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ROLE OF DIFFERENT CONCENTRATION OF PGRS(PLANT GROWTH REGULATORS) ON *IN VITRO* MULTIPLICATION OF *DENDROBIUM FIMBRIATUM* VAR.*OCULATUM* USING NODAL SEGMENTS.

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Abstract:

Dendrobium fimbriatum var.oculatum is an epiphytic orchid of of immense floricultural apppeal as well as some local medicinal values. It is a native to north-eastern India and parts of Indo-Chinese peninsula with a height of about 1m bearing beautiful bunches of bright yellow flowers having a reddish brown blotch in the middle and reddish streaks at the base, mildly scented and flowers lasting for 8-10 days. Like many other orchids of this region, this species is also found to be in the verge of extinction, due to large-scale denundation of forest areas. So in order to preserve it from possible extinction, attempts are made to multiply it through in vitro culture of nodular portions. NAA (1 mg/l) in MS medium was found to be most effective for early initiation of bud and multiple shoot induction.Full strength liquid MS medium proved to be the best for nodal explant culture.

Key Words: *in vitro technique, D.fimbriatum, nodular portions, NAA,IAA,BAP,KN,MS medium, liquid MS medium.*

Introduction:

Orchids are one of the most beautiful flowers in nature and Orchidaceae is the second largest family in India(Satish Kumar and Maninlal,1994).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). 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 fimbriatum* var.*oculatum* is a hard cane-type epiphytic orchid reaching a height of about 1m producing beautiful bunches of bright yellow flowers with orbicular fimbriate lip having a large dark reddish -brown blotch in the middle and reddish streaks at base, mildly scented and flowers remaining fresh for 8-10days. The above orchid also has some local medicinal values like for the treatment of lethargy, madness, loss of appetite, lung cancer, stomache cancer, epilepsy, etc(Quattocchu,2012).

Like many other orchids of this region, *Dendrobium fimbriatum* var.*oculatum* is also at the verge of extinction due to large-scale denundation of forest areas. The seeds are very few in number , so in order to conserve this beautiful orchid from further extinction, nodular portions 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).

Materials and methods:

Fresh and healthy nodular potions were collected from the Orchidarium of Dept. 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% $Hgcl_2$ solution for 2-3 minutes inside a laminar air flow cabinet. Again washed 2-3 times in sterile DDW. The nodular portions were then innoculated 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 were inoculated in different concentrations of IAA(3,2,1 mg/l) ,NAA(1,2,3 mg/l), BAP, KN and full, 1/2,1/4 and 1/8 strength liquid MS medium. The percentage of sucrose is 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$^{\circ c}$), 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 and photographed whenever necessary.

Results and Discussion:

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

than at higher concentrations of NAA(2mg/lt,3mg/lt).

Protuberance of first leaf and shoot was seen in 1NAA mg/lt and 2 mg/lt within 3-4 days and 6-7 days respectively, induction of a single shoot was seen in 1NAA mg/lt and 2mg/lt in within 14-15 days and 16-17days of MS medium respectively**(Table:1** and **Table:2)**. Thus 1NAA mg/lt was selected for further sub-culturing of the above species. No favourable results were found from cytokinins.

Table 1 : Response of various plant growth regulator(s) on the nodular portions of *Dendrobuim fimbriatum* var. *oculatum* in MS medium

Sl. No.	MS medium + PGR(s) (mg/l)	First bud formation within (days)	First leaf formation within (wks)	First foot formation within (wks)
1	3 IAA	23.33 c ± 1.70	7.67 d ± 0.471	8.67 d ± 0.471
2	1 NAA	14.67c ± 0.471	3.33 a ± 0.471	6.33 a ± 0.471
3	3 NAA	16.33 b ± 0.471	4 b ± 0.816	6.67 a ± 0.471
4	3 NAA	22.67 c ± 0.471	5.67 c ± 0.471	7.67 b ± 0.471
	5%	0.92132	0.38933	0.60315

