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# *IN VITRO SEED* GERMINATION AND MICROPROPAGATION OF *DENDROBIUM LINDLEYI* STEUD. AN INDIGENOUS ORCHID OF BANGLADESH

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**Abstract**

The seeds of *Dendrobium lindleyi* were germinated on 0.8% (w/v) agar solidified Murashige and Skoog (MS) as well as Phytamax (PM) basal media. The germination rate was found to be better on PM medium. The germinated seedlings underwent rapid elongation on Plant Growth Regulators (PGRs) supplemented media and the highest growth was recorded on MS with 3% (w/v) sucrose + 2.0 mg/l 6-Benzyl Amino Purine (BAP) + 0.1mg/l Indole-3-Butyric Acid (IBA). The seed derived seedlings developed strong and stout roots on half strength MS medium with 1.5% sucrose. For micropropagation the pseudobulb segments were used as explant and the lower part of pseudobulb segment produced highest number of multiple shoot buds (9-10/segment) on agar solidified MS medium supplemented with 3% (w/v) sucrose + 2.5 mg/l BAP + 0.1% (w/v) Activated Charcoal. The highest rate of elongation and rooting was found from multiple shoot bud derived seedlings as well as same seed derived seedlings. Seed and multiple shoot bud derived rooted seedlings were finally transferred to outside natural environment by successive phases of acclimatization.

*Key words*: *In vitro* germination, Micropropagation, *Dendrobium lindleyi*, Orchid

## Introduction

*Dendrobium lindleyi* Steud. is a horticulturally important epiphytic orchid having beautiful spikes of yellow flowers which persist for about one and half months. This species is sporadically distributed in Chittagong, Chittagong Hill Tracts and Sylhet forest area of Bangladesh. Its population in nature has been reduced because of ruthless collection by leading orchid growers and also for destruction of habitats. It is, therefore, considered important to use *in vitro* techniques for rapid propagation of this species to meet up the commercial demand as well as for its reestablishment in nature. *In vitro* technique of micropropagation in orchid was developed with the success of Morel (1960) for shoot tip culture in *Cymbidium* and four million plantlets could be produced in a year from a single protocorm (Morel 1964). Later on the technique of *in vitro* micropropagation was refined and made effective in a range of orchid species Sagawa and Shoji 1967 (Arditti 1977, and Hoque *et al.* 1994). The clonal propagation technique created intense interest among orchid growers and many leading orchid nurseries around the world include tissue culture technique as a routine in their operations. In most of the cases cultural techniques and media compositions have been found be to very specific to

species and to organ. Therefore, it is important to develop effective cultural technique and formulate media composition for individual species separately. The present investigation was undertaken with a view to developing an efficient and reproducible protocol for *in vitro* germination and micropropagation of *Dendrobium lindleyi,* a commercially important orchid of Bangladesh. Since this species is now becoming rare, it deserves special attention for conservation. The technique of tissue culture is now widely used for rapid propagation of rare plant species and the development efficient protocol for mass scale production of seedlings is a prerequisite for such programme.

**Materials and Methods**

The fruits of *Dendrobium lindleyi* Steud. *were* collected from the Botanical Garden of Chittagong University. The fruits were washed with running tap water, rubbed with cotton and further washed with distilled water. These were then surface sterilized by treating with 0.2% (w/v) $HgCl_2$ for 10 minutes and thereafter washed 2 - 3 times with double distilled sterile water in a laminar airflow cabinet. The fruits were dissected longitudinally with a sterile surgical blade; the seeds were separated from capsule and inoculated on to the surface of sterile germination medium taken in culture vessels. All these operations were done in a laminar airflow cabinet. Before inoculation of seeds, the culture vessels (2.5 cm × 15 cm test tubes or 100/150ml conical flasks) containing medium were autoclaved at 1.9 $kg/cm^2$ of pressure at a temperature of $121^0C$ for 20 minutes to ensure sterilization. The $p^H$ of the media was adjusted to 5.8 for MS and 5.4 for PM based media. The temperature of the culture room was maintained at $25 \pm 2^0C$, where a regular cycle of 14h continuous light and 10 h dark was maintained. MS (Murashige and Skoog 1962) and PM (Arditti 1977) basal media were used for germination. After germination the seedlings continued its growth on germination medium but the growth rate was very slow. In order to induce rapid elongation, seedlings were transferred on to a wide spectrum of plant growth regulators (PGRs) supplemented media. In elongation media the seedlings produced weak root system. In order to induce strong and stout root system, the seedlings were grown on two different kinds of 0.8% (w/v) agar solidified rooting media namely, (i) half strength MS + 1.5% (w/v) sucrose and (ii) MS + 3% (w/v) sucrose + 0.5 $mgl^{-1}$ IAA. Finally the rooted seedlings were taken out of the culture vessels and transferred to outside environment following successive phases of acclimatization. For this purpose, the culture vessels were kept open for one day in the culture room and then kept outside of the culture room for 6 h in the next day. Later on, those were kept outside of the culture room for 12 h. Finally the seedlings were taken out of the culture vessels and rinsed with running tap water for removal of agar attached to the roots. Then the seedlings were transplanted to pots containing moistened coir, saw dust and coal. Transplanted seedlings were watered regularly for about 1 - 2 months where the seedlings established and grew well.

