

Significance of different concentration of plant growth regulators(PGRs) on *in vitro* multiplication of *Dendrobium moschatum*(Buch.- Ham.)Sw. using nodular explants.

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Abstract: *Dendrobium moschatum*(Buch.-Ham.)Sw.(The Musky Smelling *Dendrobium*) is an epiphytic orchid of N.E.India which produce graceful, intricately fabricated beautiful flowers of immense floricultural appeal. It is a robust species with erect radiating branches ,bearing sweetly scented flowers with light yellow and purplish tinge on petals and sepals having reddish veins. Like other orchids of this region, this species is also endangered due to large scale denundation of forest areas. So in order to preserve it from further extinction, attempts have been made to conserve and multiply it through *in vitro* culture of nodular explants. NAA at concentration 1.0 mg/L in MS medium was found to be the most effective for early initiation of bud and multiple shoot induction. Full strength liquid MS medium has been found to produce the best result for nodular cultured explants.

Key Words: *in vitro* technique, *D.moschatum*, MS medium, nodular segments, NAA, IAA, BAP, liquid MS medium.

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I. Introduction

Orchids are one of the highly loved flowers of nature because they represent beauty, luxury and strength. These delicate and beautiful flowers add an elegance and beauty to the flower arrangements and decorations. Orchidaceae is the second largest family in India (Satish Kumar and Maninlal, 1994). The nearest estimates range between 3,000 to 3,500 species in over 800 genera. The North-East region is perhaps the richest orchid wealth in the world and about 800 species are known to occur in this region (Chadha,1994). About 350 species have been reported from Assam (Arora and Mukherjee,1983). Commercially, the orchid *Dendrobium* command a high demand in their price for extremely beautiful, intricately fabricated, highly colourful and long lasting flowers and have contributed immensely to the developement of international trade in floriculture (Lawler,1984)

Dendrobium moschatum(Buch.-Ham.)Sw. is an epiphytic orchid with erect radiating branches bearing tuft of pseudobulbs. Inflorescence arises from the apical region with laxly bearing 10-12 flowers. The flowers are 5-5.5cm across, sweetly scented, light yellow with purplish tinge on petals and sepals having reddish veins. Flowering time-May to June lasting for 6-7 days. The above mentioned orchid is also said to have some medicinal values- leaf juice are used as ear-drops for ear pain (Quattrocchi,2012). Plicatol B. is a phenanthrene (hydrocarbon) that can be isolated from *Dendrobium moschatum*(Buch.-Ham.)Sw. (Govaerts,2013).

Like other orchids of this region, *Dendrobium moschatum*(Buch.-Ham.)Sw. is also in the verge of extinction due to large-scale denundation of forest areas. Also the seeds are very few in number, so in order to conserve this beautiful from further extinction, nodular segments were cultured aseptically in MS (Murashige and Skoog)medium(1962) using *in vitro* technique for early initiation of bud and multiple shoot induction. *In vitro* techniques provide better understanding of different physico-chemical requirements that might affect growth (Adritti *et.al*,1981).

II. Materials and methods

Fresh and healthy nodular portions were collected from the Orchidarium of Department of Life Sciences, Dibrugarh University for the present study. The nodular portions so collected were first washed thoroughly, carefully under tap water, then with sterile DDW solution using 15% Teepol. The nodular portions were surface sterilized by immersing in 0.01% HgCl₂ solution for 2-3 minutes inside a laminar air flow cabinet. Again washed 2-3 times in sterile DDW. The nodular portions were then inoculated into the flasks containing MS medium (1962) with different concentrations of auxins (IAA,NAA) and cytokinins (BAP,KN). The plant growth regulators (PGRs) were taken as 1mg/10ml of DW made up with 1000ml of MS medium. The explants

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were inoculated in different concentrations of IAA (3,2 ,1 mg/l) ,NAA(1,2,3 mg/l), BAP, KN and full, $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{8}$ strength liquid MS medium. The percentage of sucrose was taken as 30mg/lt, agar 8mg/lt, pH of the medium adjusted to 5.6 before sterilization and autoclaved at 15lbs/(inch) for 10-15 minutes. The inoculated flasks were stored on racks in growth room under continuous conditions of temperature (22-24°), humidity (65%-70%) and 12hr/day light (2000-3500 lux).

