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# *In Vitro* Seed Germination and Protocorm Development of *Dendrobium aqueum* Lindl. A Rare Orchid Species from Eastern Ghats of Tamil Nadu

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**Abstract:** *Dendrobium aqueum* is an exquisite epiphytic orchid of the Kolli Hills (Eastern ghats) of Tamil Nadu. It is fast disappearing from its natural habitats. In the present investigation the undehisced capsule were used opened *in vitro* condition and the seeds were implanted. The seed development and protocorm development were recorded. The highest seed germination of 90% was observed by the 30th day on KnC basal VW media, whereas on HMS and VW media the germination percentages were 40% in HMS and 30% in VW respectively. The well-developed shoots were removed from the culture tubes and transferred to the rooting media. About 90% of the shoots rooted well after transfer to the HMS and KnC medium supplemented with 0.2 mg/l IBA. Rooting also took place in the two basic media, but duration was longer when compared with the hormone-supplemented media. The rooted plantlets were hardened. Then the hardened plantlets were relocated in natural habitats.

**Key words:** Protocorm · Orchids · MS medium · BAP and Acclimatization

## INTRODUCTION

Although population growth is not the only cause of environmental degradation it is an important exacerbating factor. It can undermine the capacity of many developing countries to conserve resources and meet basic human needs. Increasing population pressures on land and other natural resources can aggravate the intensity of natural disasters such as flood and drought. Depletion of the Earth's biological diversity has much more profound consequences than some other environmental threats. As the loss of biodiversity is irreversible the potential impact on the earth's living systems is enormous. The human species has evolved biologically and culturally in a highly diverse world [1].

Hard and intelligent decisions are needed to save the valuable taxa from the vastly changing landscape. As part of an *ex situ* conservation strategy, artificial propagation including micropropagation is successfully employed for recovering certain other species having specific problems in conventional horticulture [2,3]. Micropropagation through embryo and tissue culture has much advantage over conventional vegetative propagation and could be fielded into service to complement efforts to conserve and utilize local biodiversity on a sustainable basis. The advantage of plant tissue culture for the conservation of endemic and endangered species lies in the fact that it makes use of small units without sacrificing the mother plant, takes pressure off the wild populations and makes available large number of plants for restoration as well as for horticultural applications. Propagation in vitro has been successfully employed for the conservation of crop genetic resources, particularly with those crops which are vegetative propagated or which have recalcitrant seeds, which cannot be stored under conventional seed bank conditions [4-6] while tissue culture is essentially used as a crisis management tool for the multiplication of the existing genotypes.

*Dendrobium aqueum* is an exquisite epiphytic orchid of the Kolli Hills ( Eastern ghats ) of Tamil Nadu. It is fast disappearing from its natural habitats due to extensive collections by the orchid enthusiast. The plants produce very minute seeds, which lack endosperm. Seeds are wind dispersed. Naturally they require mycorrhizal association

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for their germination. Hence, a fast method of growing and conserving them in the green houses is an urgent need.

*In vitro* cultures of orchid seeds have shown that different species require different and often specific medium composition for optimum germination and growth. However, the sequential steps of development and histomorphological changes from embryo to seedling have been traced only in a few species, including those of Vanda [7], Cattleya [8], Cymbidium [9] and Geodorum [10].

Purpose of this article is to report our success in aseptic germination of seeds of Dendrobium aquem and to trace the sequence of developmental changes that take place from the onset of seed germination and development of protocorm- like bodies(plbs).

## MATERIALS AND METHODS

The mature undehised capsules were collected from Kolli Hills (Eastern ghats) of Tamil Nadu. For surface sterilization the freshly collected undehised capsules were first rinsed in 90%( v/v) ethanol for 90 sec, followed by soaking them in 1.0% (w/v) mercuric chloride solution for 20 min. and finally washed thrice with sterile distilled water. After opening the capsules with a scalpel, about 150 seeds inoculated in each of the 150 ml conical flask containing 50 ml culture medium. There were six replicate flasks per treatment, three for each capsule. The cultures were maintained at 25°C±2°C under a 12- hr photoperiod provided by Philips white fluorescent lights of 3000 lux intensity.

The germination media used are Knudson C and half MS medium. In KC media 2% sucrose and half MS medium 3% sucrose served as carbon source. In addition, two different plant growth regulators (PGRs) were supplemented to each type of medium in different combinations (Table 1) the pH adjusted to 5.3 in Kn C medium and 5.7 in half MS medium and the media were solidified with 0.9% agar to Knudson C medium and 0.8% agar to half MS medium and autoclaved at 121°C for 15 min. duration.

