

IN VITRO SEED GERMINATION OF *DENDROBIUM AQUEUM* : A RARE ORCHID OF WESTERN GHATS, KARNATAKA

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Abstract— The genus *Dendrobium* is one of the most important genera with distribution of eleven species in the Western Ghats, belong to the family Orchidaceae. It is known for its long lasting, showy flowers which has its significance in floriculture industry. *Dendrobium aqueum* species is being reported under Near Threatened category by the conservation assessment and management plan workshop (CAMP, 2001). *In vitro* studies will immensely aid conservation measures of this orchid species. MS, VW, B5 and KC media supplemented with various concentrations of auxins and cytokinins were used in combination for asymbiotic seed germination and plantlet formation. In the evaluation of the media VW medium supplemented with 0.5 mg BAP + 5 mg NAA was found to be most suitable for plantlet formation. VW basal medium fortified with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC found to be most suitable for *in vitro* rooting. Hardened plants were transferred to green house after *ex vitro* rooting technique. Significance of the present work is discussed here.

Keywords— *Dendrobium aqueum*, VW, B5, KC, MS, NAA, IAA, BAP, AC, CM.

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

INTRODUCTION

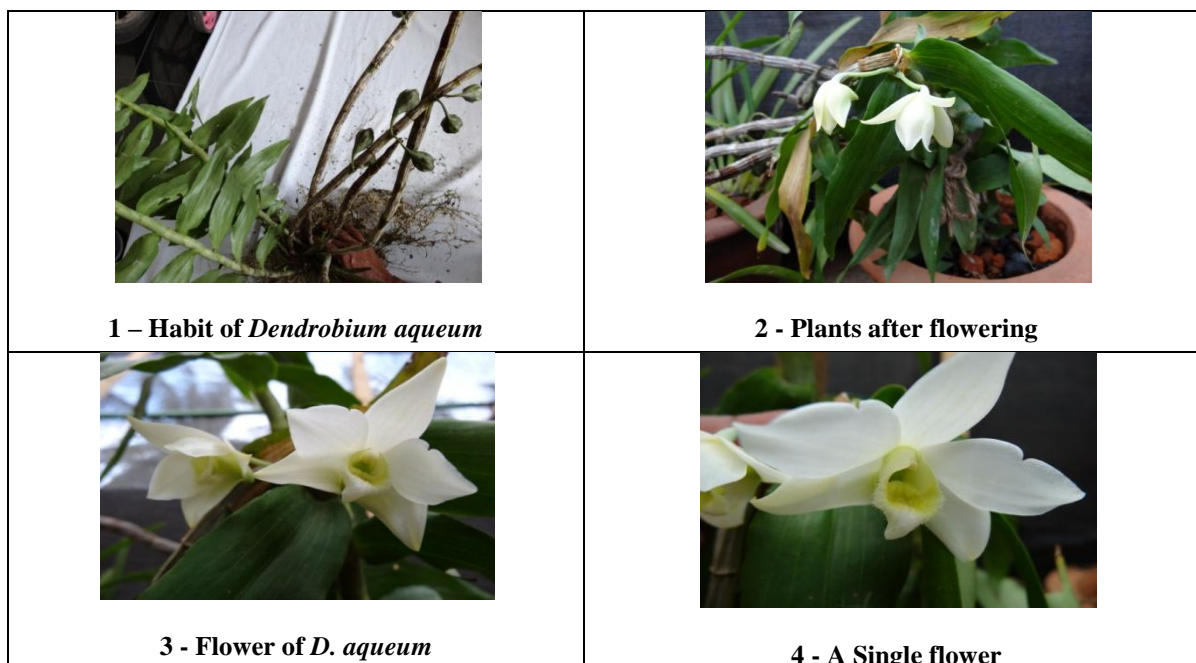
Orchidaceae is one of the largest families of flowering plants. The genus *Dendrobium* is the largest genus of Orchidaceae having mostly epiphytic or occasionally lithophytic habits and comprising of about 1500 species worldwide ⁽⁵⁾ with 116 species in India ^(12,13).

