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INFLUENCE OF PLANT GROWTH REGULATORS ON *IN VITRO* CLONAL PROPAGATION OF *DENDROBIUM* SONIA 'EARSAKUL'

Priya Kumari, I.* Sabina George, T. & Rajmohan, K[1]

Department of Pomology and Floriculture, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

[1] Department of Plant Biotechnology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Email: priyarenjith@gmail.com

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ABSTRACT

An *in vitro* propagation protocol was standardized for commercially important cutflower orchid *Dendrobium Sonia(*(Fa. Orchidaceae)*)* 'Earsakul.' Stem nodal explants were used to regenerate aseptic cultures in the culture establishment medium supplemented with auxins and cytokinins. The culture establishment medium of ½ MS supplemented with 4 mg L^{-1} BA was observed to give early bud break. In the shoot multiplication stage, treatment combinations of 2.0 mg L^{-1}kinetin and 0.1 mg L^{-1} NAA was found to give earliest (11.00) shoot multiplication and maximum numbers (4.66) of healthy shoots. The rooting media supplemented with 0.5 mg L^{-1} NAA gave earliest rooting (19.6). Charcoal and brick pieces in 1:1 proportion gave highest survival rate of 66.67 % at the planting out stage.

Keywords: *Dendrobium, BA, NAA, stem nodes*

Number of Tables : 4 **Number of References : 8**

INTRODUCTION

Orchids rank second among the top selling cut flowers in the world. Among the various orchid genera, *Dendrobium*is the most popular cut flower orchid in the international orchid trade.*Dendrobium* Sonia 'Earsakul is a highly demanding cut flower orchid noted for its long arching spike bearing dark purple color and fetching higher price than its ancestral clone *Dendrobium* Sonia 'BOM'. Though it is commercially propagated through micropropagation routed through protocorm-like bodies (PLBs), the plantlets often lack clonal fidelity, the presence of which is highly decisive in determining the quality of spikes and flowers, at international forefront. Also the propagation techniques routed through PLBs are often limited by low rate of PLB formation, low viability, longer duration for plantlet regeneration and different responses among PLB and hybrids (Tokuhara and Mii, 1993). Furthermore, the growth promoting effect of the growth regulators are species specific and hence they need to be empirically determined for each species and hybrids (Prakash*et al*., 1996). Hence the standardization of viable *in vitro* clonal propagation protocol specific for varieties in demand is highly indispensable for their large-scale multiplication and commercial cultivation.

Clonal propagation routed through enhanced release of axillary buds has been a reliable and efficient method for shoot multiplication since it does not destroy the shoot tip and in turn the entire costly mother plant. Also this method was proven to maintain the much needed clonal fidelity of mother plant thus preserving the economic floral traits.

Considering the above aspects the present study was undertaken to investigate the effects of different plant growth regulators on shoot regeneration through enhanced release of axillary buds from stem nodal explants from kiekies and its subsequent growth in *Dendrobium* Sonia 'Earsakul'.

MATERIALS AND METHODS

Shoots of 8-12 cm length having 3-5 nodes were harvested from 2 - 3 weeks old kiekies of green house grown mother plants and wiped with 100 per cent alcohol. The basal root portion and leaves were detached and shoot portion cut to 1.0 - 2.0 cm single stem nodal segments. The prepared stem nodal segments were immersed in 1000 times diluted 'Labolene' solution for 30 minutes, washed thoroughly in running tap water followed by glass distilled water. Surface sterilization was done inside laminar airflow chamber using 0.1 % mercuric chloride for 5 minutes, followed by 3-4 rinsing with sterile distilled water.

Half MS (Murashige and Skoog, 1962) fortified with 3% sucrose, 200.0 ml L^{-1} coconut water (CW), 0.5 g L^{-1} activated charcoal (AC) and 6.2 % agar was used as the basal media. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121 ^{0} C temperatures and 1.06 kg cm^{-2} pressure for 20 minutes. The cytokinins, BA and kinetin (1, 2 and 4 mg L^{-1}) alone and in combination with 0.1 or 0.5 mg L^{-1} NAA, 1 or 2 mg L^{-1} IAA were tried for culture establishment. The regenerated shoots from