The onset of germination and the percentage of seed germination on two basal media were recorded periodically at every 10-day interval after the day of initial inoculation. Seed germination was first observed after 40 days, 50 days and 60 days of inoculation on KnC, VW and HMS media respectively. The highest seed germination of 90% was observed by the 30$^{th}$ day on KnC basal VW media, whereas on HMS and VW media the germination percentages were 40% in HMS and 30% in VW respectively. In KnC basal medium complete seed germination was achieved by the 60$^{th}$ day in both the cases.

## RESULTS AND DISCUSSION

The first sign of germination on the 30$^{th}$ day on KnC basal medium was that the embryo turned into spherical form and was enclosed in dark yellow color. After a few days some adventive tissues appeared on their tips and the embryo ruptured at one of the poles and after a few days it showed 4-5 fold increase in size with abundant chloroplasts and starch cells. The embryos by the 50$^{th}$ day exhibited a prominent zone of promeristematic cells and developed a pair of leaf primordials. One of the primordials developed more rapidly than the other to produce an unequal pair of first embryogenic photosynthetic leaves. Simultaneously with the development of embryonic leaves at the proximal end of the embryo the marginal cells at the distal end of the embryo started giving rise to tubular and unicellular rhizoids.

Table 1: Media employed for seed germination of *Dendrobium aqueum*

| Media employed for seed germination | % of germination | Time taken for initiation of germination (days) | Shoot growth (%) |
|---|---|---|---|
| MS - B | - | - | - |
| MS - CW | - | - | - |
| MS - P | - | - | - |
| HMS - B | 50 | 85 | 10 |
| HMS - CW | 30 | 80 | - |
| HMS - P | 20 | 90 | - |
| KnC - B | 90 | 65 | 50 |
| KnC - CW | 80 | 70 | 50 |
| KnC - P | 70 | 75 | 40 |
| VW - B | - | - | - |
| VW - CW | - | - | - |
| VW - P | - | - | - |

Table 1: Root induction - *Dendrobium aqueum*

| Medium+Plant growth regulators | mg/l | Total no of explant | No of explant responded | % of response | No of roots per plantlets |
|---|---|---|---|---|---|
| HMS + IBA | Basal | 250 | 118f | 47.2f | 1.6f |
| | 0.1 | 250 | 209de | 83.6de | 4.8ab |
| | 0.2 | 250 | 222a | 88.8a | 5.2a |
| | 0.3 | 250 | 220ab | 88.0ab | 4.6bc |
| | 0.4 | 250 | 217bc | 86.8bc | 4.0cd |
| | 0.5 | 250 | 212cd | 84.8cd | 3.6de |
| KnC + IBA | Basal | 250 | 102ef | 40.8ef | 1.0f |
| | 0.1 | 250 | 109d | 43.6d | 1.6de |
| | 0.2 | 250 | 133a | 53.2a | 3.8a |
| | 0.3 | 250 | 126b | 50.4b | 2.4b |
| | 0.4 | 250 | 120bc | 48.0bc | 2.2bc |
| | 0.5 | 250 | 104de | 41.6de | 1.8cd |

The seeds are thin and transparent. The cells of seed coats are varied in size and shape. Such variations that were observed in the present study are in agreement with the observations noted by the Previous workers [11,12]. It has been earlier established that the nature of seed coat is of great taxonomic value within the orchidaceae [13].

Thus the early concept that the orchid seeds are sterile and or atleast incapable of germination is no more valid [14], that the germination of seeds of epiphytic orchids posed no problem has been pointed out by various workers [15-19]. However they have indicated that difficulty was encounted in the germination of terrestrial orchid seeds in contrast to those of epiphytic taxa.

The well-developed shoots were removed from the culture tubes and transferred to the rooting media. About 90% of the shoots rooted well after transfer to the HMS and KnC medium supplemented with 0.2 mg/l IBA. Rooting also took place in the two basic media, but duration was longer when compared with the hormone-supplemented media. The rooted plantlets were hardened. Then the hardened plantlets were relocated in natural habitats. In the present investigation 90% of the shoots of the three species were cultured in to basal and IBA supplemented medium, which was essential for root regeneration. Similar results were obtained in *Cymbidium kanran* [20] and *Cymbidium sinense* [21]. In *Vanda coerulea* the rooting efficiency varied with different concentrations of auxin such as IAA, IBA and NAA. The highest percentage of rooting was significant in IAA supplemented half strength Vacin and Went medium [22].

The present investigation was mainly aimed at understanding the mode of seed germination and organogenesis of the developing seedling. Although different workers have suggested several nutrient media for orchids, three well-known media were tried in the present study to probe into the germination response of seeds selected species. While Knudson medium promoted the germination of *Dendrobium aqueum*.

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