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Research Article

In Vitro Micropropagation of *Dendrobium chrysanthum* Wall. ex Lindl. –A Threatened Orchid.

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Abstract: An efficient protocol for seed germination and micropropagation of *Dendrobium chrysanthum* Wall ex Lindl. was established. Four nutrient media were used for seed germination and early protocorm development: Murashige and Skoog (MS), half –strength MS, Knudson 'C' (KC), and Vasin and Went (VW); combinations and alone of four plant growth regulators i.e. 6-benzylaminopurine (BAP), kinetin (KN), α-napthalene acetic acid (NAA), and indole-3-butyric acid (IBA) were studied. MS medium was found as most ideal for seed germination (98±0.48) and lowest in VW (71.12 ±0.42). 3 months old protocorm were sub cultured on fresh MS medium supplemented with different concentrations of BAP, KN, NAA, and IBA alone and in combination. After 30 days highest secondary protocorms (21.25±0.63) were observed in MS medium containing BAP (4.0µM). MS medium supplemented with 8µM IBA induced the maximum roots per shoot. After 16 days of transfer to green house the survival rate was 88%..
Keywords: *Dendrobium chrysanthum*, MS, protorm, micropropagation, BAP

INTRODUCTION

Dendrobium is the second largest genus of Orchidaceae with approximately 1500 species and almost one fourth of them are used as ornamental due to their beautiful flowers [1]. Out of these there are about 300 species available in India. *D. Chrysanthum* Wall. ex. Lindl. is one of the valuable ornamental orchids available in North East India. Due to its herbal medicinal value[2] and horticultural importance it is over exploited making it very rare in wild. The plant is generally propagated by vegetative method which is very slow. Its pods contain millions of tiny seeds but they rarely germinate in their natural conditions. Knudson successfully germinated orchid seeds on nutrient medium under in vitro conditions [3]. Morel developed a tissue culture procedure for clonal propagation of Dendrobium species [4].

But there is no any specific protocol for the in vitro culture of *D. Chrysanthum*, So the present study has undertaken to develop a specific media which supports the propagation of *D. Chrysanthum* Wall ex. Lindl.

MATERIALS AND METHODS

Plants of *D. Chrysanthum* Wall ex. Lindl. were collected from Garo Hills of Western Meghalaya and were cultivated in Botanical Garden, Goalapra College, Assam. From these immature capsules of almost 80 days were collected and cleaned thoroughly under tap water, disinfected with 70 per cent ethanol for 30 seconds and then surface sterilized by 3 per cent sodium hypochlorite solution for 25 minutes. The pods are then washed in sterilized distilled water and then dried. The capsules are then cut longitudinally in a plate in laminar air flow chamber; seeds were diluted in sterile water.

For the present study four different media MS (Murashige and Skoog), ½ MS, Knudson 'C' (KC), Vasin and Went media (Vasin and Went) were taken [5-7]. All these media were supplemented with 3% sucrose (w/v) and solidified with 0.8% agar. The P^H of the media was maintained at 5.8. For sterilization media was autoclaved at 121^0C at 15 psi for about 15 minutes. During culture temperature was maintained 24± 2^0C under cool fluorescent light at 50µmol m^{-2} s^{-1} with 14 hour photoperiod. The seed germination data was recorded after 60 days of inoculation and the developmental stages were observed after the interval of every 30 days from seed germination. The most efficient medium was supplemented with different concentrations of 6-benzyl amino purine (BAP), kinetin (KN), 2-napthalene acetic acid (NAA) and indol 3-butyric acid (IBA) alone and in combination to study their effect in development of plantlets. For these 60 days old protocorms were used. Data was recorded at the interval of every 30 days. For the induction of roots, plants without roots after 2 months were cultured on MS media supplemented with different concentrations (5.0 10.0 and 15.0 µM) of IBA and NAA.

Well rooted plants are rinsed carefully in water to remove the residual medium and then transplanted in plastic pots containing a mixture of coarsely crushed sterile brick, charcoal and vermicompost and kept in green house at 25± 2^oC and 80 per cent relative humidity. Plants were watered at the interval of two days and survivability was calculated after 60 days in green house. Results were studied by one way analysis of variance. Mean and Standard error were determined by Duncan's multiple range tests at probability level 0.5.