**Results and Discussion**

The seeds germinated on 0.8% (w/v) agar solidified MS and PM medium (Plate 1a). show that PM medium was better than MS medium for germination of *Dendrobium lindleyi* orchid seeds.

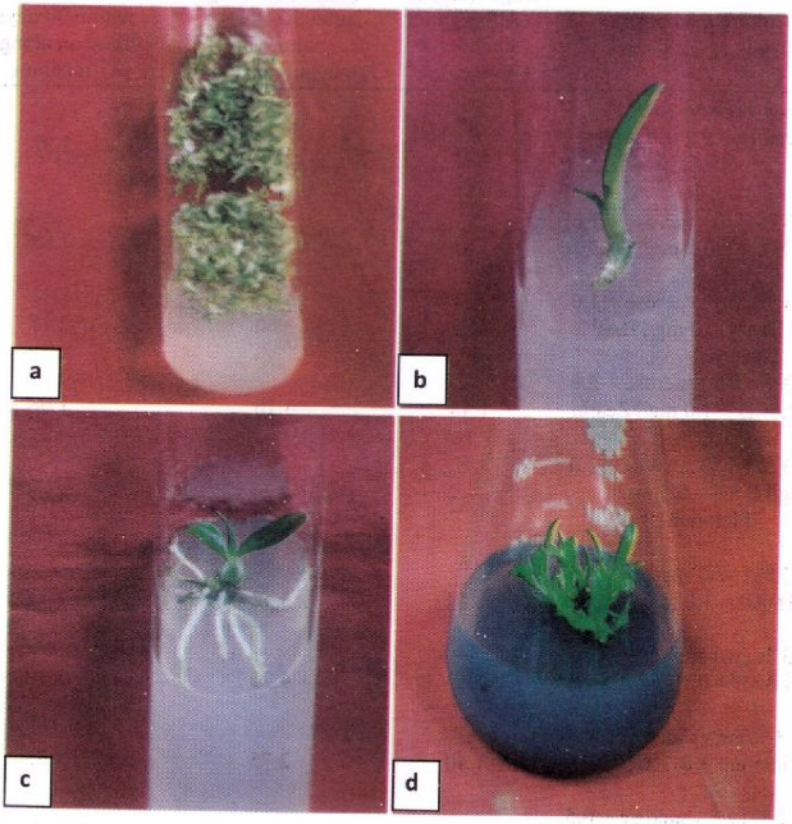


Plate 1. Axenic culture of *D. lindleyi*. a, *In vitro* seed germination; b, Elongation of germinated seedling; c, Rooting of elongated seedling; d, Multiple shoot buds derived from lower part of pseudobulb segment.

The germinated seedlings underwent elongation when subcultured on the same germination medium but the growth rate was very slow. For rapid elongation, the tiny seedlings were transferred to a medium containing different plant growth regulators (6-Benzyl Amino Purine, Indole-3-Acetic Acid, Indole-3-Butyric Acid, α-Naphthalene Acetic Acid, Kinetin and Picloram) supplemented with casein hydrolysate (CH) and activated charcoal (AC). The highest rate of elongation of seedling was achieved on MS medium fortified with 2.0 mg/l BAP + 0.1 mg/l IBA (Plate 1b) followed by that on MVW medium with 1.5 mg/l Kn + 3.0 mg/l BAP + 1.0 mg/l NAA (Table 1).

Table 1. Elongation of germinated seedlings* of *D. lindleyi* on 0.8 % (w/v) agar solidified MS medium supplemented with different combination and concentration of PGRs.

