Effective use of synthetic seed technology in the regeneration of *Cymbidium aloifolium* using protocorm-like bodies

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Synthetic seed technology offers tremendous potential in micropropagation. It deals with *in vitro* conservation and storage of rare and endangered plant species along with their easy handling and transportation. This technology is becoming prevalent due to its wide applications in germplasm conservation and for exchanges between countries in floricultural trade. The present study examines the regeneration and conversion capabilities of *Cymbidium aloifolium* using protocorm-like bodies when stored at different temperatures. The propagules showed high proliferative potential by multiplication and complete plantlets were obtained in 58 days on basal M medium supplemented with 1 mg l⁻¹ of indole-3-acetic acid.

**Keywords:** *Cymbidium aloifolium*, protocorm-like bodies, regeneration, synthetic seeds.

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**ACKNOWLEDGEMENTS.** We thank DST-NISA (BDID/01/23/2014-HSRS/20) and SAC-ISRO-AVIRIS-NG-AO for financial assistance. We also thank Dr Bimal Bhattacharya (SAC-ISRO, Ahmedabad, India) for support, and Philip Townsend, Adam Chlus and Zhiwei Ye (University of Wisconsin, USA) for sharing topographic and BRDF-corrected AVIRIS-NG images for the four forest covers.

Received 16 October 2019; revised accepted 1 December 2020
doi: 10.18520/cs/v120/i3/567-570
**Table 1.** Effect of different growth additives on time taken for initiation response and plantlet formation (days) in synthetic seeds immediately after their preparation in *Cymbidium aloifolium*

| Additives (1 mg l⁻¹) | Time taken for initiation response (days) | Time taken for plantlet formation (days) | Remarks |
|---------------------|------------------------------------------|------------------------------------------|---------|
| M                   | 25                                       | 45                                       | Protocorm-like bodies (PLBs) multiplication |
| M + IAA             | 38                                       | 58                                       | Formation of plantlets with long roots |
| M + 2,4-D           | 34                                       | 54                                       | – |
| M + BAP             | 30                                       | 51                                       | PLBs multiplication |
| M + IAA + KN        | 40                                       | 60                                       | PLBs multiplication |
| M + IBA + BAP       | 42                                       | 62                                       | PLBs multiplication |

M, Mitra medium; IAA, Indole-3-acetic acid; 2,4-D, 2,4-Dichlorophenoxyacetic acid; BAP, 6-Benzylaminopurine; IBA, Indole-3-butyric acid; KN, Kinetin.

**Figure 1.** Synthetic seeds in *Cymbidium aloifolium*: a, Spherical, non-leaky and firm seeds with 3% sodium alginate and 100 mM calcium chloride. b, Multiple shoot formation (M + BAP (1 mg l⁻¹)). c, Formation of protocorm-like bodies (PLBs) (M + IBA (1 mg l⁻¹) + BAP (1 mg l⁻¹)). d, e, Formation of long roots and complete plantlet formation (M + IAA (1 mg l⁻¹) + KN (1 mg l⁻¹)). f, g, Multiplication of PLBs (M + IAA (1 mg l⁻¹) + KN (1 mg l⁻¹)). h, i, Formation of leaf primordia (M + 2, 4-D (1 mg l⁻¹)). j, Multiplication of PLBs and complete plantlet formation (M). k, Complete plantlet formation with well-developed roots (M + BAP (1 mg l⁻¹)).

**Figure 2.** Effect of temperature and storage on the conversion frequency of synthetic seeds in *Cymbidium aloifolium*.

interval) to ascertain the maximum period for which the seeds could remain viable. The per cent viability of seeds was calculated by dividing the live seed count by total seed count. The seeds were stored at two different temperature regimes, viz. 4°C and 25°C.

Freshly prepared seeds were inoculated on basal M medium with and without different plant growth regulators (PGRs) into 20 × 150 mm culture tubes which were maintained at 25 ± 2°C under 35 μE m⁻² s⁻¹ light intensity and 50–60% relative humidity. One set of encapsulated PLBs was kept in a refrigerator at 4°C and another set were kept at 25°C. Each treatment consisted of eight replicates and observations were made by taking the average time of all replicates. The experiment was repeated twice.

In the present experiment, synthetic seeds were successfully prepared in *C. aloifolium*. Their physical characteristics such as size, shape and firmness varied with the concentration of the gelling agent and quantity of calcium chloride used. An encapsulation matrix of 3% sodium alginate and 100 mM calcium chloride yielded spherical, non-leaky and firm seeds. Lower concentrations (sodium alginate; 2.0%, 2.5% and CaCl₂; 50 mM) were not suitable for encapsulation as the beads formed were irregularly outlined, soft and leaky. The effect of different PGRs on the time taken for initiation response and subsequent plantlet development (days) in synthetic seeds, immediately after their preparation was analysed. Table 1 and Figure 1 a–k provide a summary of the results. Freshly encapsulated PLBs (i.e. control) converted with 90% frequency after 25 days, when directly inoculated on M medium supplemented with different growth additives. The encapsulants, i.e. PLBs multiplied and differentiated into complete plantlets in 45 days. The propagules showed high proliferative potential by their multiplication and complete plantlets were observed in 58 days on basal medium supplemented with indole-3-acetic
acid (IAA) (1 mg l⁻¹). However, a combination of indole-3-butyric acid (IBA) and kinetin (KN) at 1 mg l⁻¹ each resulted in delaying the initiation response and subsequent plantlet formation, which is in accordance with the results of Vij and Aggarwal¹⁸ in *Vanda coerulea*. According to Vij, the exogenous requirement of plant hormones depends on their endogenous level in the plant system, which varies with the phase of plant growth.

The conversion frequency of seeds was also observed to vary with time and temperature of storage (Figure 2). The freshly prepared seeds converted readily on M medium and showed proliferation. Synthetic seeds stored at 12°C and showed proliferation. Synthetic seeds stored at 25°C in *C. aloifolium*. Synthetic seeds retained 60% viability after 15 days, which gradually reduced to 45% after 30 days, 25% after 45 days and only 15% seeds converted after 60 days. However, seeds when stored at 25°C completely lost their viability after 45 days. Similar results were observed by Sarmah et al.¹⁹ and Pehwal et al.²⁰ for seeds stored at 4°C. This is possibly due to low metabolic rates at low temperatures in accordance with an earlier suggestion⁵. According to Sakamoto et al.,²¹ synthetic seeds dry quickly and are difficult to store for longer periods unless kept in humid environment and/or coated with a hydrophobic membrane, coating of substances like wax, resin, polyorganosilicane, etc. has been used by some workers.²²-²³ However, we did not perform such experiments due to paucity of time. Hence, if we want to store synthetic seeds for longer period, coating of substances like wax, resin, etc. should be used and then stored in refrigerators for their longer viability.

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**ACKNOWLEDGEMENT.** Financial assistance from the University Grants Commission, New Delhi to S.V. is acknowledged.