The flasks were observed at regular intervals for the development and growth of buds, multiple shoot induction, roots, leaves etc. They were repeatedly sub-cultured at regular intervals.

III. Results and Discussion

MS medium supplemented with NAA 1 mg/lt was found to be the most suitable for nodular explant culture in comparison to other auxins (IAA) and cytokinins (BAP,KN). Nodular explants in MS, when inoculated with 1mg/lt NAA, the number as well as the time taken for the growth and development of bud, leaves, roots, multiple shoot induction, plantlet height were faster and better than at higher concentration of NAA(2mg/1,3mg/lt)(Saikia and Borua,2017).

Protuberance of first leaf and shoot was seen in 1NAA mg/lt and 2 mg/lt within 3-5 days and 5-7 days respectively, induction of first shoot was seen in 1 NAA mg/lt and 2 mg/lt within 14-15 days and 18-19 days of MS medium respectively. Thus 1NAA mg/lt was selected for further sub-culturing of the above species. No effective results were found from cytokinins (as well as their combinations with other growth regulators).

IAA was slightly effective in promoting bud growth in *Dendrobium moschatum*(Buch.-Ham.)Sw. The same effect of the hormones was reported in *Vanda* (Rao and Avadhani,1963) and *Spathoglottis plicata* (Chennaaveeriah and Patil,1975) (**Table:1 and Table:2**)

Table 1 : Response of various plant growth regulator(s) on the nodular portions of *Dendrobium moschatum*(Buch.-Ham.)Sw. in MS medium

Sl. No.	MS medium + PGR(s) (mg/l)	First bud formation within PLB(s)(days)	First leaf formation within (wks)	First shoot formation within (wks)
1	3 IAA	16.33a ± 0.471	5b ± 0.816	7.67 b ± 0.471
2	1 NAA	14.33a ± 0.471	3.33 a ± 0.471	5.67 a ± 0.471
3	2 NAA	19 b ± 1.414	4.67b ± 0.471	6.33a ± 0.471
4	1NAA+1BAP	15.33a ± 0.943	3.67 a ± 0.471	6.33a ± 0.471
	5%	2.51714	0.74551	1.333

Means followed by common letter(s) are not significantly (p<0.05) different as determined by Duncan’s New Multiple Range Test (DMRT).

1NAA+1BAP mg/lt was slightly effective in promoting bud growth in *Dendrobium moschatum*(Buch.-Ham.)Sw. but the parameters (**Table:1 and Table: 2**) of leaf and root growth was not as favourable in comparison to only 1 NAA mg/lt MS medium. The combined effect of 1 BAP mg/lt+ 1 NAA mg/lt showed good growth of leaf and root as well as their length and plant height in the beginning as reported in *Phalaeanopsis* and *Doritaneopsis* by sub-culturing shoot-tips of flower stalks having 1 or 2 leaf primordial in New Dogashime medium (NDM) containing (0.1 mg/lt) NAA and (1 mg/lt) BAP (Tokuhara *et.al*, 1993). Cytokinin stimulated formation of numerous protocorms and shoots, it inhibited root formation (Kulkulezanke *et.al*, 1997) (**Table:2**).

Table 2 : Effect of different concentrations of plant growth regulator(s) and their response to the different parameters upon nodal explants of *Dendrobium moschatum*(Buch.-Ham.)Sw. (3 months/ 12 weeks) in MS medium

Sl. No.	MS medium + PGR(s) (mg/l)	Leaf length (cm)	Root length (cm)	No. of leaves per plantlet	No. of roots per plantlet	Plantlet height (cm)	No. of multiple shoot per plantlet
1	3 IAA	0.6a ± 0.082	1.30 a ± 0.082	1.67 a ± 0.471	3.67 a ± 0.471	2.60 a ± 0.082	1.33 a ± 0.471
2	1 NAA	0.93 b ± 0.047	1.57 b ± 0.047	2.33 a ± 0.471	5.67 b ± 0.471	2.87 c ± 0.125	2.33 a ± 0.471
3	2 NAA	0.83 b ± 0.047	1.57 b ± 0.047	1.67 a ± 0.047	3.67 a ± 0.471	2.60 a ± 0.082	1.67 a ± 0.471
4	1 NAA+1BAP	0.87 b ± 0.094	1.47 b ± 0.047	2.33 a ± 0.471	4.67 ab ± 0.471	2.80 b ± 0.082	1.67 a ± 0.471
	5%	0.14910	0.16330	1.20210	1.00021	0.057740	1.2021

Means followed by common letter(s) are not significantly (p<0.05) different as determined by Duncan’s New

Multiple Range Test (DMRT).

