J. Phytol. Res. **12**(1-2) : 21-23, 1999

PLANTLET REGENERATION FROM ENCAPSULATED PROTOCORMS OF THE ENDEMIC ORCHID *COELOGYNE ODORATISSIMA* VAR. *ANGUSTIFOLIA* LINDL.

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Regeneration after encapsulation in sodium alginate matrix of protocorms from the endemic orchid *Coelogyne odoritissima* var. *angustifolia* Lindl. was evaluated. The protocorms propagated *in vitro* were used as source material. Encapsulation with sodium alginate and subsequent regrowth was compared to encapsulation with nutrient enriched alginate capsule followed by regrowth on nutrient medium.

Keywords : *Coelogyne odoratissima* Var. *angustifolia* Lindl; Endemic; Protocorms.

Introduction

Application of synthetic seed technology in the field of micropropagation, storage and transport has been well recognised in several agronomically important crops and forest tree[1]. In orchids the protocorms produced in culture were found to be suitable for encapsulation[2]. Encapsulated protocorms may be useful for transportation of hybrid orchids and for conservation of orchid germplasm[3]. *Coelogyne odoritissima* var. *angustifolia* is an orchid endemic to Nilgiri Biosphere Reseve. Though it produces many seeds, less than 5% are germinable in nature and require mycorrhizal association. Successful propagation by culturing the embryos in a simple defined medium under *in vitro* through a protocorm phase has been developed (Unpublished). Production of encapsulated protocorm and their successful regeneration after storing at different temperatures has been described in the present study.

Material and methods

Approximately 60-70 days old protocorm obtained via seed culture were used for encapsulation. The protocorms were encapsulated in 3% alginate, MS medium containing sucrose (3%) and in different concentration of sucrose (2, 4, 6 & 9%) along with 3% alginate. The protocorms were picked and mixed with 3% alginate solution and dropped in $CaCl_2 . 2H_2O$ solution (1.016g/150ml) for 30 minutes and were recovered by decanting $CaCl_2 . 2H_2O$ solution and washed thrice with sterile distilled water, blotted and germinated on different substrata. Ten beads were cultured in each test tubes at 25±1°C with a RH of 70% and 2000 lux intensity for 12 hrs a day. Three replicates were maintained in each combination. One set of encapsulated protocorms was (prepared in MS medium without sucrose) stored at 25±1°C and 7° C in empty petriplate for 60 days, after the storage the beads were cultured on nutrient media.

Results and Discussion

The beads prepared in 3% alginate initially remained viable for 10 days but showed no sign of growth and finally turned brown. Whereas the beads prepared in MS medium and cultured on MS medium showed the shoot and root emergence after 30 days but the growth was slow. Encapsulated protocorms cultured on MS medium, supplemented with 0.5mg/1NAA and BAP showed 100% germination and well developed plantlets were obtained within 60 days.

The protocorms encapsulated in

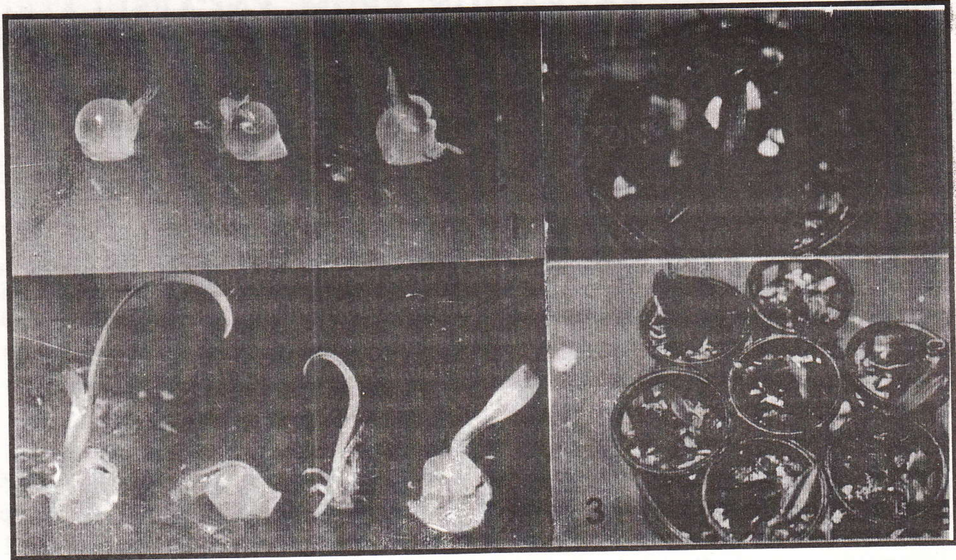

Fig. 1-3 *COELOGYNE ODORATISSIMA* VAR. *ANGUSTIFOLIA* LINDL.

Fig. 1. Protocorms encapsulated with 3% alginate in 2% sucrose.

Fig. 2 Protocorms encapsulated with 3% alginate in 9% sucrose.

Fig. 3 (A&B). Seedling showing 100% survival in potting medium containing Brick pieces : Vermiculiate : Charcoal : Drymoss in the ratio of 1:1:1:1

Table 1. Effect of Sucrose on the germination of the encapsulated protocorms.

Sl. No.	Concentration of Sucrose(%)	Germination % (after development of first leaf)	Seedling height (shoot & root) (cm) mean ±S.E.*
1	2	92	0.86 ± 0.12
2.	4	100	1.08 ± 0.16
3.	6	100	1.40 ± 0.17
4.	9	100	1.56 ± 0.17

Observation made after 45 days

* Values are means (n=10) ± S.E.

different concentration of sucrose were cultured on MS basal medium. Irrespective of the concentration of sucrose the protocorms germinated, but the growth and emergence of first leaf was very slow in 2% sucrose (Fig. 1). The maximum shoot length (1.56cm) was observed in the protocorms encapsulated in 9% sucrose (Fig. 2 & Table 1).

Encapsulated protocorms stored for 60 days at 25±1° C in petriplate differentiated into complete plantlet within 15 days (100%) on MS medium. Whereas there was a gradual reduction (88%) in the conversion in the protocorms stored at 7°C. The seedling obtained from the encapsulated protocorms were transferred to the potting medium containing Brick pieces, vermiculate, charcoal and dry moss in the ratio of 1:1:1:1: at 80% RH the plantlets showed 100% survival (Fig.3A&B).

In the present study protocorms obtained via seed culture were encapsulated in alginate, MS basal medium and also with various concentration of sucrose. Protocorms encapsulated with 3% alginate was found to be suitable, whereas 4% was found to be effective in *Cymbidium giganteum*[4] and *Phaius tankervilliae*[3]. The encapsulated protocorm in *Coelogyne odoratisima* var. *angustifolia* required an average of 15-20 and 40-60 days for shoot and root emergence on the contrary in *P.tankervilliae* took 14-35 and 95-125 days for shoot and root emergence[3].

Drew[5] found that sucrose was essential for carrot somatic embryo germination and the development of plantlets. In the present study all the concentration of sucrose tried induced germination (92-100%). However the growth rate (shoot and root formation) was found to vary with different concentration (Table 1). Encapsulated protocorms stored at 25±1°C showed 100% germination whereas regeneration frequency of encapsulated protocorms was reduced when stored at low temperature. Similar response was also observed in *Phaius tankervilliae*[3].The encapsulated protocorm may be useful for easier handling, transporting and *in vitro* conservation of orchid germplasm.

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