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Original Article

Cryopreservation of PLBs of *Brassidium* Fly Away Using Encapsulation-Dehydration Technique

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Abstract

Key words: Encapsulation-dehydration, PLBs, cryopreservation

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In vitro grown protocorm-like bodies (PLBs) of *Brassidium* Fly Away orchid hybrid were cryopreserved using encapsulation- dehydration technique. The viability of the cryopreserved cells was determined by 2,3,5-triphenyltetrazolium chloride (TTC) assay. For the preculture treatment, the PLBs were excised into two standard sizes of 1-2 and 4-5 mm and were precultured on half-strength Murashige and Skoog (MS) semi solid medium supplemented with different concentrations of sucrose (0, 0.2, 0.4, 0.6, 0.8 and 1.0M). The PLBs size 4-5 mm and 0.6 M sucrose concentration was selected based on highest viability obtained in TTC assay. The PLBs were encapsulated for 30 minutes using 3% (w/v) liquid sodium alginate medium supplemented with 0.4M sucrose and 0.1M calcium chloride and osmoprotected in 0.75M sucrose solution for 24 hours at 25°C. The beads were then dehydrated using 50g heat-sterilised silica gel for four hours, cryopreserved for 24 hours, thawed in a 40±2°C water bath for 90 seconds, and regenerated in semi-solid half-strength. Biochemical analyses were conducted and the cryopreserved PLBs had produced lower content of chlorophyll while the highest specific peroxidase activity was observed in cryopreserved PLBs.

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Introduction

The Orchidaceae is one of the largest families of flowering plants and become popular in floricultural industry because of their colours, shapes, sizes, and bloom persistence (Yu & Xu, 2007). The growing demands for orchid cut flowers act as a boost to the various breeding programmes of orchids. Orchids are threated because of the unstoppable harvesting of wild type orchids, which harm the existence of the wild species of orchids. The genetic resources of ornamental plants, especially orchids are required to be stored due to their increasing of extinction.

Cryopreservation is an alternative or a duplicate storage for the traditional *in situ* and *ex situ* germplasm conservation (Engelmann *et al*., 2000). The successful cryopreservation of biological tissues can be achieved by avoiding the intracellular ice crystal formation due to an irreversible damage to cell membranes will occured and thus destroying their semi-permeability (Panis *et al*., 2005). Cryogenic technique such as vitrification, encapsulation-dehydration and encapsulation-vitrification has been developed and the number of species or