culture establishment media were subjected to different treatments of BA and kinetin (0.5, 1 and 2 mg L^{-1}) alone and in combination with auxins (0.1 and 0.5 mg L^{-1} NAA, or 1 mg L^{-1} IAA for multiple shooting. Healthy single microshoots of 2.0 – 3.0 cm height were subcultured to rooting media supplemented with auxins (IAA, NAA and IBA at 0.5, 1 and 2 mg L^{-1}). The cultures were incubated at 26 +/. 2^{o} C and photoperiod of 15 hours at light intensity of 3000 lux provided by cool white fluorescent tubes. Before planting out, the culture vessels containing rooted plantlets were transferred to room temperature and kept near window to get diffused sunlight for two weeks. The cotton plugs were removed, sterile water added to the vessel and kept for 10 to 15 minutes. The plantlets were taken out carefully from culture vessels using forceps. The agar adhering to the roots were completely removed by thorough washing with running tap water. The plantlets were treated with Carbendazim (Bavistin 50 WP), 0.1 % solution for 30 minutes before planting. The different planting media tried were soilrite, charcoal pieces + brick pieces and charcoal pieces + brick pieces + soilrite which were autoclaved and transferred to perforated plastic containers. Polythene covers finely perforated were tied over each individual container to maintain humidity around the plants. Daily mist spraying with water was done. Weekly spraying of plants with ½ MS solution was also carried out. Completely Randomized Design was the experimental design employed. The data was analysed using ANOVA (Analysis of Variance). Each treatment for culture establishment and multiple shooting were replicated three times and for *in vitro* rooting were replicated six times.

RESULTS AND DISCUSSION

The treatments tried to assess the effect of plant growth regulators on shoot initiation showed significant difference with the mean number of days for bud initiation varying from 9.67 to 22.5 days. The earliest bud initiation was observed in 4.0 mg L^{-1}BA (9.67). Similar early response was obtained in treatments with 2.0 or 4.0 mg L^{-1} kinetin alone. The combinations of 1.0, 2.0 or 4.0 mg L^{-1} kinetin with 0.1 mg L^{-1} NAA also induced early bud break of less than 13 days. The combinations of 1.0 and 2.0 mg L^{-1} BA with NAA at 0.1 and 0.5 mg L^{-1} also induced early bud initiation. Kinetin and BA at higher levels of 4.0 mg L^{-1} along with 1.0 mg L^{-1} IAA too produced early bud break. On the other hand, presence of IAA at higher levels of 1.0 or 2.0 mg L^{-1} alone and in combination with 1.0 mg l^{-1} kinetin delayed bud break. The highest shoot number (4.33 shoots) however, was obtained in the medium with 2.0 mg L^{-1}BA and 0.1 mg L^{-1}NAA. Comparatively early shoot multiplication (11.00 days) was initiated in treatment combination of kinetin 2.0 mg L^{-1} and NAA 0.1 mg L^{-1}. Among the other treatments tried, the early shoot

Table1: *Effect of plant growth regulators on culture establishment of Dendrobium Sonia 'Earsakul'.*

Culture period: 4 weeks

Treatments (mg L^{-1})	Survival %	Days for bud initiation	No: of shoots
CONTROL	100.00	22.33	1.33
BA 1.0	100.00	17.33	1.33
BA 2.0	100.00	13.67	1.67
BA 4.0	100.00	9.67	2.33
kinetin 1.0	100.00	14.67	1.00
kinetin 2.0	100.00	12.33	1.33
kinetin 4.0	66.67	11.33	1.67
NAA 0.1	66.67	16.00	1.00
NAA 0.5	66.67	17.50	1.00
IAA 1.0	66.67	21.00	1.00
IAA 2.0	66.67	22.50	1.00
BA 1.0 + NAA 0.1	100.00	12.00	1.50
BA 2.0 + NAA 0.1	100.00	12.67	4.33
BA 4.0 + NAA 0.1	100.00	15.33	1.33
BA 1.0 + NAA 0.5	100.00	14.33	1.67
BA 2.0 + NAA 0.5	66.67	11.67	1.50
BA 4.0 + NAA 0.5	100.00	19.50	1.00
kinetin 1.0 + NAA 0.1	100.00	13.00	2.67
kinetin 2.0 + NAA 0.1	100.00	10.00	3.00
kinetin 4.0 + NAA 0.1	66.67	11.33	2.67
kinetin 1.0 + NAA 0.5	66.67	16.50	1.50
kinetin 2.0 + NAA 0.5	100.00	15.00	2.00
kinetin 4.0 + NAA 0.5	100.00	13.33	2.00
BA 1.0 + IAA 1.0	100.00	17.33	1.00
BA 2.0 + IAA 1.0	100.00	14.00	1.33
BA 4.0 + IAA 1.0	66.67	12.33	2.33
BA 1.0 + IAA 2.0	100.00	13.50	1.00
BA 2.0 + IAA 2.0	100.00	13.33	1.00
BA 4.0 + IAA 2.0	66.67	10.67	2.33
kinetin 1.0 + IAA 1.0	100.00	22.00	1.50
kinetin 2.0 + IAA 1.0	100.00	16.00	1.33
kinetin 4.0 + IAA 1.0	66.67	12.67	1.67
kinetin 1.0 + IAA 2.0	66.67	20.00	1.00
kinetin 2.0 + IAA 2.0	100.00	17.50	1.50
kinetin 4.0 + IAA 2.0	100.00	14.00	2.00
F		6.49 * *	2.61 * *
£ CD (2,2)		4.39	1.49
(2,3)		4.00	1.37
(3,3)		3.58	1.22

