess their effect on development and spread of callus. Observations were recorded on spread, nature and colour of the callus at 45 DAI.

Protocorm Induction

After the formation of callus, the calli masses were transferred to MS media containing seven different

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concentrations of BAP *i.e*., 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mgl^{-1} for protocorm formation. Observations were recorded on number of protocorms formed, spread, nature and colour of protocorm mass at 30 and 45 DAI.

Results and Discussion

The results revealed that germination process was initiated in seeds obtained from green capsule and inoculated on MS media fortified with various combinations of IAA and BAP, after 25 days of culture and the media fortified with 1.0 mgl^{-1} IAA and 2.0 mgl^{-1} BAP showed green colouration in 15 out of 20 cultures within 35 days of culture, all the cultures showed light green coloration indicating the completion of germination process (Table 1).

Auxins and cytokinins are most frequently used growth regulators for nutrient media to initiate and increase the germination process of seeds. Observations as made during the present study have also been reported earlier by Mitra (1986) who found that cytokinin alone or in combination with auxin IAA stimulated germination in orchid seeds. It was observed that the maximum spread of callus was obtained on MS medium fortified with 3.0 mgl^{-1} BAP in combination with 0.5 mgl^{-1} NAA which produced a compact dark green callus mass (Table 2). This treatment was followed by the combination of 2.5 mgl^{-1} BAP and 1.0 mgl^{-1} NAA. On the other hand, MS medium fortified with1.5 mgl^{-1} BAP and 1.0 mgl^{-1} NAA produced the minimum spread of callus.

The effect of cytokinins on orchid seed germination, callus formation and seedling growth was reported to be different in different species (Arditti, 1967). In some species it promoted callus formation and increased fresh weight while in others it induced formation of numerous shoots without affecting fresh weight. Similarly, the role of growth substances in propagation of *Cymbidium* was studied by Fonnesbech (1972) who

Table 1. Effect of PGRs on germination on *in vitro* seed culture of *Dendrobium* orchid cv. Banyat Pink in culture tubes.

Treatment Number	Treatments		Time taken in days for germination (per cent germination)		
	IAA(mg/l)	BAP(mg/l)	25 DAI	30 DAI	35 DAI
1	0.5	1.5	5 (25%)	12 (60%)	16 (80%)
2	0.5	2.0	6 (30%)	13 (65%)	18 (90%(
3	0.5	2.5	5 (25%)	12 (60%)	16 (80%)
4	0.5	3.0	5 (25%)	11 (55%)	15 (75%)
5	1.0	1.5	6 (30%)	13 (65%)	15 (75%)
6	1.0	2.0	8 (40%)	15 (75%)	20 (100%)
7	1.0	2.5	7 (35%)	14 (70%)	18 (90%)
8	1.0	3.0	6 (35%)	13 (65%)	17 (85%)

Figures in the parentheses indicate the corresponding percentage of cultures showing germination.

Table 2. Effect of PGRs on spread, nature and colour of callus on *in vitro* seed culture of *Dendrobium* orchid cv. Banyat Pink.

Treatment Number	Treatments		Spread of callus (cm)		Nature of the Callus	Colour of the Callus
	BAP(mgl^{-1})	NAA(mgl^{-1})	Length	Breadth		
1	1.5	0.5	1.00	0.50	Compact	Dark green
2	2.0	0.5	1.06	0.50	Compact	Green
3	2.5	0.5	1.10	0.56	Friable	Green
4	3.0	0.5	1.23	0.93	Compact	Dark green
5	1.5	1.0	1.20	0.40	Compact	Dark green
6	2.0	1.0	1.20	0.53	Compact	Dark green
7	2.5	1.0	1.10	0.66	Friable	Dark green
8	3.0	1.0	1.00	0.50	Friable	Dark green

Table 3. Effect of BAP on protocorm formation, multiplication, nature and colour of protocorm mass on *in vitro* seed culture of *Dendrobium* orchid cv. Banyat Pink.

Treatment Number	Treatments (BAP)mgl-1	Number of protocorms /culture		Spread of protocorm mass(cm^2)		Nature of protocorm mass		Colour of protocorm mass	
		30DAI	45DAI	30DAI	45DAI	30DAI	45DAI	30DAI	45DAI
T1	0	2.94	3.50	0.86×0.42	0.98×0.47	Compact	Compact	Green	Green
T2	0.5	4.83	5.17	0.86×0.46	1.06×0.56	Friable	Friable	Green	Green
T3	1.0	5.00	5.50	0.94×0.54	1.22×0.55	Friable	Friable	Green	Green
T4	1.5	5.00	6.83	0.93×0.56	1.20×0.70	Friable	Friable	Green	Green
T5	2.0	4.67	5.05	0.98×0.52	1.25×0.63	Friable	Friable	Green	Green
T6	2.5	4.00	4.67	0.86×0.53	0.94×0.62	Friable	Friable	Green	Green
T7	3.0	2.94	3.33	0.73×0.34	0.92×0.46	Compact	Friable	Green	Green
SE(m)±	-	0.32	0.25						
CD(5%)	-	0.97	0.76						

reported that Knudson's medium supplemented with BA promoted callus formation and increased fresh weight while higher concentration of NAA caused inhibition of chlorophyll synthesis. The results of the present investigation are in close agreement with the findings of earlier workers as stated above.

With respect to protocorm formation, it was observed that the number of protocorms per culture were higher (5.0 in each case) on MS medium supplemented with BAP 1.0 mgl^{-1} and 1.5 mgl^{-1} after 30 days of inoculation (Table 3). However, after 45 days of inoculation, the protocorm number was significantly higher with the supplement of 1.5 mgl^{-1} BAP (6.83), followed by 1.0 mgl^{-1} BAP (5.5). On the other hand, the least number of protocorms per culture were formed on MS medium supplemented with 3.0 mgl^{-1} of BAP during both the observations. The result is in conformity with Rasmussen (1995) who reported a positive effect of adding cytokinin (*e.g.,* Benzyl Adenine or Kinetin) to the media on protocorm development. It might be due to enhancement of cell division, normally observed by the action of cytokinin. However, in the present investigation, a concentration of 1.5 mgl^{-1} BAP was most effective while higher or lower concentrations of BAP were not much effective for protocorm development. BAP at 1.5 mgl^{-1} not only improved the number and size of protocorms but also produced a green compact mass of protocorm.

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