

## Regeneration of *Dendrobium wardianum* Warner (Orchidaceae) from synthetic seeds

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Protocorm-like-bodies (plbs) of *D. wardianum* were successfully encapsulated in calcium alginate beads to get synthetic seeds and 100% conversion frequency was recorded after 45 days of culture when germinated on MS medium. The beads could be stored at 4°C up to 180 days. However, the germination declined with increase in duration of storage. The regenerants were phenotypically similar to controls.

Mericlone has opened new vistas in orchid tissue culture<sup>1-3</sup>. It becomes particularly important for orchids as their genotypes are highly heterozygous and vegetative propagation by division is slow<sup>4</sup>. Natural regeneration of orchids through seeds is very poor due to their minute size, lack of embryo and/or presence of a reduced endosperm<sup>5</sup>. Plant regeneration *in vitro* can occur via adventitious bud development, somatic embryogenesis or axillary/apical meristem proliferation. Recently, production of synthetic seeds by encapsulating somatic embryos (SE) has been reported in a few plants<sup>6,7</sup>. However, this technique has not been amenable for use in case of orchids which do not produce SE in true sense rather give rise to protocorm-like-bodies (plbs) in culture offering the most potential method for clonal propagation. *Dendrobium wardianum* Warner, a beautiful, epiphytic, commercially favoured orchid has become endangered in its natural habitat due to large scale denudation of lands and ruthless exploitation to cater its demand in the cut flower industry. In the present study an attempt has been made to produce synthetic clonal seeds by encapsulating plbs of *D. wardianum*, preserve them under cold condition over a considerable period and study the conversion frequency.

Sodium alginate (Sigma) was mixed with Murashige and Skoog<sup>8</sup> (MS) liquid medium with 3% sucrose without growth regulator and the solution pH was adjusted to 5.8. For formation of insoluble calcium alginate, 100 mM calcium chloride was prepared in liquid MS medium and the autoclaving carried out at 1.05 kg/cm<sup>2</sup> for 15 mins.

Plbs were obtained by culturing shoot apices of *D. wardianum* on MS medium with 3% sucrose, 0.8% agar and 2.5 mg/l 6-benzylaminopurine (BAP) as reported<sup>9</sup>. These plbs were separated, blot dried, mixed with sodium alginate solution, and dropped into CaCl<sub>2</sub>.2H<sub>2</sub>O solution using a wide mouth dropping pipette. The drops, each containing a single plb, when left in CaCl<sub>2</sub>.2H<sub>2</sub>O solution for 40 min on gyratory shaker (75 rpm) formed round, firm beads as a result of the ion exchange reaction between Na<sup>+</sup> and Ca<sup>2+</sup> ions. Later these beads were recovered by recycling and decanting CaCl<sub>2</sub>.2H<sub>2</sub>O solution and washed thrice with autoclaved MS liquid medium. The beads were then plated on basal MS medium for further growth and development studies.

The cultures were maintained at 24 ± 1°C under 16 hr photoperiod (fluorescent light 30 Em<sup>-2</sup>S<sup>-1</sup>). Encapsulated plbs (10 sets of 20 beads each) were stored at 4°C. Non-encapsulated plbs stored at 4°C served as control. The regeneration potential was recorded at every 15 day interval up to 180 days and the complete experiment was repeated twice.

Encapsulated plbs (without storage) when germinated directly on medium (Fig. 1a) with or without BAP showed the conversion frequency of 100% after 45 days of culture. On the other hand, a conversion frequency of 60% was recorded in case of non-encapsulated plbs (without storage) under the same cultural conditions. The synthetic seeds stored at 4°C remained viable and germinated up to day 120. However, a poor germination of 30% was recorded which further declined to 10% by day 180. Higher germination percentage in case of synthetic seeds (without storage) could be due to the matrix which not only facilitates regular nutrient supply but also protects the dispersed, delicate tissue from any mechanical injury during handling and from desiccation. Synthetic seeds plated on MS medium free of growth regulators eventually developed into multiple plantlets (Fig. 1b) whereas the same when cultured on medium containing BAP 5 mg/l) resulted in their multiplication only. The multiple plantlets obtained from synthetic seeds were cultured in flasks containing MS medium without growth regulators. Within 16 weeks of transfer, plantlets of 6-8 cm height with 3-4 nodes and well developed root system were obtained. These were potted in earthen pots containing charcoal pieces, brickbats, coconut fibres and a layer of moss at the surface and hardened under glass house conditions

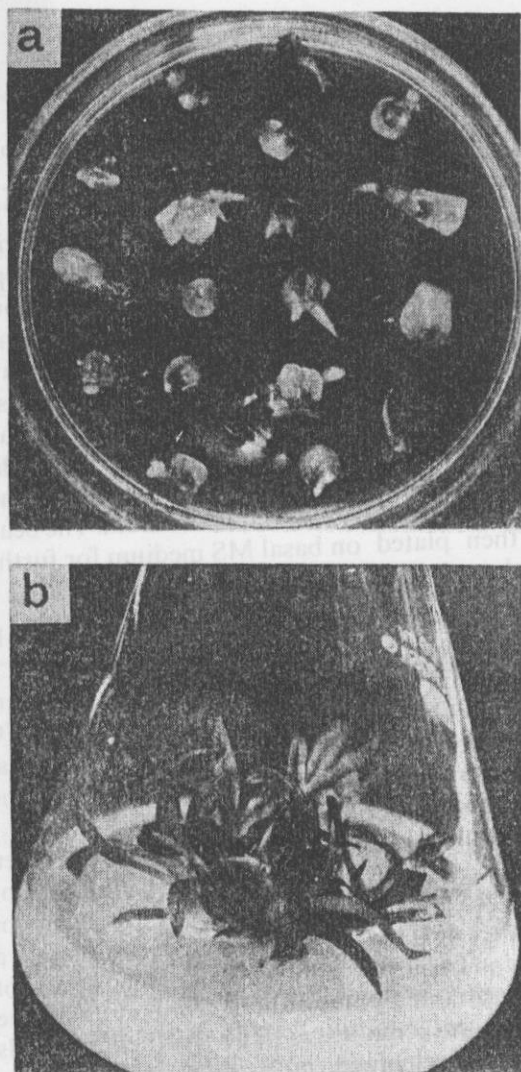


Fig. 1—(a) Germinating synthetic seeds on MS basal medium; and (b) multiple plantlets obtained from synthetic seeds

(temperature 20°-25°C, RH 80-90%). About 65% of the regenerants survived.

Tissue culture techniques for clonal propagation of plants that have a high per unit value, like ornamentals and fruits are of great significance. High volume propagation potential combined with formation of synthetic seeds for low cost delivery is expected to open new vistas. Recently, efforts have been intensified in this area by encapsulating SE<sup>6</sup> and shoot bud explants<sup>10,11</sup> in various matrices and growing them in different substrata. A significant outcome of the study was the observation that the synthetic seeds retained their viability even after 120 days at 4°C and the plants recovered also maintained their clonal identity as there were no morphological differences observed among the regenerants. Attempts are underway to directly transfer the synthetic seeds to natural substrata by adding antifungal and antibacterial compounds in the matrices.

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