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Micropropagation of *dendrobium barbatulum lindl.* From axillary buds

Sr. Sagaya Mary .B and Dr. Divakar K.M.

Plant Tissue Culture Division, Department of Botany, St. Joseph's Post-Graduate Studies and Research Centre, Langford Road, Bangalore-560 027

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Abstract

One of the most exciting and important aspects of In vitro cell and tissue culture is the capability to regenerate and propagate plants from cultured cells and tissues. The simplest type of In vitro plant propagation is the stimulation of axillary bud development, illustrated in the present chapter. This technique exploits the normal ontogenetic route for branch development by axillary buds. The axillary buds are treated with hormones to break dormancy and produce shoot branches. The shoots are then separated and rooted to produce plants. Alternatively, the shoots are used as propagules for further propagation.

Abbreviations: *VW - Vacin and Went medium, B5 - Gamborg B5 medium, MS- Murashige and Skoog medium, KC – Knudson C, NAA – Naphthalene Acetic Acid, IAA – Indole Acetic Acid, BAP – Benzyl Amino Purine, AC – Activated Charcoal & CM–Coconut Milk*

Introduction

Dendrobium barbatulum Lindl.

Dendrobium barbatulum Lindl. is an epiphytic herb commonly seen in many parts of the Southern Western Ghats in India. This species usually flowers in the winter months of January-February. All the axillary buds would have dried by the time it starts giving out new inflorescence and when it flowers it flowers profusely. The flowers are white and once open flowers last for around two weeks. Though the plant itself is not very big and has shorter canes the flower are quite big, sometime reaching a width of 4+cms. The racemes are lateral.

This species loves bright sunlight. One can always find the plant on branches open to wind and bright sunlight. With dried sheath covering the cane plant does not appear active until a new shoot starts.

Plants are perennial, and the new growth is very susceptible to water. It takes only a little amount of watering during a "wrong" time is enough to kill the new growth. Like all *Dendrobiums*, one thing to keep in mind is that never to over water and never water when its giving out new shoots (wrong time) also good amount of sunlight must be provided.

Though the flowers are attractive and many it is not found much in cultivation. Habitat destruction due to various reasons is one of the main reasons for decline of the orchid species. This is true for not just this particular species, but it is true for all other species both orchids and non-orchids alike.

Materials and Methods

The healthy plants of *Dendrobium barbatulum Lindl.* were collected from somavarpet / coorg and raised in pots containing soil and farm yard manure (1:1) in green house in the department of botany, . Axillary Bud explants were surface sterilized by cleaning thoroughly under running tap water and washed with a solution of Tween 20(Two drops in 100 ml water) for 5 min, and again washed with sterile distilled water. The cleaned explants were finally treated with Hg cl**2** (0.1%) for 3 min under aseptic conditions and washed six times with sterile distilled water to remove traces of Hg cl**2**.

Surface sterilization using Bud culture

A Protocol for standardization of surface sterilization of explants and nutrient media

1. Protocol for standardization of surface sterilization of explants: Axillary buds were sterilized using several sterilants like Hydrogen peroxide and Mercuric chloride and bleach. Treatment with $HgCl_2$ concentration of 0.1% has produced the neat cultures from the inoculation.
2. Various nutrient media – Vasin and vent medium, Knudsson C medium and MS medium were used for standardization purpose, along with various concentrations of phytohormones.

After surface sterilization, axillary bud explants were trimmed and inoculated on B5 basal medium supplemented with different concentrations and combinations of 0.5mg BAP+3 mg NAA +50ml CM shoot

induction after 30 days small Plb were developed and give rise to plantlet. Fully developed plantlets transferred to B5 basal medium with plant growth regulators for root induction. For shoot elongation, small shoots were transferred to B5 basal medium supplemented with 1 mg BAP, 3mg IAA after 40days; the cultures containing the rooted plantlets were transferred to the greenhouse at 60-70 per cent humidity.

***In vitro* rooting**

In vitro rooting was successful with B5 medium supplemented with 1 mg BAP, 3mg IAA, 50 ml CM and 500 mg AC.

***Ex vitro* rooting**

The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of NAA (made in distilled water) then planted in small pots containing solrite (potting mix) sprayed with bavistin to avoid fungal infection. *In vitro* rooted plants in the pot trays containing potting mixture maintained under mist chamber and covered with perforated plastic cups.

Hardening

Well grown shoots were directly transferred to small pots containing soil, sand and solrite (mixture of pearlite and peat moss) and were kept in the green house. Successfully established plantlets were subsequently transferred to field condition.