After 15 weeks, some of the plantlets were transferred to liquid 1 NAA mg/l (for 3 weeks) showed better response in all the above parameters in comparison to solid 1 NAA mg/l MS medium. The number of multiple shoots per plantlet was found to be more in liquid medium than solid medium (**Table: 3**)

Table 3: Comparison of different parameters of liquid and solid medium of the same concentration tried in MS medium of *Dendrobium moschatum*(Buch.-Ham.)Sw. months/ 20 weeks)

Sl. No.	MS medium + PGR(s) (mg/l)	Leaf length (cm)	Root length (cm)	No. of leaves per plantlet	No. of roots per plantlet	Plantlet height (cm)	No. of multiple shoot per plantlet
1	1 NAA (liquid)	1.30 a ± 0.082	1.8 a ± 0.082	2.33 a 0.471	8.0 b ± 0.082	3.20 a ± 0.082	8.33 b ± 0.471
2	1 NAA (solid)	1.30 a +0.047	1.63 a ± 0.047	4.3 a ± 0.471	5.67 a ± 0.471	2.97 a ± 0.125	2.33 a ± 0.471
	5%	0.379224	1.22460	1.2900	1.4333	0.37922	2.48260

Means followed by common letter(s) are not significantly ($p < 0.05$) different as determined by Duncan's New Multiple Range Test (DMRT).

It was found that liquid MS medium leads to an increase in the total number of roots/plantlet. Liquid culture was better suited for root cultures (Street, 1969). NAA enriched medium (MS) favoured multiple shoot bud formation (Vij and Kaur, 1998). The quantity and quality of roots and leaves, plantlet height and multiple shoot induction was found to be the best in full strength liquid MS medium (with 3% sucrose) in comparison to other strengths ($1/2, 1/4, 1/8$) of liquid MS medium (**Table:4**). The growth and development of *Dendrobium moschatum*(Buch.-Ham.)Sw. was more vigorous and faster, roots developed at a faster rate and the medium devoid of any hormonal supplements was found to be the best as reported by Haque et.al (1994). Total number of multiple shoots per node is -19.3 shoots /nodal explant (average).

Table 4 : Comparison of different parameters of liquid medium without PGR(s) for standardisation further to potting medium in *Dendrobium moschatum*(Buch.-Ham.)Sw. under in vitro conditions. (5 months 3 weeks)

Sl. No.	MS liquid medium in different strengths (mg/l)	Leaf length (cm)	Root length (cm)	No. of leaves per plantlet	No. of roots per plantlet	Plantlet height (cm)	No. of multiple shoot per plantlet
1	Full	1.53 b ± 0.047	1.97 b ± 0.094	2.67 b ± 0.471	10.33 c ± 0.471	3.43 c ± 0.094	8.00 c ± 0.816
2	$1/2$	1.33 a ± 0.047	1.90 a ± 0.082	2.00 bc ± 0	8.33 b ± 0.471	3.27 b ± 0.047	8.00 b ± 0.816
3	$1/4$	1.30 a ± 0.082	1.80 a ± 0.082	1.33 ab ± 0.471	8.00 b ± 0.816	3.13 a ± 0.047	2.67 b ± 0.471
4	$1/8$	1.30 a ± 0.082	1.80 a ± 0.082	1.00 a ± 0	5.67 a ± 0.471	3.13 a ± 0.047	0.67 a ± 0.471
	5%	0.06965	0.20820	1.94300	1.37460	0.12912	1.66701

Means followed by common letter(s) are not significantly ($p < 0.05$) different as determined by Duncan's New Multiple Range Test (DMRT).

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