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MORPHOLOGICAL DEVELOPMENT, IN VITRO STORAGE, AND REGENERATION OF PROTOCORM-LIKE BODIES OF ORCHID HYBRID Dendrobium Shavin White

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By

SURYANTI BUSTAM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of Master of Science

MORPHOLOGICAL DEVELOPMENT, *IN VITRO* STORAGE, AND REGENERATION OF PROTOCORM-LIKE BODIES OF ORCHID HYBRID *Dendrobium* Shavin White

By

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May 2013

Chairman : Associate Professor Uma Rani Sinniah, PhD

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This study was carried out to establish a method for storage of orchid protocorm-like bodies (PLBs) in order to improve the handling and transport of the propagules. Identifying the developmental stages of PLBs is an important criteria in ensuring increased plantlet regeneration potential while the ability to store PLBs allows the accumulation of large number of PLBs which can facilitate the production of uniform plants for commercial planting.

Initially, observation on growth and development of PLBs were made using stereomicroscope and variable pressure scanning electron microscopy (VPSEM) to identify the time taken for growth and development to occur. The study revealed that secondary PLBs were formed from single isolated PLBs after 4 – 7 weeks of culture

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upon the initiation of embryos after 2 - 3 weeks of culture. Shoots were found to be initiated from newly formed PLBs after 5 – 7 weeks of culture. This was followed by selection of the best PLBs developmental stage as at one time PLBs varying in size and maturity stage can be obtained. The PLBs were categorized into five based on size and presence or absence of shoot namely ≤ 2 mm (S1), >2 – 4 mm (S2), >4 – 6 mm (S3), >2 – 4 mm with shoot (S4) and >4 – 6 mm with shoot (S5). Results from this experiment showed that PLBs with shoot (S4 and S5) gave significantly higher conversion percentage (85% and 90% respectively) as compared to the PLBs without shoot (S1, S2 and S3) when cultured on semi-solid ½ MS basal medium devoid of plant growth regulators irrespective of size.

In the second part of the study naked or encapsulated PLBs were kept in air tight container stored in darkness at different temperatures namely 5°C, 10°C, 25°C ± 2 and 30°C ± 2. Naked PLBs can be efficiently stored at 25°C ± 2 where high viability percentages (88 – 100%) were obtained even after 135 days of storage. Meanwhile encapsulated PLBs also showed the best response when stored at 25°C ± 2. At 25°C ± 2, encapsulated PLBs stored for 75 days retained 80 – 92% conversion while 52% was obtained after 135 days of storage.

Subsequently, the feasibility of germinating synthetic seeds of *Dendrobium* Shavin White in different substrates was tested. The different substrates include M1 (semi-solid ½ MS basal medium), M2 (cotton irrigated with sterilized liquid ½ MS basal medium), M3 (cotton irrigated with sterilized distilled water) and M4 (cotton irrigated with non-sterilized distilled water). The encapsulated PLBs regenerated well in M1 where 96% of

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encapsulated PLBs germinated and 76% of them converted into plantlets. Results from this study revealed that although optimum results were recorded by inoculating encapsulated PLBs in M1, they can also be regenerated using M2 or M3 with 72% and 56% germination and 64% and 44% conversion respectively.

The present study established that selection of PLBs is important as it ensures rapid conversion into plantlet. PLBs varying in size ranging for 2 – 6 mm can be used provided the shoot initials are present. Suitable PLBs can be effectively stored at 25°C ± 2 for 135 days in the naked form in air tight container, retaining 80 – 92% of PLBs converted into plantlet or in the encapsulated form with 52%.

In addition this study showed that encapsulated PLBs can technically function as normal seeds in relation to germination whereby encapsulated PLBs can germinate with the supply of sterilized distilled water.

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PERKEMBANGAN MORFOLOGI, PENYIMPANAN SECARA *IN VITRO,* DAN PERTUMBUHAN JASAD MENYERUPAI PROTOKOM BAGI ORKID HIBRID *Dendrobium* Shavin White

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Kajian ini dilakukan untuk membentuk kaedah penyimpanan jasad menyerupai protokom (PLBs) orkid bagi mempertingkat kaedah pengurusan dan pemindahan bahan. Pengenalpastian peringkat perkembangan PLBs merupkan kriteria penting bagi memastikan potensi pertumbuhan anak pokok yang tinggi manakala keupayaan untuk menyimpan PLBs akan membolehkan PLBs dikumpulkan dalam kuantiti yang banyak bagi membantu pengeluaran tumbuhan yang seragam untuk tujuan komersial.

