

## PROPAGATION OF *DENDROBIUM AGGREGATUM* BY GREEN CAPSULE CULTURE

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**ABSTRACT.** An efficient protocol for propagation of *Dendrobium aggregatum* using the axenic immature seeds, derived from green capsule, was developed. The immature embryos from 120 days old capsules after pollination were germinated on Murashige and Skoog (MS) medium supplement with various concentration of BAP alone or in combination with NAA along with coconut water, and the same media were used for induction, multiplication, elongation and rooting *in vitro* shoots. MS medium with the addition of 3% sucrose 1.5 mg L<sup>-1</sup> Benzyl amino purine (BAP) and 15% coconut water (CW) favoured the higher rate of germination, more number of protocorm like bodies, production of maximum number of shoots, elongation of shoots, as well as root formation. During acclimatization, 95% of the plantlets survived after one month.

**KEY WORDS:** *Dendrobium*, protocorm like bodies, acclimatization, *in vitro*

**Introduction.** Orchids occupy top position among all flowering plants and are valued for cut flower production and as potted plants, often with a very high price in the international market. The genus *Dendrobium* (Family: Orchidaceae) exhibits a vast diversity in vegetative and floral characteristic and is of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity (Jones *et al.* 1998). Though orchids produce a large number of seeds, very few (<5%) of them germinate under natural condition because the seeds are non-endospermic, minute and require a mycorrhizal association (Rao 1977). The conventional method of vegetative propagation of orchids is a time consuming and tedious process (Sagawa & Kunisaki 1984).

*Dendrobium aggregatum* Roxb. (= *D. lindleyi* Steud.)<sup>1</sup> is a mild fragrant sympodial orchid, grown in many counties especially in developed temperate countries of the world as pot plants or cut flowers

(Aktat *et al.* 2007). It has graceful, pendulous racemes of medium sized flowers usually in white colour. The traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year. Orchid is well known for their exploitation as major trade in developed countries (Sagawa & Kunisaki, 1984). *In vitro* culture has proved particularly useful with groups of plants, which are difficult to propagate using conventional techniques (Fay 1994). When mass propagation of a new hybrid or a variety is needed within a short time, tissue culture is the only method (Goh *et al.* 1992).

The orchid resource of the world in general and Western-Ghats region of India in particular is depleting day by day due to habitat loss. The collection of wild *Dendrobium* continues at levels ranging from hobbyist to large-scale illegal trade. Endemic orchids of the Western-Ghats India are facing the grim possibility of extinction under intense biotic pressures like jhum cultivation, forest fires, indiscriminate wild collection and illegal trade by the local people. Hence conservation and sustainable utilization assume greater importance to save the dwindling orchids (Kishor *et al.* 2006). Satisfying the interest of the hobbyist and demand of the traders through large-scale micropropagation is one the preferable options to prevent illegal collection from wild (Sunitibala & Rajikumar 2009).

<sup>1</sup> The name *Dendrobium aggregatum* Roxb. (1832) is illegitimate, being a later homonym of *D. aggregatum* Kunth (1816), the basionym of the species presently known as *Ornithidium aggregatum* (Kunth) Rehb.f. As *D. lindleyi* is still amply cultivated and traded under the name of *D. aggregatum* Roxb., we have maintained the latter name throughout the article. [Note by the Editor]

A perusal of available literature reveals that micropropagation has been achieved using immature or mature embryos, shoot tip explants and from axenic nodal segments in *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch., *D. candidum* Wall. ex Lindl. and other hybrids of *Dendrobium* (Zhang *et al.* 1993, Liu *et al.* 1988, Shiao *et al.* 2005, Xie *et al.* 2010, Nambiar *et al.* 2012). However, there is no report on clonal propagation of *D. aggregatum* using different explant sources. In this study, authors report the development of an efficient simple and reproducible one step protocol for multiple *D. aggregatum* seedlings, rooting of the microshoots and successful transplantation.

