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CRYOPRESERVATION OF *Dendrobium* Shavin White USING SUCROSE PRECULTURE - ENCAPSULATION VITRIFICATION METHOD

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BY

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CERTIFICATION

This project report entitled 'CRYOPRESERVATION OF *Dendrobium* Shavin White USING SUCROSE PRECULTURE - ENCAPSULATION VITRIFICATION METHOD' is prepared by Pugalanthi Nagappan and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticltural Science.

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LIST OF ABBREVIATIONS

CRD: Complete Randomized Design

ES: PLBs were encapsulated with alginate matrix before pre treated with sucrose

LN: Liquid Nitrogen

LS1: Loading Solution 1

MS: Murashige and Skoog

PLB: Protocorm like bodies

PVS: Plant Vitrification Solution

RCBD: Randomized Complete Block Design

SE: PLBs pre treated with sucrose prior to encapsulation

ABSTRACT

Dendrobium Shavin White is a hybrid from the crossing between *Dendrobium* Walter Oumae and *Dendrobium* Queen Florist which has vigorous growth and durable. *Dendrobium* Shavin White produces flowers all year around with high number of flowers in a single inflorescence. The bloom is usually greenish white. PLB, the somatic embryo of orchid is one of the tissues with high ability to become plantlets when cultured. Growth and development of PLBs cannot be arrested due to active cellular divisions. Therefore a suitable protocol to preserve the PLBs is required for efficient handling of the planting material. This experiment is conducted to determine the survival of naked PLB exposure to different concentrations of sucrose, followed by exposure to PVS2 after encapsulation upon cryopreservation. It is also conducted to determine the survival of encapsulated PLB exposure to different concentrations of sucrose, followed by exposure to PVS2 upon cryopreservation. Prior to cryopreservation of PLBs, viability test was carried out. The experimental design used was CRD with 4 replications. Data collected were percentages of PLBs regeneration and colour of PLBs. Data show that, the highest survival percentage of PLBs in LN was recorded by encapsulated PLB pre treated with 0.2 M sucrose concentration in 15 minutes soaking of PVS2 (31.25%).

ABSTRAK

Dendrobium Shavin White adalah sejenis orkid kacukan Dendrobium Walter Oumae dan Dendrobium Queen Florist yang mempunyai pertumbuhan yang memberangsangkan dan tahan lama. Dendrobium Shavin White boleh mengeluarkan bunga setiap tahun dalam kuantiti yang banyak dalam satu kuntum. Orkid yang mekar berwarna hijau keputihan. PLB, adalah sejenis tisu orkid iaitu somatik embrio yang boleh menjadi sebuah anak pokok apabila di kultur. Pertumbuhan dan perkembangan PLBs tidak boleh dikawal kerana pembahagian cellularnya yang aktif. Oleh itu, protokol yang sesuai untuk menyimpan PLBs di perlukan untuk pengendalian yang lebih efektif dalam penanaman. Justeru itu, objektif experimen ini adalah untuk mengkaji kehidupan PLB yang didedahkan kepada pelbagai kepekatan sukrosa dan di ikuti dengan pendedahan dalam PVS2 selepas di selaput dan akhirnya proses krioawetan. Experimen kedua bertujuan untuk mengkaji kehidupan PLB yang diselaput dan didedahkan kepada pelbagai kepekatan sukrosa dan di ikuti dengan pendedahan dalam PVS2 dan akhirnya proses krioawetan. Untuk mengetahui pengaruh setiap prosedur, ujian percambahan dijalankan selepas pendedahan kepada sukrosa, selepas pendedahan kepada sukrosa dan PVS2 dan akhirnya di ikuti pendedahan kepada LN. Reka bentuk eksperimen yang digunakan adalah CRD dengan 4 replikasi. Data yang dikumpul ialah peratus percambahan PLB dan warna PLB. Data menunjukan, peratus kehidupan PLB yang paling tinggi dalam LN ialah PLB yang diselaput dan di dedahkan dalam kepekatan sukrosa 0.2M dan 15 minit rendaman PVS2 (31.25%).

CHAPTER 1

INTRODUCTION

Orchidaceae is one of the largest families of flowering plants, consisting more than 800 genera and approximately 26,000 species (Ersan *et al.,* 2013).Orchid is well known for its fascinating beauty and great diversity in size, colour, shape and fragrance. In horticulture, orchids have vast importance both as potted plants and cut flowers (Khoddamzadeh *et al.,* 2011). Some of the more popular orchids belong to the genera *Phalaenopsis*, *Dendrobium* and *Aranda*. *Dendrobium* is the second largest genus which consists of more than 1,100 species worldwide, and its major distribution regions range from Southeast Asia to New Guinea and Australia (Puchooa 2004; Xu *et al*., 2006). Today numerous hybrids are being produced with unique characteristics.