RESULTS AND DISCUSSION

Percentage of seed germination was observed maximum in MS (98± 0.48) % followed by half strength MS (89.34± 0.51) %, KC (76.82 ± 0.43) % and VW (71.12± 0.42) %. The germination was marked by swelling and emergence of embryo from seeds within three months. After three weeks these embryos develop into protocorms. 90 days old protocorms were then subcultured on fresh MS medium supplemented with different concentrations of BAP (2.0, 4.0, 8.0 µM), KN (2.0, 4.0, 8.0 µM), NAA (2.0, 4.0, 8.0 µM) and IBA (2.0, 4.0, 8.0 µM) alone and in combination. After 30 days in supplemented medium the highest secondary protocorms (21.25 ±0.63) were observed in MS medium containing BAP (4.0 µM) and NAA (2.0 µM) followed by BAP (4.0 µM) alone. Within 30 to 40 days all the protocorms were converted into plantlets giving rise to multiple numbers of shoots within the same media.

For the induction of roots plantlets were supplemented with different concentrations of auxins. MS medium supplemented with 8µM IBA induced the maximum roots (6.84 ± 0.05) per shoot followed by 12 µM IBA. Among the three NAA was much less effective in inducing initiation of roots. After 16 days of transfer to green house in pots containing sterile mixture of brick and charcoal and vermin compost, the survival rate was 88%.

In vitro seed germination is an effective tool for mass propagation and conservation of *Dendrobium chrysanthum*. So, by commercially adopting this process we can reduce the pressure on demand of wild *Dendrobium* thus helping in conservation of natural population. The different media used in this investigation with different composition and concentrations of minerals, organic compounds, vitamins shows different results for in vitro germination. The full strength MS media has been found more suitable for in vitro germination in comparison to other media used in the investigation. Nitrogen has an important role in the germination of seeds [7, 8]. Nitrogen induces the growth and differentiation of cells [9]. Nitrogen in the form of ammonium nitrate in MS medium is most suitable for seed germination and plant development in Dendrobium species [10]. The presence of vitamins nicotinic acid, pyridoxine and thiamine promote seed germination in *Dendrobium chrysanthum*. These finding are similar with those observed in Cymbidium sp.[11].