| Culture medium | Average initial length (cm) of seedlings after 60 days of germination | Average length (cm) of seedlings after 30 days of culture on elongation medium | Increased length (cm) of seedlings within 30 days of culture on elongation medium |
|---|---|---|---|
| MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 2.0 mg/l NAA | 1.42 | 3.74 | 2.32 |
| MS + 3 % (w/v) sucrose + 1.0 mg/l NAA + 0.5 mg/l BAP | 1.55 | 3.53 | 1.98 |
| MS + 3 % (w/v) sucrose + 1.0 mg/l IAA + 2.0 mg/l BAP | 1.45 | 3.60 | 2.15 |
| MS + 3 % (w/v) sucrose + 2.0 mg/l 2,4- D + 1.0 mg/l Zeatin | 1.48 | 3.16 | 1.68 |
| MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 0.5 mg/l Picloram | 1.52 | 3.75 | 2.23 |
| MS + 3 % (w/v) sucrose + 0.5 mg/l BAP + 1.0 mg/l IAA | 1.40 | 3.52 | 2.12 |
| MS+ 3 % (w/v) sucrose + 2.0 mg/l BAP + 0.1 mg/l IBA | 1.50 | 4.20 | 2.70 |
| MS + 3 % (w/v) sucrose +1.0 mg/l Picloram + 0.5 mg/l BAP | 1.40 | 2.75 | 1.35 |
| PM + 2 % (w/v) sucrose + 0.5 mg/l IAA + 1.0 mg/l BAP | 1.45 | 3.70 | 2.25 |
| MVW + 2 % (w/v) sucrose + 1.5 mg/l Kn + 3.0 mg/l BAP + 1.0 mg/l NAA | 1.52 | 3.05 | 2.43 |

*Based on observations recorded from 50 seedlings taking 5 at random from each of 10 culture vessels.

The elongated seedlings produced weak root system on the elongation media. For induction of strong and stout root system the elongated seedlings (2 - 3 cm) were transferred to rooting media. Two different kinds of 0.8 % (w/v) agar solidified rooting media namely, (i) half strength MS + 1.5 % (w/v) sucrose and (ii) MS + 3 % (w/v) sucrose + 0.5 mg/l.IAA were used for induction of well-developed root system (Table 4).

The efficiency of the rooting media was evaluated based on the increase in length and number of roots developed per seedling. Half strength MS + 1.5 % (w/v) sucrose was proved more effective for the development of better root system (Plate 1c). This finding indicates that less nutrient containing medium was more effective for induction of strong and stout root system than auxin supplemented full strength media. Similar results were reported by Agarwal *et al.* (1992) in *Vanilla walkeriae* by Barua and Bhadra (1999) in *Cymbidium aloifolium* and *Spathoglottis plicata* by Bhadra *et al.* (2002) in *Dendrobium aphyllum* and by Sinha and Roy (2004) in *Vanda teres*.

Table 2. Development of multiple shoot buds* from pseudobulb segments in *D. lindleyi* when cultured on 0.8% (w/v) agar solidified media supplemented with different PGRs.

| Culture medium | Explants | Time (d) required for sprouting of multiple shoot buds | Average number of multiple shoot buds sprouted in each pseudobulb segment |
|---|---|---|---|
| MS + 3 %(w/v) sucrose + 2.0 mg/l NAA+ 2.0 mg/l BAP | PSL** | 25 - 30 | 10.14 |
| | PSU*** | 25 - 30 | 8.17 |
| MS + 3 % (w/v) sucrose + 1.0 mg/l BAP + 0.5 mg/l Kn + 2 g/l CH | PSL** | 30 - 35 | 7.65 |
| | PSU*** | 30 -35 | 5.23 |
| MS + 3 % (w/v) sucrose + 0.5 mg/l BAP +1.0 mg/l NAA | PSL** | 25 - 30 | 7.73 |
| | PSU*** | 25 - 30 | 5.33 |
| MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 1.0 mg/l IAA | PSL** | 25 - 30 | 6.92 |
| | PSU*** | 25 - 30 | 4.37 |
| MS + 3 % (w/v) sucrose + 2.5 mg/l BAP + 0.1 % (w/v) AC | PSL** | 25 - 30 | 10.52 |
| | PSU*** | 25 - 30 | 8.37 |
| MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 0.5 mg/l Picloram | PSL** | 30 - 35 | 7.94 |
| | PSU*** | 30 - 35 | 5.23 |
| PM + 2 % (w/v) sucrose + 1.0 mg/l BAP + 0.5 mg/l IAA | PSL** | 25 - 30 | 6.78 |
| | PSU*** | 30 - 35 | 4.28 |
| MVW + 2 % (w/v) sucrose + 3.0 mg/l BAP + 1.0 mg/l NAA + 1.5 mg/l Kn | PSL** | 30 - 35 | 7.32 |
| | PSU*** | 30 - 35 | 5.24 |

*Based on observations recorded from 20 cultured segments in each medium.
**PSL = Pseudobulb Segment Lower part.
***PSU = Pseudobulb Segment Upper part.

