UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF SHORT TERM AND LONG TERM STORAGE PROTOCOLS FOR OIL PALM (*Elaeis guineensis* Jacq.) POLYEMBRYOIDS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment 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 fulfilment of the requirement for the degree of Master of Science

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March 2013

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In vitro culture of oil palm (*Elaeis guineensis* Jacq.) from suspension culture into plantlet is a time consuming process which has been reported to be highly heterogeneous resulting in embryoids differing in sizes, colours and developmental stages. The ability to select embroids at the right stage of development allows the selection of high quality materials. Due to fact that conversion of embryoids is a continuous process, the establishment of suitable storage method is necessary for the selected material. Thus, this study focus on establishment of short term and long term storage protocols for oil palm polyembryoids. In order to establish a successful storage technique, it is essential to identify the right stage of embryoids prior to plantlet formation which will aid in simultaneous plantlet production whereby it can be stored adequately and recommenced for growth only when needed. In the first experiment, the micromorphological changes during conversion of suspension culture into polyembryoids were observed. Morphological observations revealed

that, matured polyembryoids with the presence of globular structures, haustorium and secondary somatic embryo (SSE) as the appropriate stage for rapid conversion into plantlets hence it was chosen for storage. In the second experiment, determination of suitable gelling agent was conducted using two types of gelling agent with different concentration (Agar Type 900 at 8g/L, 10g/L and 12g/L or Gelrite® at 1.5g/L, 2.5g/L and 3.5g/L) for better conversion of selected polyembryoids. Gelrite® with 3.5g/L concentration was chosen as the effective gelling agent for higher conversion of polyembryoids into plantlets.

Prior to storage, the samples were pretreated with sucrose to condition the polyembryoids for better adaptation to storage condition. Thus, in the third experiment, the effect of encapsulation and gradual sucrose preculture on polyembryoids was undertaken. Results show that exposure of naked polyembryoids to sucrose preculture led to lethal damage while the encapsulated polyembryoids were able to withstand the treatment and gave better survival. Hence, for both short term and long term storage study, encapsulated and sucrose precultured polyembryoids were used. In the fourth experiment, method for short term storage was established by manipulating storage temperature whereby the samples were stored at 5°C, 10°C and 25°C. In this study, the highest survival (73.3%) was obtained at 5°C after 60 days of storage. The results showed that storage in higher temperature reduced the survival rate due to increasing metabolic activity that denatured the proteins.

In the fifth experiment, the encapsulated polyembryoids were subjected to long term storage through cryopreservation method after gradual sucrose preculture and Laminar Air Flow (LAF) desiccation using encapsulation dehydration technique. In this technique, encapsulated materials were subjected to cryopreservation after desiccation in order to avoid freezing injury upon liquid nitrogen (LN) exposure. Based on the results obtained, 73.3% survival percentage was obtained with 23.3% water content for encapsulated polyembryoids that subjected to gradual sucrose preculture and cryopreservation. The experiments showed that, oil palm polyembryoids acquired tolerance for successful storage either short term or long term.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBENTUKAN PROTOKOL PENYIMPANAN UNTUK JANGKA MASA PENDEK DAN JANGKA MASA PANJANG BAGI POLIEMBRYOIDS KELAPA SAWIT (*Elaeis guineensis* Jacq.)

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Penghasilan anak pokok daripada kultur *in vitro* kelapa sawit (*Elaeis guineensis* Jacq.) menggunakan kultur suspension merupakan proses yang memerlukan tempoh masa yang lama. Pertumbuhan embryoids yang terlibat sebelum penghasilan anak pokok telah dilaporkan tidak seragam sehingga menyebabkan perbezaan yang ketara dari segi saiz, warna dan peringkat pertumbuhan. Keupayaan memilih embryoids pada peringkat pertumbuhan yang tepat boleh memastikan pemilihan bahan yang berkualiti tinggi. Disebabkan proses penukaran embryoid yang berterusan, pembentukan kaedah penyimpanan yang sesuai amatlah diperlukan untuk menyimpannya. Oleh itu, kajian ini memberi tumpuan kepada pembentukan protokol penyimpanan jangka pendek dan jangka panjang bagi poliembryoid kelapa sawit. Dalam usaha membentuk teknik penyimpanan yang sesuai, untuk mengenalpasti peringkat pertumbuhan yang tepat bagi pembentukan anak pokok yang cepat adalah