**Materials and methods.** Plants of *D. aggregatum* were collected from their natural habitat and kept under shade net (75%) house environment at the campus of the Government Arts College, Coimbatore. After flowering, several flowers were hand pollinated on the second of anthesis. The pollinated flowers were bagged with butter paper for one week. Several capsules of *D. aggregatum* were harvested 120 days after pollination and brought to the laboratory for *in vitro* seed germination.

**Establishment of shoot cultures by *in vitro* germination of immature embryos.** The harvested capsules were soaked in aqueous solution of commercial detergent (labolene) for 10 minutes followed by 0.5 mg/L<sup>-1</sup> Bavistin (Himedia) for 20 minutes. The capsules were surface disinfected in 70% (v/v) ethyl alcohol for 30 seconds followed by 0.12% (w/v) mercuric chloride solution for 10 minutes and then rinsed 3-4 times sterile distilled water before air drying in a laminar air flow chamber for 5 minutes. Green capsules were dissected longitudinally with a sterile surgical blade. The immature seeds were scooped out of the sterilized capsules and small mass of the aggregated seeds were germinated in culture bottles (60 mm × 105 mm) each containing 30 ml of full strength Murashige and Skoog (MS) basal medium. The basal medium was comprised of full strength MS medium 30 mg/ L<sup>-1</sup> sucrose and gelled with 8 g/ L<sup>-1</sup> Difco bacto agar (Himedia, India). It was then supplemented with different concentration and combination of naphthalene acetic acid (NAA) and benzyl aminopurine (BAP) along with coconut water (CW) at pH 5.8 (Table 1). The cultures were

Table 1. Effect of different concentration of plant growth regulators on *in vitro* development of plantlets from immature seeds of *Dendrobium aggregatum*

Concentration of BAP and NAA in MS medium + CW 150 ml/L	No. of green pods used per bottle	Capability of immature seeds forming protocorm like bodies	No. of shoots per bottle	No. of shoots with roots	Percentage of shoot forming the roots
<b>BAP</b>					
0.5	1	+	35	14	40.0
1.0	1	++	50	35	87.5
1.5	1	+++	75	75	100.0
2.0	1	+	41	28	68.3
2.5	1	+	32	14	36.8
3.0	1	+	20	5	25.0
<b>BAP + NAA</b>					
0.5 + 0.5	1	+	23	13	56.5
1.0 + 1.0	1	+	27	20	74.0
1.5 + 1.5	1	+++	37	37	100.0
2.0 + 2.0	1	++	26	15	57.8
2.5 + 2.5	1	+	20	8	40.0
3.0 + 3.0	1	+	14	3	21.4
<b>MS Basal</b>	1	-	-	-	-

+ -- Very less number of protocorm like bodies formation

++ -- Less number of protocorm like bodies formation

+++ -- More number of protocorm like bodies formation

incubated at  $25 \pm 2^\circ\text{C}$  under cool white fluorescent light with 14 hours photoperiod.

After 15 days culture, various concentration of BAP and NAA ranging from 0.5–3.0 mgL<sup>-1</sup> individually (BAP) and combined (BAP + NAA) along with coconut water effect were analyzed through the parameters such as production of amount of protocorm like bodies (PLBs), numbers of shoots and number of shoots with roots.

#### **Subculture of protocorm like bodies and shoots.**

Shoots and protocorm like bodies were transferred to the same media composition produced more protocorm like bodies, multiple shoots and roots.

**Hardening.** Well rooted shoots were removed from culture vessels and thoroughly washed with tap water to remove residual medium and transferred to plastic pots containing a mixture uniform, small charcoal pieces and brick pieces (1:1). They were then kept in the shade house 25% light and mist irrigated.

**Experiment design and data analysis.** Experiments were set up in completely randomized design. Each treatment had 10 replicates. Significance of treatment effects was determined using DMRT analysis.

