UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF POTENTIAL MARKERS FOR PROLIFIC EXPLANT TISSUES OF OIL PALM (*Elaeis guineensis* Jacq.)

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ISOLATION AND CHARACTERIZATION OF POTENTIAL MARKERS FOR PROLIFIC EXPLANT TISSUES OF OIL PALM (*Elaeis guineensis* Jacq.)

By

NORASHIKIN SARPAN

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

August 2011

Dedicated to my family

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

ISOLATION AND CHARACTERIZATION OF POTENTIAL MARKERS FOR PROLIFIC EXPLANT TISSUES OF OIL PALM (*Elaeis guineensis* Jacq,)

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August 2011

Chairman : Parameswari Namasivayam, PhD

Faculty : Biotechnology and Biomolecular Sciences

Tissue culture is a promising technology for the mass propagation of elite oil palm. Although this approach has been widely used in the local oil palm plantation companies, the callogenesis and embryogenesis rates remain low. Therefore, the application of markers for early diagnosis of embryogenic potential would help in reducing the cost for tissue culture of oil palm. The first part of this study was aimed to identify DNA markers for oil palm prolific explant tissues via representational difference analysis (RDA), a technique whereby the differences between two highly related genomes can be identified. In this part, a total of five forward and reverse RDA were performed using the explant tissues possessing different proliferation ability; measurement was based on the previous tissue culture data of respective oil palm tissue culture agencies. Among the difference products identified, 13:14 C58 which was putatively enriched in non-prolific

tissues, possesses a few nucleotide differences when aligned with the equivalent DNA region of the highly prolific tissues. However, further verification is needed to confirm its ability as a marker for distinguishing the non-prolific explant tissues from the highly prolific explant tissues. The second part of this study was aimed to characterize one of the previously identified putative embryogenic markers known as Eg707 (FJ196136) using a technique called *in situ* hybridization. The gene expression of Eg707 was detected in all the tested tissues committed to embryogenic pathway as early as in embryogenic callus up to the germinating embryo. Since the formation of proembryo in embryogenic callus is regarded as the first key factor in oil palm somatic embryo development, Eg707 could be used as a potential molecular marker for early detection of oil palm somatic embryogenesis. The third part of this study was aimed to validate the efficiency of previously identified promising candidate embryogenic markers. Expression profile of five oil palm embryogenic-related genes were generated in this study using real time PCR with mRNA from oil palm leaf explant tissues that exhibit different proliferation ability in tissue culture. Two of the transcripts possess a higher potential to be used as a marker as early at the leaf explant stage of the oil palm tissue culture process. The two transcripts were grouped into two categories based on their expression profiles of either continuous or time-dependent. However, the expression profile of each gene did not correlate very well across samples possessing similar proliferation ability from various agencies. Thus, more oil palm genotypes should be analyzed to obtain a more robust selection of the markers for screening of oil palm leaf explants within a particular agency.

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

PEMENCILAN DAN PENCIRIAN PENANDA YANG BERPOTENSI BAGI TISU EKSPLAN PROLIFIK KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

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Ogos 2011

Pengerusi : Parameswari Namasivayam, PhD

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Kultur tisu ialah teknologi yang menjanjikan pengeluaran besar-besaran kelapa sawit elit. Walaupun kaedah ini telah banyak digunakan oleh syarikat pengeluaran kelapa sawit dalam negara, kadar kalugenesis dan embriogenesis masih rendah. Oleh itu, penggunaan penanda untuk diagnosis awal potensi embriogenik dapat membantu mengurangkan kos kultur tisu kelapa sawit. Bahagian pertama dalam kajian ini bertujuan untuk mengenal pasti penanda DNA untuk tisu eksplan prolifik kelapa sawit melalui 'Representational Difference Analysis (RDA)', iaitu satu teknik di mana perbezaan di antara dua genom yang hampir sama dapat dikenalpasti. Dalam bahagian ini, sebanyak lima RDA kehadapan dan kebelakang telah dijalankan dengan menggunakan tisu eksplan yang berbeza kadar proliferasinya; pengukuran dibuat berdasarkan data pada kultur tisu yang sebelumnya oleh agensi tisu kultur kelapa sawit

yang berkenaan. Di kalangan produk yang dapat dikenalpasti melalui RDA, 13:14 C58 yang mana ianya ialah putatif jujukan tidak prolifik yang telah diperkayakan, memiliki beberapa perbezaan pasangan bes selepas dijajarkan bersama dengan kawasan DNA yang sama dalam tisu yang sangat prolifik. Walaubagaimanapun, verifikasi selanjutnya adalah sangat diperlukan untuk mengesahkan keberkesanannya sebagai penanda dalam membezakan tisu eksplan yang tidak prolifik dengan tisu eksplan yang sangat prolifik. Bahagian kedua dalam kajian ini adalah bertujuan untuk mencirikan salah satu daripada penanda embriogenik putatif yang telah dikenalpasti sebelum ini yang juga dikenali sebagai Eg707 (FJ196136) dengan menggunakan teknik kajian hibridisasi secara *in situ*. Ekspresi gen Eg707 dapat dikesan di kesemua tisu yang terlibat dalam laluan embriogenik seawal kalus embriogenik sehinggalah ke peringkat embrio yang sedang bercambah. Disebabkan pembentukan proembrio di peringkat kalus embriogenik telah dianggap sebagai penunjuk pertama dalam perkembangan somatik embrio kelapa sawit, Eg707 dapat digunakan sebagai penanda molekul untuk mengesan peringkat awal somatik embriogenesis kelapa sawit. Bahagian ketiga dalam kajian ini adalah bertujuan untuk mengesahkan kecekapan beberapa calon yang berpotensi tinggi sebagai penanda embriogenik yang juga telah dikenal pasti sebelum ini. Profil ekspresi lima gen-gen yang berkaitan dengan embriogenik kelapa sawit telah dihasilkan dengan menggunakan ‘PCR maya nyata’ terhadap tisu eksplan daun kelapa sawit yang mempamerkan perbezaan kadar prolifik di dalam kultur tisu. Terdapat dua transkrip memiliki potensi yang tinggi untuk digunakan sebagai penanda seawal peringkat eksplan daun di dalam kultur tisu kelapa sawit. Kedua-dua transkrip telah dikategorikan dalam dua kategori berdasarkan profil ekspresi masing-masing samada berterusan ataupun bergantung kepada masa. Walaubagaimanapun, profil ekspresi oleh setiap gen tidak dapat dikaitkan

dengan sampel yang memiliki kadar proliferasi yang sama dari agensi yang lain. Oleh yang demikian, lebih banyak genotaip kelapa sawit perlu dianalisa untuk mendapatkan penanda yang sesuai untuk digunakan dalam sesuatu agensi untuk penyaringan tisu eksplan kelapa sawit.

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I certify that a Thesis Examination Committee has met on 26th August 2011 to conduct the final examination of Norashikin Binti Sarpan on her thesis entitled "Isolation and Characterization of Potential Markers for Prolific Explant Tissues of Oil Palm (*Elaeis guineensis* Jacq.)" 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 candidate be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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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 duly acknowledge. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

NORASHIKIN SARPAN

Date: 26 August 2011

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