sangat penting. Ini membolehkan pengeluaran anak pokok yang seragam di mana ia boleh disimpan dan dirangsangkan untuk pertumbuhan semula apabila diperlukan. Dalam eksperimen pertama, pemerhatian telah dibuat terhadap perubahan mikro morfologi semasa pertukaran kultur suspension kepada poliembryoid. Pemerhatian morfologi mendedahkan bahawa poliembryoids matang dengan kehadiran struktur globular, haustorium dan somatik embrio sekunder (SSE) adalah peringkat yang paling tepat untuk memastikan pertumbuhan anak pokok yang cepat jadi embryoid ini telah dipilih untuk disimpan. Eksperimen kedua telah dijalankan bagi menentukan agen pembentuk gel yang sesuai dengan menggunakan dua jenis agen pembentuk gel dengan kepekatan yang berlainan (8 g/L,10 g/L dan 12 g/L Agar Jenis 900 atau 1.5 g/L, 2.5 g/L dan 3.5 g/L Gelrite®) bagi memastikan pertumbuhan anak pokok yang lebih baik daripada poliembryoids yang terpilih. Gelrite® pada kepekatan 3.5g/L dipilih sebagai agen pembentuk gel yang sesuai untuk pertumbuhan yang lebih baik daripada poliembryoid kepada anak pokok.

Sebelum penyimpanan, sampel telah dirawat terlebih dahulu dengan sukrosa bagi menyesuaikan polyembryoids kepada keadaan penyimpanan. Oleh itu, dalam eksperimen ketiga, kesan pengkapsulan dan rawatan sukrosa secara berterusan terhadap poliembryoid kelapa sawit telah dijalankan. Kajian menunjukkan bahawa apabila polyembryoids yang tidak dikapsul didedahkan kepada rawatan sukrosa ia menyebabkan kematian. Manakala, poliembryoid yang dikapsulkan dapat menahan rawatan sukrosa dan berkebolehan untuk terus hidup lebih baik berbanding poliembryoid yang tidak dikapsul. Oleh itu, bagi kajian penyimpanan jangka masa pendek dan jangka masa panjang, poliembryoid yang dikapsul dan dirawat dengan sukrosa telah digunakan. Dalam eksperimen keempat, kaedah penyimpanan untuk

jangka masa pendek telah dilakukan dengan memanipulasikan suhu di mana sampel disimpan pada suhu 5°C, 10°C dan 25°C. Dalam kajian ini, kebolehan poliembryoid untuk terus hidup yang tertinggi (73.3%) telah diperolehi pada suhu 5°C selepas disimpan selama 60 hari. Hasil kajian menunjukkan bahawa penyimpanan sampel pada suhu yang lebih tinggi mengurangkan kadar kebolehan untuk terus hidup disebabkan peningkatan aktiviti metabolik yang menguraikan protein lebih awal.

Dalam eksperimen kelima, poliembryoid yang dikapsulkan disimpan untuk jangka masa panjang melalui krioawetan selepas rawatan sukrosa secara berterusan dan pengeringan dengan kabinet Aliran Udara lamina (LAF). Ia dikenal sebagai teknik dehidrasi pengkapsulan. Dalam teknik ini, bahan yang dikapsulkan didedahkan dengan krioawetan selepas pengeringan bagi mengelakkan kecederaan pembekuan yang disebabkan oleh pendedahan tehadap cecair nitrogen (LN). Berdasarkan keputusan kajian, 73.3% kebolehan poliembryoid untuk terus hidup telah diperolehi apabila kandungan air poliembryoid yang dikapsulkan adalah sebanyak 23.3% setelah dirawat dengan sukrosa secara berterusan dan dikrioawet. Kajian yang dijalankan menunjukkan bahawa, poliembryoid kelapa sawit memerlukan daya ketahanan yang cukup untuk penyimpanan sama ada jangka masa pendek atau jangka masa panjang.

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I certify that a Thesis Examination Committee has met on 14 March 2013 conduct the final examination of Sharrmila Rengeswari a/p Palanyandy on her thesis entitled "Establishment of Short Term and Long Term Storage Protocols for Oil Palm (*Elaeis guineensis* Jacq.) Polyembryoids" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

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

SHARRMILA RENGESWARI PALANYANDY

Date: 14 March 2013

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