Dendrobium aqueum Lindl ^(1,3,4) is an epiphytic, sympodial orchid found at an altitude of 1300 to 1600m on Shola trees in deep shade ^(11,13). Also Common along Western Ghats in wet deciduous forests of Karnataka, Kodagu, Kerala, Tamil Nadu and Maharashtra. It is a robust pendulous leafy epiphyte, flowers leaf opposed 2 or 3 together, whitish green or pale yellow with faint fragrance. Lip three lobed, midlobe triangular. Flowering and fruiting takes place between May to June or July to December. Its spot characters are Leaf when flowering. Lip white with yellow colour in the throat. Stem a brown, covered with white scales, clavate ^(1,7).

Several species of *Dendrobium* have also been used in Chinese medicine ⁽⁶⁾ and in Indian system of medicine ⁽¹²⁾ to cure cough, cold etc. It has antiglycation and antioxidant properties which could serve as a traditional medicine in treatment of Diabetes ⁽¹⁴⁾

This species is being reported under Near Threatened category ⁽¹³⁾ by the conservation assessment and management plan workshop. *D. aqueum* population has been declining due to loss of habitat, indiscriminate collections by the orchid enthusiasts and due to minute, seeds which lack endosperm which shows inherent lower germination rate in nature ⁽¹⁰⁾. Hence, a fast method of growing combinations and conserving them in the green houses is an urgent need. *In vitro* studies will immensely aid conservation measures of this orchid species ⁽⁷⁾.

Asymbiotic germination on basal nutrient medium ⁽⁹⁾ and a combination of various growth regulators ⁽²⁾ are a gift to the Orchid industry. *In vitro* asymbiotic seed germination is thus considered much faster and effective method for conservation and mass multiplication of threatened and endangered orchids. Hence this investigation was undertaken for judicious use of growth regulators during *in vitro* seed germination ⁽⁸⁾ of *Dendrobium aqueum* Lindl.



MATERIALS AND METHODS

Plant specimen collection was made from dry deciduous forest around Somwarpet taluk in the north-east of the Coorg district between 1st July 2012 and 6th July 2012. Somwarpet is located at 10.42°N 74.73°E latitude. It has an elevation of 1525 metres (5003 feet). During the visit, the average temperature was about 16°C, Wind NW at 3 km/h and 93% humidity.

Plant specimens were collected from the natural environment in perforated, clean, polythene bags. Care was taken to ensure to retain the mother plant intact in its natural epiphytic habitat. The plants were taken to the department of Botany, St. Joseph's Post-Graduate and Research centre for planting in the green house. Standard floras were referred for authentication of the genus and species. Herbaria were prepared using standard protocol and voucher specimens were deposited in the herbaria of St. Joseph's college Post-Graduate and Research Centre, Bangalore.

Green capsules of wild were collected and then rinsed thoroughly three times with sterile distilled water, followed by dipping them in 70% ethanol for 30s. Sterilized capsules were dried and then split longitudinally with sterile surgical blade. Seeds were inoculated on different nutrient media like MS medium, B₅ medium, KC medium, VW medium which were prepared with various concentrations and combinations of phytohormones and other additives. VW medium gave the best results in comparison to all other media. So VW was standardized for *Dendrobium aqueum*.

Seed cultures were placed in growth chamber at 25 ± 20 C and 70 –80% relative humidity under 24h-light and under 16h-light/8h-dark with light provided by cool white fluorescent lamps for 70 days.