**Results and discussion.** The seeds taken from the green capsules were sown on the MS medium (Table-1) containing various concentrations of two plant growth regulators, namely BAP and NAA along with CW. Invariably all the embryos transferred to the MS medium with various concentrations of BAP, NAA and CW germinated within two weeks. Swelling and glistening of the embryos were first noticed within 10 days. The swelling of the embryo was followed by pigment synthesis. The embryos turned from yellow to yellowish green and finally becoming green as they grew.

Due to the non-endospermic nature of the seed, the germination in nature is a unique phenomenon and requires fungal infection. Germination is much more successful in *in vitro*. The production of orchid seedling from seed involves, sequential phases of germination, protocorm formation and seedling development. In the present investigation also same sequence of seedling development was observed when the selected orchid, *D. aggregatum* was grown on the medium. As the embryos development into globouse

protocorms, seed coat (testa) got ruptured and rhizoids and shoot initials were getting formed. Among the six different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l<sup>-1</sup>) individually in combination with NAA (0.5, 1.0 1.5, 2.0, 2.5 and 3.0 mg l<sup>-1</sup>) along with CW was used. MS medium contain BAP + CW (1.5 mg/l + 15% in combination) was found to be the most suitable, which supported higher rate of germinations, more number of protocorm bodies, shoots and roots. Shoots and protocorm like bodies were transferred to same media composition produced more protocorm like bodies, multiple shoots and roots. Incorporation of coconut water to basal medium induces and enhances early differentiation of PLBs. Earlier Talukdar (2001) reported similar observation in *D. aphyllum*. Leetham (1974) reported that a plant growth hormone like cytokinin is present in coconut water. PLBs developed from the germinating seeds after 45-50 days of culture were allowed to differentiate in the same medium. The pear shaped PLBs with tiny leaf sprouting were developed after 20-25 days of germination.

Auxin was the first plant growth hormone added to the seed culture. In majority of the cases auxins (mostly NAA, IAA and IBA) enhanced the germination and seedling growth (Nasiruddin *et al.* 2003). In the present study BAP and CW stimulated shoot and root growth in *D. aggregatum* as reported in *D. microbulbon* A. Rich. (Urvashi Sharma *et al.* 2007). More number of protocorm like bodies were produced by the medium, which contained 1.5 mg l<sup>-1</sup> BAP with CW and 1.5 mg/l + 1.5 mg/l BAP and NAA with CW. Very poor result was observed in the medium contained 3.0 + 3.0 mg l<sup>-1</sup> BAP and NAA. (Table-1). In the present investigation the seedling development of *D. aggregatum* was best on the MS medium supplemented with 1.5mg l<sup>-1</sup> BAP + 15% CW. These finding are in agreement with Urvashi Sharma 2007, who observed that BAP induced better shoot and root growth in *D. microbulbon*.

A well-developed cluster were selected and transferred to second subculture for root induction (Fig. 1). These subculture were grown using the same concentration and combination of the same plant growth regulators. These cultures media were used to study their stimulatory effect of the number of shoots and roots per shoot. The number of shoots and roots were counted. After maintaining for 2-3 passages on the