£ Since the effective number of replications varies, separate CD values were computed

* * Significant at 1 % level

Table2: *Effect of plant growth regulators on multiple shooting of Dendrobium Sonia 'Earsakul'.*

Culture period: 4 weeks

Treatments (mg L^{-1})	Survival %	Days for shoot initiation	No: of shoots
CONTROL	100.00	20.67	1.33
BA 0.5	100.00	16.33	1.00
BA 1.0	100.00	15.67	1.33
BA 2.0	100.00	12.33	1.67
kinetin 0.5	100.00	14.00	1.33
kinetin 1.0	100.00	13.67	1.33
kinetin 2.0	100.00	11.33	2.00
NAA 0.1	66.67	17.00	1.00
NAA 0.5	66.67	21.00	1.50
IAA 1.0	66.67	22.50	1.00
BA 0.5 + NAA 0.1	100.00	16.67	1.33
BA 1.0 + NAA 0.1	100.00	15.67	1.67
BA 2.0 + NAA 0.1	100.00	13.67	1.67
BA 0.5 + NAA 0.5	66.67	16.00	1.50
BA 1.0 + NAA 0.5	100.00	13.00	1.00
BA 2.0 + NAA 0.5	100.00	11.67	2.66
BA 0.5 + NAA 1.0	66.67	17.00	2.00
BA 1.0 + NAA 1.0	100.00	14.33	1.67
BA 2.0 + NAA 1.0	66.67	12.00	2.00
kinetin 0.5 + NAA 0.1	66.67	14.00	1.50
kinetin 1.0 + NAA 0.1	100.00	11.67	2.66
kinetin 2.0 + NAA 0.1	100.00	11.00	4.66
kinetin 0.5 + NAA 0.5	100.00	11.33	3.33
kinetin 1.0 + NAA 0.5	100.00	14.33	2.66
kinetin 2.0 + NAA 0.5	100.00	11.33	2.33
BA 0.5 + IAA 1.0	66.67	19.00	1.50
BA 1.0 + IAA 1.0	66.67	17.50	1.00
BA 2.0 + IAA 1.0	66.67	17.00	1.50
BA 0.5 + IAA 2.0	66.67	19.50	1.00
BA 1.0 + IAA 2.0	66.67	18.50	1.00
BA 2.0 + IAA 2.0	66.67	22.50	2.00
kinetin 0.5 + IAA 1.0	66.67	18.00	1.00
kinetin 1.0 + IAA 1.0	66.67	14.50	1.00
kinetin 2.0 + IAA 1.0	66.67	16.00	1.50
kinetin 0.5 + IAA 2.0	66.67	22.00	1.00
kinetin 1.0 + IAA 2.0	66.67	21.00	1.00
kinetin 2.0 + IAA 2.0	66.67	19.50	1.00
F		5.90* *	2.04 *
£ CD (2,2)		4.46	1.81
(2,3)		4.06	1.66
(3,3)		3.64	1.48

£Since the effective number of replications varies, separate CD values were computed

* * Significant at 1 % level

* Significant at 5 % level

Table 3: *Effect of plant growth regulators on in vitro rooting of Dendrobium Sonia cv. Earsakul*