Sub-culturing was regularly done every 15 days and observations were made. Each experiment was repeated twice and consisted of 3 replicates of 10 explants for each treatment

In vitro rooting

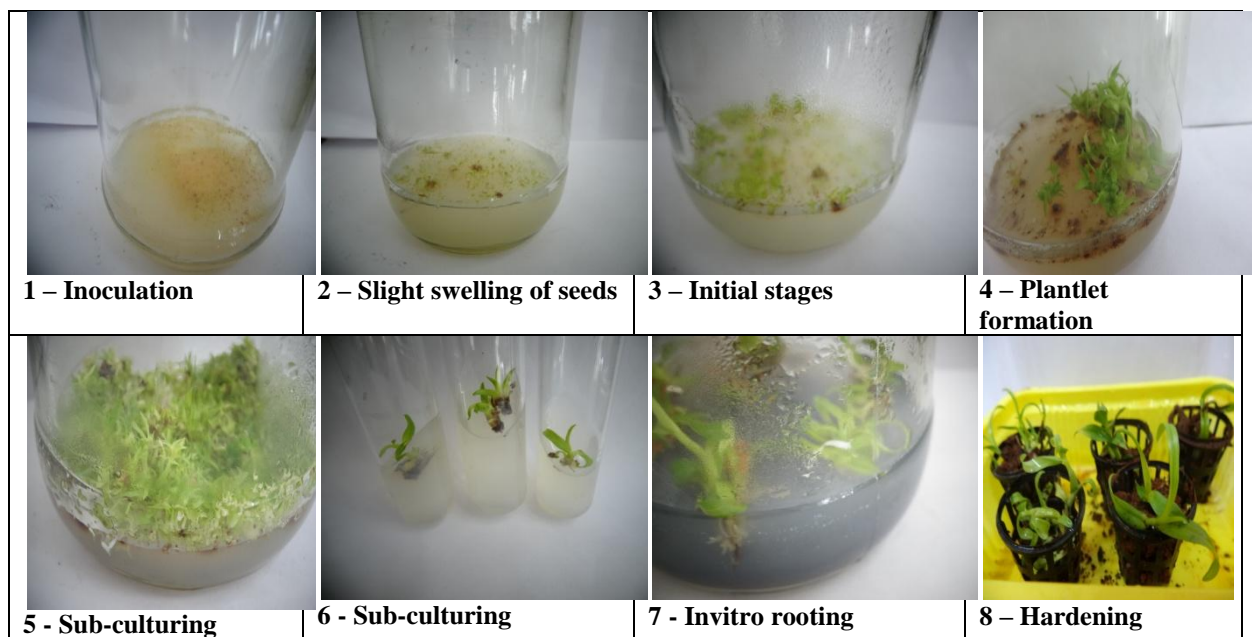
In vitro rooting was most successful with VW media supplemented with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC.

Ex vitro rooting

The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of IAA (distilled made in water) then planted in small pots containing solrite (potting mix) sprayed with bavistin to avoid fungal infection. *In vitro* rooted plants in the thumbpots containing potting mixture maintained under mist chamber and covered with perforated plastic cups. All these above protocols were followed inside greenhouse conditions and further allowed to acclimatize.

Hardening

Well grown shoots were directly transferred to small pots containing perlite, peat moss, soil and sand. They were kept in the green house. Successfully established plantlets were subsequently transferred to field condition.



RESULTS AND DISCUSSION

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

Media used	Media composition	Average plantlet formation (percentage)	
MS	Basal MS media + 1mg BAP + 1mg IAA	20%	
	Basal MS media + 2mg BAP + 1mg IAA	30%	
	Basal MS media + 3mg BAP + 1mg IAA	35%	

B₅	Basal B ₅ media + 1mg BAP + 1mg IAA Basal B ₅ media + 2mg BAP + 1mg IAA Basal B ₅ media + 3mg BAP + 1mg IAA	25% 30% 40%	<p style="text-align: center;">B₅</p> <p>Axis Title: 50, 0 Legend: B5 Data: 1 (25), 2 (30), 3 (40)</p>
KC	Basal KC media + 1mg BAP + 3mg IAA Basal KC media + 2mg BAP + 5 mg IAA Basal KC media + 3mg BAP + 10 mg IAA	25% 40% 35%	<p style="text-align: center;">KC</p> <p>Axis Title: 50 Legend: KC Data: 1 (25), 2 (40), 3 (35)</p>