Table 3. Elongation of multiple shoot buds* developed from pseudobulb segments of *D. lindleyi* when grown on 0.8% (w/v) agar solidified media supplemented with different PGRs.

| Culture medium | Average initial length (cm) of individual multiple shoot bud | Average length (cm) of individual multiple shoot bud after 30 days of culture on elongation medium | Increased length (cm) of shoot bud within 30 days of culture in elongation medium |
|---|---|---|---|
| MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 2.0 mg/l NAA | 1.42 | 3.87 | 2.45 |
| MS + 3 % (w/v) sucrose + 0.5 mg/l BAP + 1.0 mg/l NAA | 1.40 | 3.44 | 2.04 |
| MS + 3 % (w/v) sucrose + 0.1 mg/l IBA + 2.0 mg/l BAP | 1.45 | 4.13 | 2.68 |
| MS + 3 % (w/v) sucrose + 2.5 mg/l BAP + 0.1 % (w/v) AC | 1.45 | 3.86 | 2.41 |
| MS + 3 % (w/v) sucrose + 0.5 mg/l BAP + 1.0 mg/l IAA | 1.38 | 3.54 | 2.16 |
| MS+ 3 % (w/v) sucrose + 2.0 mg/l BAP + 0.5 $mgl^{-1}$ Picloram | 1.40 | 4.52 | 2.12 |
| PM + 2 % (w/v) sucrose + 1.0 mg/l BAP + 0.5 mg/l IAA | 1.35 | 3.48 | 2.13 |
| MVW + 2 % (w/v) sucrose + 3.0 mg/l BAP + 1.0 mg/l NAA + 1.5 mg/l Kn | 1.42 | 3.90 | 2.48 |

*Based on observations recorded from 20 cultured shoot buds.

For *in vitro* micropropagation pseudobulb segments (0.5 - 1.0 cm) of the aseptically raised seedlings were cultured on 0.8 % (w/v) agar solidified MS and PM medium supplemented with various combinations and concentrations of PGRs (Table 2). The upper and the lower part of pseudobulb segments underwent direct organogenesis producing multiple shoot buds (Plate. 1d). The number of shoot buds developed per segment was dependent on plant growth regulator (PGR) combinations and concentration

of the nutrient media. The efficiency of a medium was assessed on the basis of number of multiple shoot buds developed in each pseudobulb segment. Maximum number of multiple shoot buds (10 - 12/segment) was developed in lower part of segment on MS medium supplemented with 2.5 mg/l BAP and 0.1% (w/v) AC. This finding indicates

Table 4. Development of roots in *D. lindleyi* seedlings* when cultured on 0.8% (w/v) agar solidified rooting media.

| Rooting medium | Average initial length and number of roots in each seedling before culture on rooting medium | | Average length and number of roots after 30 days of culture on rooting medium | | Average increased length and number of roots after 30 days of culture on rooting medium | |
|---|---|---|---|---|---|---|
| | Length (cm) | No. of roots/ Seedling | Length (cm) | No. of roots/ seedling | Length (cm) | No. of roots/ seedling |
| ½ MS + 1.5 % (w/v) sucrose | 1.18 | 2.12 | 2.63 | 4.26 | 1.45 | 2.14 |
| MS + 3 % (w/v) sucrose + 0.5 mg/l IAA | 1.12 | 2.08 | 2.15 | 3.16 | 0.96 | 1.08 |

*Based on observations recorded from 50 seedlings taking 5 at random from each of 10 culture vessels.

Table 5. Development of root system in individual shoot bud* of *D. lindleyi* when grown on 0.8% (w/v) agar solidified rooting media.

| Rooting medium | Average number of roots/ individual shoot bud after 30d of culture on rooting medium | Average length (cm) of roots after 30d of culture on rooting medium |
|---|---|---|
| ½ MS +1.5% (w/v) sucrose | 4.95 | 2.78 |
| MS+3% (w/v) sucrose + 0.5 mg/l IAA | 3.16 | 2.24 |

*Based on observations recorded from 50 cultured individual shoot bud.

that same species responses differently for different organs (Yam and Weatherhead 1991 and Aggarwal 2001). Similar results were also noted by Gupta and Bhadra (1998) in *Cymbidium aloifolium by* Sheelavantmath *et al.* (2000) in *Geodorum densiflorum* and by Bhadra and Bhowmik (2005) in *Arundina graminifolia.* The growth promoting ability of AC which generally happens because of adsorption of excess nutrients by AC. A second possibility is that AC adsorbs phytotoxic metabolites, which may be released by the tissues (Yam *et al.*, 1990). In order to induce rapid elongation and thereafter rooting, multiple shoot buds developed from rhizome segments were further cultured on

elongation and rooting media respectively (Tables 3 and 5). MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 0.1 mg/l IBA was found most effective for enhancing elongation. Whereas rooting was best on half strength MS + 1.5 % (w/v) sucrose. The seedlings at a stage of 5 - 7 cm were taken out of the culture vessel and transferred to pots outside the culture room following successive phases of adjustment.

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