Pada awalnya, pemerhatian terhadap pertumbuhan dan perkembangan PLBs telah dibuat menggunakan mikroskop stereo dan mikroskop pengimbasan elektron tekanan berubah-ubah (VPSEM). Kajian ini mendedahkan bahawa PLBs sekunder terbentuk dari PLBs tunggal selepas 4 - 7 minggu dikultur iaitu selepas pembentukan embrio pada 2 - 3

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minggu selepas dikultur. Pucuk terbentuk daripada PLBs sekunder selepas 5 - 7 minggu dikultur. Seterusnya diikuti dengan pemilihan peringkat PLBs yang terbaik disebabkan wujudnya PLBs pelbagai saiz dan peringkat kematangan yang ada dalam satu-satu masa. PLBs dikategorikan kepada lima berdasarkan saiz dan kewujudan pucuk iaitu ≤ 2 mm (S1), >2 - 4 mm (S2), >4 - 6 mm (S3), >2 - 4 mm dengan pucuk (S4) dan >4 - 6 mm dengan pucuk (S5). Keputusan daripada eksperimen ini menunjukkan bahawa PLBs dengan pucuk (S4 dan S5) memberikan peratusan pembentukan anak pokok yang jauh lebih tinggi (85% dan 90% setiap satu) berbanding dengan PLBs tanpa pucuk (S1, S2 dan S3) apabila dikultur pada media asas MS separa pejal berkepekatan separa tanpa dibekalkan dengan hormon pertumbuhan tumbuhan, tanpa berdasarkan saiz.

Dalam bahagian kedua kajian, PLBs yang tidak dikapsul dan yang telah dikapsul dimasukkan ke dalam bekas kedap udara dan disimpan dalam keadaan gelap pada suhu yang berbeza iaitu 5°C, 10°C, 25°C ± 2 dan 30°C ± 2. PLBs tanpa kapsul boleh disimpan secara efisyen pada 25°C ± 2 di mana peratusan kebernasan yang tinggi (88 - 100%) telah diperoleh walaupun disimpan selama 135 hari. Selain itu, PLBs yang dikapsul juga menghasilkan keputusan terbaik apabila disimpan pada 25°C ± 2. Pada 25°C ± 2, 80 – 92% PLBs yang dikapsul yang disimpan selama 75 berupaya untuk membentuk anak pokok manakala 52% pula diperoleh selepas disimpan selama 135 hari.

Seterusnya, kemungkinan percambahan biji benih sintetik *Dendrobium* Shavin White di substrat yang berbeza telah diuji termasuk M1 (media asas MS separa pejal berkepekatan separa), M2 (kapas dibasahkan dengan media asas MS cair berkepekatan

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separa yang steril), M3 (kapas yang dibasahkan dengan air suling yang steril) dan M4 (kapas yang dibasahkan dengan air suling yang tidak steril). PLBs yang telah dikapsul bertumbuh semula dengan baik dalam M1 di mana 96% daripada PLBs yang telah dikapsul bercambah dan 76% daripadanya membentuk anak pokok. Keputusan daripada kajian ini mendedahkan bahawa walaupun keputusan yang optimum direkodkan oleh PLBs di dalam M1, ianya juga boleh bertumbuh semula menggunakan M2 atau M3 dengan kadar percambahan adalah 72% dan 56% dan kadar pembentukan anak pokok adalah 64% dan 44% bagi setiap satu.

Kajian ini membuktikan bahawa pemilihan PLBs adalah penting kerana ia memastikan pembentukan anak pokok dengan cepat. PLBs dengan saiz antara 2 – 6 mm boleh digunakan sekiranya ia mempunyai pucuk awal. PLBs yang tidak dikapsul boleh disimpan secara efektif pada 25°C ± 2 selama 135 hari di dalam bekas kedap udara, mengekalkan 80 – 92% daripada PLBs membentuk anak pokok ataupun yang telah dikapsul dengan 52%.

Selain itu, kajian ini menunjukkan bahawa PLBs yang dikapsul boleh berfungsi secara teknikal seperti biji benih biasa berkenaan percambahan di mana PLBs yang dikapsul berupaya untuk bercambah apabila dibekalkan dengan air suling yang steril.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been dully acknowledged. I also declare that it has not been previously, and concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

SURYANTI BINTI BUSTAM

Date: 3 May 2013

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TABLE OF CONTENTS

© COPYRIGHT UPM

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LIST OF TABLES

© COPYRIGHT UPM

LIST OF FIGURES

© COPYRIGHT UPM

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LIST OF ABBREVIATIONS

*	Significant at P<0.05
**	Significant at P<0.01
ANOVA	Analysis of variance
CRD	Completely Randomized Design
DMRT	Duncan's Multiple Range Test
h	hour
MS media	Murashige and Skoog media
mM	milimolar
ns	Non-significant
PLBs	Protocorm-like bodies
rpm	Rotary per minute
SAS	Statistical Analyses System Software
VPSEM	Variable pressure scanning electron microscopy
w/v	Weight over volume

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CHAPTER 1

INTRODUCTION

Orchids are one of the largest flowering plant families among the higher flowering plant with 700 to 800 described genera and 22,000 to 35,000 species (Fadelah et al., 2001). Orchids have been popularly grown as ornamental plant for its exotic beauty and also the long shelf life of the flowers compared to many other flowers. The orchid industry is highly potential as to date, the international business covers around 8% of world floriculture trade (Chugh et al., 2009).