Observations

1. *Dendrobium barbatulum Lindl.blooming in green house*

2. *Dendrobium barbatulum Lindl.from Sagar*

3.Dendrobium barbatulum Lindl. flowers

4. *Dendrobium barbatulum Lindl.*with buds

5. Bud culture

6. Plantlet formation

7. Bud Sub culturing

8. *In- vitro* Rooting

9. *Ex-vitro* Rooting

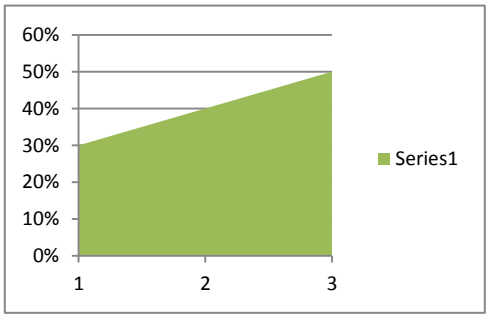
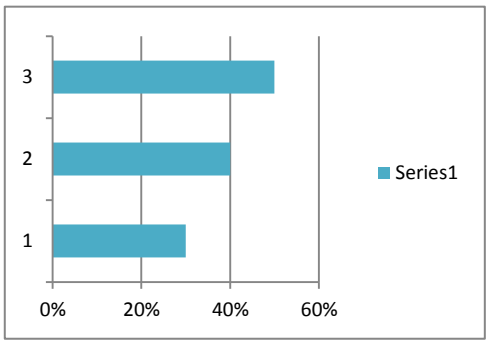
10.Hardening

11. Hardening in Green House

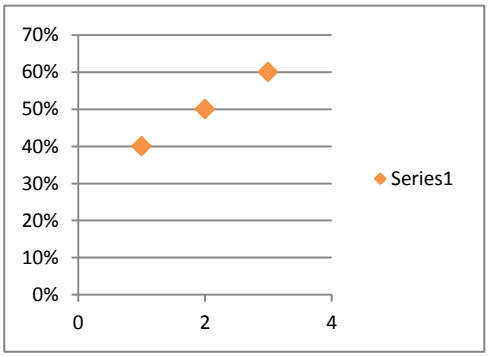
12 .Transfer to Green House

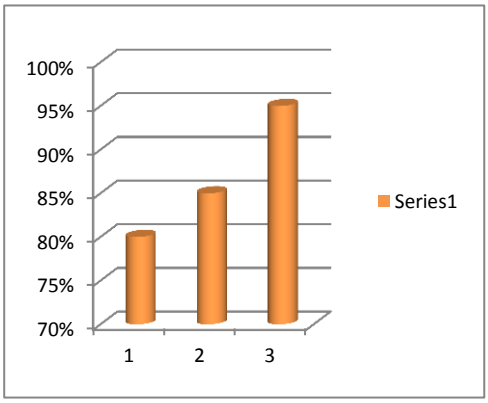
Results and Discussion

MS, B5, VW and KC media was used (Table 1)

Media used	Media composition	Average plantlet formation (percentage)	
VW	2 mg BAP +1.5 mg NAA + 50 ml CM 1 mg BAP + 0.5 mg NAA + 50 ml CM 2.5mg BAP + 2 mg NAA + 50 ml CM	40% 30% 50%	
KC	1mg BAP + 1.5 mg NAA + 50ml CM 1.5mg BAP + 0.5mg NAA+50ml CM 2mg BAP+ 1mg NAA + 50 ml CM	30% 40% 50%	
MS	2 mg BAP + 1 mg NAA + 50 ml CM 1.5 mg BAP + 0.5 mg NAA + 50 ml CM 1 mg BAP +1.5 mg NAA + 50 ml CM	50% 30% 40%	

B5 - For plantlet formation (Table 2)

Media used	Media composition	Average plantlet formation (percentage)	
B5	1mg BAP + 1.5 mg NAA + 50ml CM+500 mg AC 1.5mg BAP + 0.5 mg NAA+50ml CM +500 MG AC 0.5 mg BAP + 3mg NAA + 50 ml CM+ 500 mg AC	40% 50% 60%	

Media Used	Media Composition	Results The average rooting (percentage)	
B5	0.5mg BAP + 1.5 mg IAA + 50ml CM+500 mg AC 1.5mg BAP + 0.5mg IAA+50ml CM +500 MG AC 1 mg BAP + 3mg IAA + 50 ml CM+ 500 mg AC	80% 85% 95%	

Conclusion

The results showed the ability of the Axillary Bud explants to produce higher number of shoot lets without intervening callus phase, where all the plantlets were uniform in height and growth. This study establishes a simple, rapid, high frequency micropropagation method for *Dendrobium barbatulum Lindl*. from axillary bud explants and also this present system of normal root culture would be beneficial for the sustainable utilization of this rare endemic medicinal plant for its bioactive ingredients, thereby providing an alternative method rather than destroying whole plants that are not under cultivation. From these studies it can be concluded that B5 medium suitable for *Dendrobium barbatulum Lindl.. This* study also revealed that a low concentration of 0.5 mg BAP + 3 mg NAA + 50 ml CM was found to be more suitable for plantlets and multiple plantlets. B5 medium supplemented with Basal B5 Medium + 1 mg BAP+ 3mg IAA+ 50 ml CM +500 AC giving highest percentage was found to be suitable for *In vitro* Rooting.

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