Culture period: 4 weeks

Treatments (mg L^{-1})	Rooting %	Days for Roots Initiation	No of roots
CONTROL	83.33	26.40	2.00
IBA 0.5	66.67	20.75[1]	2.25
IBA 1.0	100	22.00[1]	3.00
IBA 2.0	83.33	23.60	3.20
NAA 0.5	83.33	19.60[1]	2.60
NAA 1.0	100	21.33[1]	3.00
NAA 2.0	100	23.17	2.50
IAA 0.5	66.67	21.75[1]	1.75
IAA 1.0	66.67	20.75[1]	2.50
IAA 2.0	100	23.17	1.67
F		3.97 * *	NS
[£]CD		(6,6) - 2.50	
		(6,5) - 2.63	
		(6,4) - 2.79	
		(5,5) - 2.74	
		(5,4) - 2.91	
		(4,4) - 3.07	

[£] Since the effective number of replications varies, separate CD values were computed

Table 4: *Survival rate of plantlets after 1 month*

Plant out media	Survival rate (%)
Soilrite	-
Charcoal + Brick pieces + soilrite	-
Charcoal + Brick pieces	66.67

multiplication was noticed in the media with 2.0 mg L^{-1}BAor 0.5, 1.0 or 2.0 mg L^{-1} kinetin and combination of 1.0 and 2.0 mg L^{-1} BA with 0.1 and 0.5 mg L^{-1} NAA. The combination of 0.5, 1.0 and 2.0 mg L^{-1} kinetin along with 0.1 and 0.5 mg L^{-1} NAA also induced earliness in shoot multiplication. (Table 1)

The optimum shoot multiplication and regeneration of healthy, vigorous shoots is dependent on plant growth regulators and media supplements used. In addition, the interaction and balance between the growth regulators in the medium and the growth substances produced endogenously by cultured cells influence growth and morphogenesis *in vitro* (Krikorian, 1982). In general, the balance between auxins and cytokinins is critical for shoot proliferation. Cytokinins at higher concentration have deleterious effect on shoot growth, while the presence of auxins can nullify the suppressive effect of cytokinins on shoot growth (Lundergan and Janick, 1980).

In the present study, significant difference was obtained for the number of shoots produced at fourth week. (Table 2). The mean shoot number varied from 1.00 to 4.66. The maximum shoot number (4.66) was obtained for the treatment with 2.0 mg L^{-1}kinetin + 0.1 mg L^{-1} NAA. Such optimum combination of kinetin and NAA producing highest number of multiple shoots of *Dendrobium* var. Betty Ho was reported by Kurup*et al*. (2005), supporting the present study.

It was noticed that microshoots produced in treatments involving both cytokinins and auxins were healthier than in treatments with either auxin or cytokininalone. In treatments involving combinations of 0.5 - 2.0 mg L^{-1} BA and 0.5 -1.0 mg L^{-1} NAA , the shoot buds were proliferating, short, non-distinct and pale green with delayed leaf emergence. On the other hand, in treatments involving combinations of 0.5 - 4.0 mg L^{-1} kinetin and 0.5 -1.0 mg L^{-1} NAA, the shoot buds were non- proliferating, few in number, distinct, dark green, vigorous, healthy with fully emerged leaves. Similar effect of kinetin added media producing better shoot growth (>2.0 cm) and BA added media producing short (<1.5 cm) shoots was reported during the *in vitro* propagation of *Dendrobium Sonia* 'BOM17' and 'BOM28' (Martin *et al*., 2005).

The mean number of days for root initiation from microshoots exhibited significant variation. The minimum days (19.60) for root initiation were noticed in rooting media supplemented with 0.5 mg L^{-1} NAA. It was observed that the root initiation was delayed when the concentration of auxins was increased from 0.5 to 2.0 mg L^{-1}. (Table 3)

The composition of the media into which *in vitro* rooted plantlets are transplanted is important for their survival (Jones, 1982). In the present study the maximum survival rate (66.67 %) of rooted plantlets was obtained in media with charcoal and brick pieces in equal proportion. (Table 4). This combination might have provided the ambient condition of good drainage, sufficient moisture retention and also good aeration, which are very much essential for orchid growth.

In conclusion the present investigation analyzed the effect of various plant growth regulators on *in vitro* clonal shoot and root morphogenesis and subsequent plantlet

development of commercially important *Dendrobium* Sonia 'Earsakul'. Using this protocol it is possible to clonally produce viable, healthy, uniform planting materials that can be used for commercial cultivation of *Dendrobium* Sonia 'Earsakul'.

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