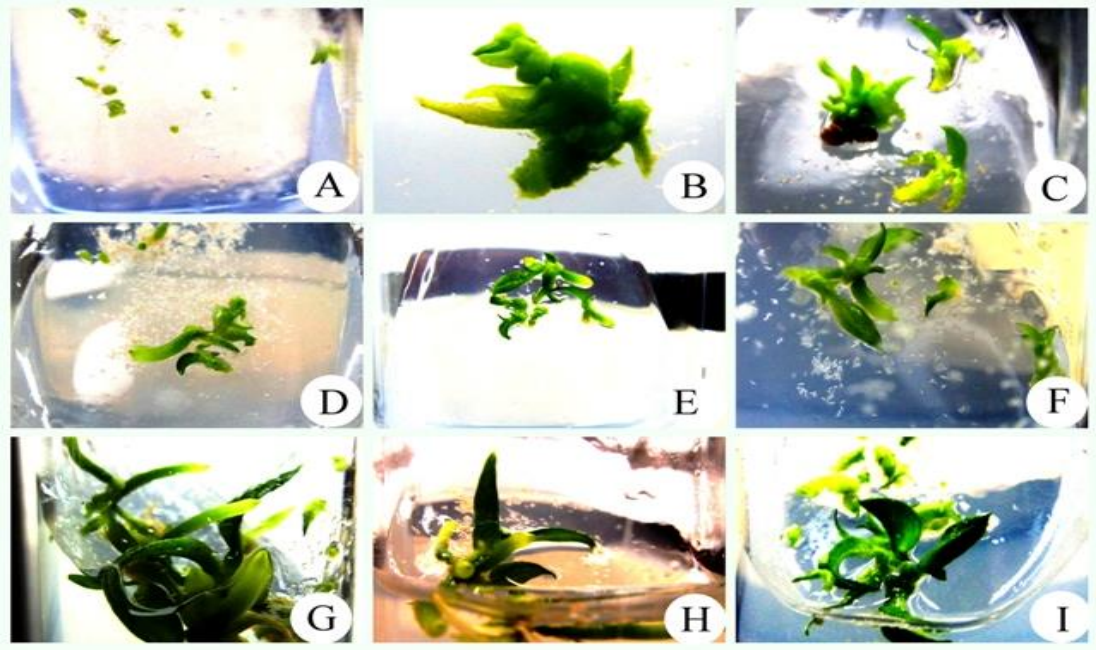

Fig.1: A-C Development of PLB bodies and callus formation; D-I Shoots and roots formation in MS medium.

Table No.1 Comparison account of seed germination and protocorm development in Dendrobium chrysanthum.

Medium	% of seed germination	Time required for seed germinaiton	Protocorm development		
			Vegetative apex stage	Leave stage	Root stage
Half MS	71.12± 0.42	9-10	78.83± 1.02	67.81± 1.04	56.91± 0.67
Kc	76.82± 0.43	8-9	59.23± 0.26	52.06± 0.22	49.73± 0.32
Ms	98.01± 0.48	10-11	47.09± 0.33	37.28± 0.72	32.76± 0.52
Vw	89.34± 0.51	10-12	43.21± 0.65	-	-

Table No.2 Development of secondary protocorm form primary protocorm of *D. chrysanthum*.

Plant Growth Regulators (µM)				Number of secondary protocorms (mean ± SE)
BAP	**NAA**	**KN**	**IBA**	
0.00	0.00	0.00	0.00	1.89 ± 0.18
2.00	-	-	-	5.09 ± 0.12
4.00	-	-	-	16.25 ± 0.69
8.00	-	-	-	9.53 ±0.65
-	2.00	-	-	7.45 ± 0.26
-	4.00	-	-	2.59 ± 0.63
-	8.00	-	-	2.10 ± 0.32
-	-	2.00	-	2.65 ± 0.36
-	-	4.00	-	10.52 ± 0.62
-	-	8.00	-	13.72 ± 0.23
-	-	-	2.00	6.25 ± 0.64
-	-	-	4.00	5.20 ± 0.21
-	-	-	8.00	4.25 ± 0.26
4.00	2.00	-	-	21.25 ± 0.63
4.00	4.00	-	-	16.42 ± 0.24
4.00	8.00	-	-	12.56 ± 0.45
4.00	-	-	2.00	12.52 ± 0.69
4.00	-	-	4.00	8.50 ± 0.16
4.00	-	-	8.00	8.22 ± 0.36
-	2.00	8.00	-	5.50 ± 0.64
-	4.00	8.00	-	4.80 ± 0.93
-	8.00	8.00	-	4.60 ± 0.23
-	-	8.00	2.00	5.65 ± 0.12
-	-	8.00	4.00	4.25 ± 0.20
-	-	8.00	8.00	4.70 ± 0.25

Table No. 3 Effect of NAA and IBA on root induction in *D. chrysanthum*

NAA (µM)	IBA (µM)	Number of root	Root length (cm) (Mean ± SE)
4.00	-	02.50 ± 0.36	1.20 ± 0.08
8.00	-	3.60 ± 0.40	2.40 ± 0.06
12.00	-	4.80 ± 0.35	1.46 ± 0.05
-	4.00	5.20 ± 0.15	2.36 ± 0.08
-	8.00	6.84 ± 0.05	3.50 ± 0.04
-	12.00	5.65 ± 0.25	3.64 ± 0.05

CONCLUSION

The result clearly shows that the seeds of *Dendrobium chrysanthum* Wall. ex. Lindl. can be used for micropropagation of the plant effectively. This protocol can be used to produce viable, uniform and healthy plants of *Dendrobium Chrysanthum* to restore the significantly decreasing no. of populations of this beautiful rare orchid in nature and mass scale propagation for its commercial use.

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