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

IAA was slightly effective in promoting bud growth in *Dendrobium fimbriatum* var. *oculatum.* The same effect of the hormones was reported in *Vanda* (Rao and Avadhani,1963) and *Spathoglottsis plicata* (Chennaaveeriah and Patil,1975).**(Table:1** and **Table:2)**

Table 2 : Effect of different concentrations of plant growth regulator(s) and their response to the different parameters upon nodal explants of *D. fimbriatum* var. *oculatum* (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	0.7 a ± 0.082	0.57 a ± 0.047	4 a ± 0.816	2.33 a ± 0.471	1.4 a ± 0.082	1.0 a ± 0.816
2	1 NAA	1.23 a ± 0.125	2.3 b ± 0.163	7.3 b ± 0.471	4.3 b ± 0.471	2.83 b ± 0.125	3.0 a ± 0.816
3	2 NAA	1.03	2.17 b	3.3 a	4.3 b	2.47	2.0 a

		b ± 0.125	± 0.094	± 0.471	± 0.471	b ± 0.125	± 0.816
	5%	0.30265	0.23926	2.00183	1.311050	0.37067	2.07208

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 when transferred to liquid 1 NAA mg/lt MS medium(3 weeks) showed better response in all the above parameters in comparison to solid 1 NAA mg/lt 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 *D. fimbriatum* var. *oculatum* (5 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)	1.4 a ± 0.082	2.5 a ± 0.163	5.3 b ± 0.471	11.3 a ± 0.943	3.13 a ± 0.125	11.3 b ± 0.471
2	1 NAA (solid)	1.23 a ±0.047	2.3 a ± 0.170	4.3 a ± 0.471	7.67 a ± 0.471	2.83 a ± 0.047	3 a ± 0.816
	5%	0.37922	0.33100	0	8.7574	0.4300	0

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 fimbriatum* var.*oculatum* 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 -36.9 shoots /nodal explant(average).

Table 4 : Comparison of different parameters of liquid medium without PGR(s) for standaradisation further to potting medium in *D. fimbriatum* var. *oculatum* under *in vitro* conditions. (5 months / 20weeks)

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 ± 0.125	2.63 a ±0.170	5.67 b ±0.471	14.67 c ±1.247	3.37 a ±0.125	9.67 d ± 0.471
2	½	1.40 ab ± 0.082	2.57 a ±0.047	5 ab ±0	12 bc ±1.632	3.10 a ±0.082	4.67 c ±0.471
3	¼	1.40 ab ±0.082	2.47 a ± 0.094	4.3 ab ±0.471	10 b ± 1.414	3.10 a ± 0.125	1.67 b ± 0.471
4	$^1/_8$	1.30 a ±0.082	2.53 ± 0.125	3.67 a ±0.943	4.3 a ± 0.471	3.10 a ±0.125	1 a ± 0
	5%	0.1527	0.31270	1.4902	3.24960	0.29250	0.57741

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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Different types of explants, concentrations and combination of plant growth regulators play a remarkable role during In vitro propagation of Cymbidium orchid (Tao et al., 2011). SEM and TEM analysis were determined in order to justify that PLBs is a meristematic tissue and contains actively dividing cells that is suitable as a target material for In vitro system work. ...Â In vitro seedlings of Dendrobium ellipsophyllum Tang & F.T.Wang were cultured on MS medium supplemented with cytokinin (BA, kinetin, TDZ) at 0 0.1 0.5 1.0 or 2.0 mg/L for 12 weeks. The result showed that the highest percentage of shoot formation (47.5%), shoot numbers (1.85 shoots/plant) and leaf numbers (4.45 leaves/plant) were obtained when cultured on the medium with 0.1 mg/L TDZ. supplemented with different concentrations. of plant growth harmones (6-. Benzylaminopurine, Indole-3-acetic acid). alone and in combination were used.Â In vitro propagation of rose has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. Micropropagation using shoot tip segments from 2 years old plants of cv. Heritage of rose using different combinations of TDZ, Kinetin and NAA and rooting using full, Â½ and Â¼ strength of MS macro, micro elements and vitamins was investigated.