One of the most popular orchid genera being traded in the world is *Aranda* with *Dendrobium* spp. following relatively close. In Thailand and Malaysia, *Dendrobium* is important which 80% total cut-flower production is from Thailand, and 40% from Malaysia (Ching *et al*., 2012). One of the commercially grown *Dendrobium* hybrids is *Dendrobium* Shavin White, extensively multiplied via tissue culture.

Through tissue culture procedures, the plant cells are able to regenerate whole plants in relatively short period of time. Virus-free plants are produced through meristem

culture and other tissue culture techniques based on the principle of totipotency (Purohit, 2005).

Plant tissue culture can be initiated from almost any part of a plant. Orchid tissue culture can be initiated using leaf segments and produce protocorm-like bodies (PLBs). PLBs can be isolated and subcultured for further multiplication or regenerated into new plants (Park *et al*., 2000).

(PLB's), the somatic embryos of orchids are highly regenerable propagules (Teixeira da Silva and Tanaka, 2006). PLBs continue to grow while in culture forming new PLBs or convert into plants. Hence frequent subculture is normally required, whereby PLBs converting to plantlets have to be transferred to appropriate media while others into multiplication media. This normal protocol maintains the quality of PLBs as prolonged subculture durations can lead to accumulation of phenolic compounds and cause stunted growth of PLBs. The method results in wastage, both in PLBs as well as time and media for maintenance of the PLBs. Hence, establishment of a method for storage of PLBs can be useful in managing the resources more efficiently and production of PLBs only when required. A number of studies on attempts to store orchid PLBs have been reported. PLBs are highly hydrated making it difficult to store. A study by Bustam *et al*., (2012) on *Dendrobium* Shavin White PLBs was reported to store naked PLBs for 135 days at 4°C with minimal loss in viability. Although this is very encouraging, it is only a short term measure.

According to Hirano *et al.* (2004), an important tool for long term storage of plant germplasm is cryopreservation. Cryopreservation is an effective protocol where vegetatively propagated materials such as PLBs are stored using liquid nitrogen (LN) (Engelmann, 2000).This method can be used to store and arrest the growth of PLB's by exposure to LN until the explants are needed. In LN with temperature of -196°C, all cellular divisions and metabolic processes are stopped or minimized. Thus the plant materials can be stored without modification for a theoretically unlimited period of time (Engelmann, 2006).

Preservation of diverse species of plants have been developed using various techniques such as simple freezing, vitrification, encapculation-vitrification (Xue *et al*.2008) and encapsulation-dehydration (Engelmann, 2000). Among these diverse techniques, a method that is suitable for PLBs is the encapsulation-vitrification developed by Tanoury *et al*. (1991) due to the advantages of combination between vitrification and encapsulation dehydration.

For a successful cryopreservation the most important step is pretreatment of tissues and organs, for acquisition of dehydration and freezing tolerance (Sugawara *et al*., 2005) during exposure in liquid nitrogen. Therefore, cryoprotectants such as sucrose (Tsukazaki *et al*., 2000) and concoction of chemicals termed as loading solutions (Sakai and Engelmann, 2007) are used to treat or preculture the propagules.An optimum condition for the PLBs in the pretreatment concentration and duration need to be identified before LN exposure (Ching *et al*., 2012) because unsuitable concentration of

sucrose can cause harmful effect by causing tissue blackening and retard the proliferation of PLB's (Panis *et al*., 1996).

The use of PLBs directly for storage is the easiest but may not be appropriate because direct exposure to toxic chemicals can cause death to the cells. Protection in the form of encapsulation is recommended to reduce chemical toxicity of vitrification solution and prevent death of cells (Wang *et al*., 2002). Thus, a better method for both propagation and short term to mid-term storage is through synthetic seed production technology via alginate-encapsulation for a number of commercially important orchids (Mohanraj *et al*., 2009). Synthetic seeds production also makes it easy to handle during storage and transportation.

This study was carried out with the main objective of establishing a protocol for storage of *Dendrobium* Shavin White orchid PLB using cryopreservation technique.

The specific objectives of this study are:

I) To determine the survival of naked PLB, exposed to different concentrations of sucrose, followed by exposure to PVS2 after encapsulation prior to cryopreservation.

II) To determine the survival of encapsulated PLB exposed to different concentrations of sucrose, followed by exposure to PVS2 prior to cryopreservation.

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