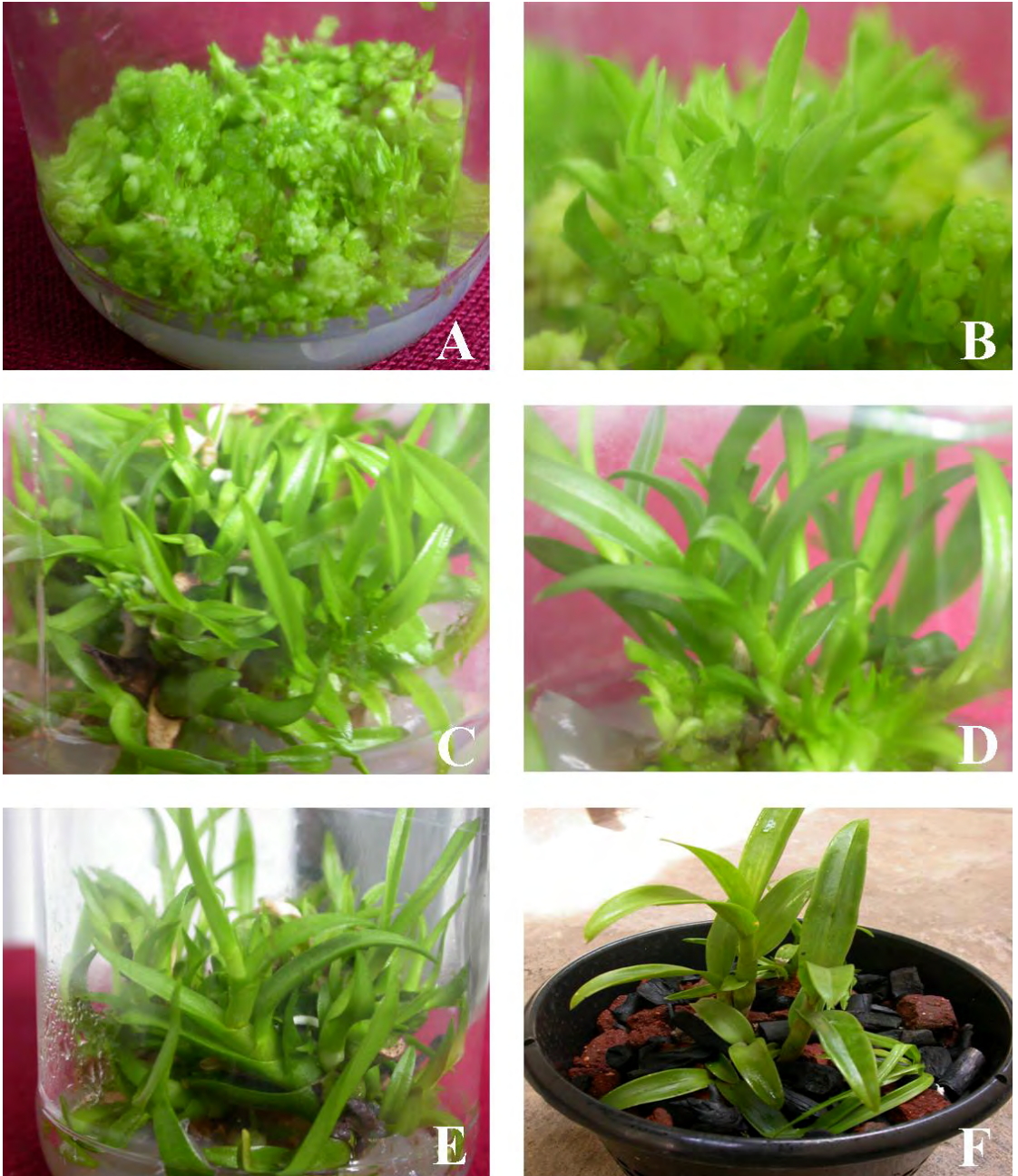


FIGURE 1. Different stages of immature seed germination and regeneration of plants of *Dendrobium aggregatum* on MS medium. A — PLBs. B — PLBs and young plants. C, D — Developed plantlets. E, F — Potted plant.

medium, the plants were taken out, washed thoroughly to remove traces of agar and transplanted to perforated plastic pots containing pieces of charcoal and bricks (Fig. 1). About 95% of the potted plants survived after one month in the shade house. The transplanted plants

were acclimatized in the shade house for 1-2 months and transferred to the environmental condition.

The present investigation revealed that concentrations of BAP and ANA along with CW in a MS medium influenced *in vitro* seed germination,

production of PLBs, shoot multiplication and root initiation. The *in vitro* raised seedlings were successfully established in the potting medium. Further growth and development of seedlings will be observed in further.

In conclusion, a simple, efficient and commercially viable protocol for germination and mass clonal propagation of *D. aggregatum* from green capsule has been established. Using this protocol, viable, uniform and healthy plants with maximum survival rate can be produced for large scale cultivation and conservation.

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