Media Composition For Plantlet Formation (Table 2)

Media used	Media composition	Average plantlet formation (percentage)	
VW	Basal VW Media + 0.5 mg BAP + 0.5 mg NAA Basal VW Media + 1.0 mg BAP + 1 mg NAA Basal VW Media + 2.0 mg BAP + 2 mg NAA	70% 60% 50%	<p style="text-align: center;">VW (1)</p> <p>Legend: VW (1) Data: 1 (70), 2 (60), 3 (50)</p>
VW	Basal VW Media + 0.5 mg BAP + 5 mg NAA Basal VW Media + 1 mg BAP + 1 mg NAA Basal VW Media + 2 mg BAP + 2 mg NAA	95% 70% 80%	<p style="text-align: center;">VW (2)</p> <p>Legend: VW (2) Data: 1 (95), 2 (70), 3 (80)</p>

In comparison to MS medium, B₅ medium and KC medium, VW medium gave best results and was standardized. Basal VW medium supplemented with 0.5 mg BAP and 5 mg NAA was found to be most suitable for plantlet formation.

Media Composition For *In vitro* Rooting (Table 3)

Media used	Media composition	Average plantlets showing rooting
VW	Basal VW Media + 1.5 mg BAP + 3 mg NAA + 500 ml CM + 200 AC	80%
	Basal VW Media + 1 mg BAP + 4 mg NAA + 50 ml CM + 250 AC	85%
	Basal VW Media + 0.5 mg BAP + 5 mg NAA + 50 ml CM + 500 mg AC	90%
VW	Basal VW Media + 2.5 mg BAP + 2.5 mg NAA + 50 ml CM + 150 mg AC	80%
	Basal VW Media + 2 mg BAP + 2 mg NAA + 50 ml CM + 250 mg AC	85%
	Basal VW Media + 1.5 mg BAP + 1.5 mg NAA + 50 ml CM + 500 mg AC	95%

	1	2	3
■ VW (1)	80	85	90
■ VW (2)	80	85	95

Germination was faster on the VW medium when compared to MS medium, KC and B5 media (Ref table 1). Concentration of VW medium supplemented supplemented with 0.5 mg BAP and 5 mg NAA was found to be most suitable for plantlet formation (Ref table 2). VW medium supplemented with *in vitro* rooting was most successful with VW media supplemented with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC (Ref table 3). Ex-vitro rooting was done by spraying with IAA and potting in solorite. The plants with good rooting were transferred to community pots and then to greenhouse conditions.

CONCLUSION

From these studies it can be concluded that the VW medium is most suitable for *Dendrobium aqueum Lindl* seed germination. This study also revealed that a low concentration of 0.5 mg BAP and 5 mg NAA was found to be more suitable for plantlets and multiple plantlets. The plant was found to have phytochemical significance. Experimental work is under progress.

SCOPE

- [1]. Use of nano biotechnology in control of bacterial and fungal contaminations.
- [2]. *In vitro* micropropagated plants can be shifted to natural habitats of Western Ghats to facilitate *In situ* conservation of *Dendrobium ovatum*.
- [3]. Using elicitors (From biological origin) for enhanced plantlet formations.
- [4]. Several species of *Dendrobium* have also been used in Chinese medicine (Hu, 1970) and in Indian system of medicine (Misra, 2007) to cure cough, cold etc. "... Our finding might open a new arena of research in traditional herbal medicine with *D. aqueum* orchid which could be utilized in diabetes due to its antiglycation and antioxidant properties" - (Sourav Mukherjee, Devyani Phatak, Juhi Parikh, Suresh Jagtap, Shamim Shaikh, Rashmi Tupe. 2012. Antiglycation and antioxidant activity of a rare medicinal orchid *Dendrobium aqueum* Lindl. *Medicinal Chemistry & Drug Discovery* 2012, 2(2) 17-29.)
- [5]. Biochemical characterization of the plants is under progress.

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