The orchid industry plays an important role in Malaysian economy. Malaysia is currently ranked third in the world ranking as orchid-producing country behind Thailand and Singapore. The export value of orchids in Malaysia during 2009 is about RM46 million (Ho, 2010).

Dendrobium is one of the popular orchid genus because it can flower all year round with high number of flowers in a single inflorescence (Martin and Madassery, 2006). One of them is *Dendrobium* Shavin White a hybrid between *Dendrobium* Walter Oumae x *Dendrobium* Queen Florist which is known to be a vigorous and durable orchid (Fadelah et al., 2001).

In order to sustain the industry, production of high quality planting materials is important. There are several methods of propagation, namely natural seed propagation,

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aseptic seed propagation, conventional vegetative propagation and micropropagation. Seed propagation in nature has not been successful due to heterozygosity of seeds and also low germination rate due to reduced endosperm in the seeds which require mycorrhizal fungi for germination (Saiprasad, 2001). Hence, aseptic seed propagation has been practiced as a means of orchid propagation. But, seedlings are not able to retain the desired characters of the mother plant completely because orchids are out breeders (Mohanraj et al., 2009). On the other hand, propagation through conventional vegetative techniques are time consuming, limited by mother plant size and require high cost per plantlet (Saiprasad, 2001)

Today, micropropagation is preferred for commercial propagation of all types of orchids as they have the advantage of producing true to type as well as virus-free plants. Micropropagation is a mass propagation method which has potential to produce many plants within a short period of time (Chugh et al., 2009). Micropropagation through initiation of protocorm-like bodies (PLBs) is considered as an efficient method because it can be rapidly proliferated to produce large number of PLBs in a short period of time where each of them can be regenerated to produce a new plant (Sheelavanthmath et al., 2005).

A single PLBs, when placed in an appropriate multiplication medium will induce the production of secondary PLBs resulting in a clump consisting of PLBs at different developmental stages. Upon transfer into conversion medium, PLBs at the right stage of development and maturity will convert into single plantlet forming root and shoot while pre mature PLBs at the early stage of development will either give rise to small multiple

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shoot or proliferate new secondary PLBs. Therefore, selecting PLBs at the right stage for conversion is important in order to produce good and healthy plantlets. PLBs maturity has been related to size with large PLBs (3 – 4 mm) designated as mature and is said to perform better as compared to small PLBs (1 – 2 mm) (Antony et al., 2010). The formation of first true leaf also could be an indicator of maturity (Chung et al., 2007).

The ability to identify propagules of high quality can be highly advantageous to the industry as this will relate to the production of uniform plants with synchronized flowering. One of the drawbacks in micropropagation of orchids is the asynchronous growth and the inability to arrest the PLBs growth requiring continuous subculture. This is due to the lack of quiescence in the PLBs growth (Ara et al., 2000). Hence, PLBs at the right stage need to be arrested through storage and subjected to growth once needed. This will allow better management of the micropropagation system, reduce wastage and allow the production of batches of uniform plants. PLBs can be stored in the naked form or in the form of synthetic seeds produced via encapsulation of the PLBs. Ability to store is often related to placing the propagules at low temperature as this condition often reduces the metabolic rate thus prolonging the shelf life. However, this is not an universal phenomenon, as Gantait et al. (2012) reported that encapsulated shoot tips of *Mokara* orchid stored better at 25°C. In addition, the response to low temperature can differ significantly due to increament or reduction of a few degree celcius in temperature. Hence the conditions required for storage has to be determined for different types of materials.

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Synthetic seed is defined as artificially encapsulated tissue that can be used for sowing as a natural seed which possess the ability to convert into a plant under *in-vitro* or *ex-vitro* conditions and it can retain this potential also after storage (Redenbaugh et al., 1993). Since the encapsulated PLBs has the potential to be germinated under *ex-vitro* condition, establishment of an easy method for regeneration of encapsulated PLBs would greatly enhance the propagation system by eliminating regeneration using the tissue culture procedure.

Generally, PLBs are kept in a culture flask in order to have stock of planting materials. In this condition, the PLBs growth cannot be stopped resulting in the requirement of frequent subculture. In order to provide frequent subculture, high labor cost is required. In addition, the increase in frequency of subculture can induce somaclonal variation and also reduce the quality of the PLBs (Minoo et al., 2006). Given the importance of orchid PLBs as planting materials and establishment of an easy storing method, this study was undertaken with the following objectives:

1. To identify the optimum PLBs stage for rapid and efficient conversion into plantlet.
2. To study the storage ability of naked and encapsulated PLBs under various temperatures.
3. To determine the regeneration ability of encapsulated PLBs for germination and conversion into complete plantlet when inoculated on different substrates.

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