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DIFFERENTIAL EXPRESSIONS OF PROTEOMES OF OIL PALM (ELAEIS GUINEENSIS JACQ.) AND ARABIDOPSIS THALIANA TISSUES DURING CALLOGENESIS

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By

ROZITA BINTI ZAMRI

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 is presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman : Assoc. Prof. Mohd Puad Abdullah, PhD

Faculty : Biotechnology and Biomolecular Sciences

In *in vitro* propagation, callus is initiated by exposing explants to the appropriate types and concentrations of plant growth regulators, and the use of suitable medium. The efficiency of callogenesis varies from plant to plant, and is dependent on the ability of each and every key player in the particular cells or tissues to respond to the inductive stimuli. The key players in this process are active functional proteins required, and should be available on time and in the right amount for completing the process. This study focuses on differentially expressed proteins in oil palm and *A. thaliana* tissues during callogenesis. Micropropagation is commercially desired for cloning and multiplication of a very high yielding planting material with elite characteristics. However, oil palm micropropagation is rather a slow and inefficient process. Oil palm callogenesis

rate is low in comparison to other plants. Using *A. thaliana* as a model plant, this study aims to discover the active candidate proteins which have the potential to trigger *in vitro* callogenesis. The first step in the investigation using Proteinchip®-SELDI-TOF-MS approach was to generate complete protein profiles of both plants tissues during callogenesis based on distinct morphological appearances. Ten oil palm ortets, 4 replicates each were sampled. Two hundred explants per replicate cultured, producing a total of 800 explants from each lines. A time course protein extraction and profiling were conducted at monthly intervals at month 0 (before media treatment), month 1, 2 and 3 until callus was formed at month 4 (after media treatment). At least 37 protein peaks were up-regulated when most of the explants induced callus during the fourth month. Out of the 37 proteins, 14 are differentially expressed only at callus stage. Ten replicates of *A. thaliana* samples were cultured. Explant development and callus induction were observed until callus was formed at 20th day. Protein profiling were conducted at five distinct developmental stages, which are at day 0 (before media treatment), day 5, day 10, day 15 and day 20 (after media treatment). Six potential up-regulated protein peaks were recognized to be present at the critical stage of callusing in *A. thaliana*. Differentially-expressed proteins from both oil palm and *A. thaliana* were compared. Six potential callogenesis protein candidates were identified. The proteins were not present in the leaf explants suggesting that they were induced as a result of *in vitro* callus induction.

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

EKSPRESSI BERBEZA PROTEOM TISU KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.) DAN *ARABIDOPSIS THALIANA* SEMASA KALOGENESIS

Oleh

ROZITA BINTI ZAMRI

Julai 2010

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Di dalam propagasi *in vitro*, kalus diperolehi secara mendedahkan eksplan kepada jenis dan kepekatan pengaruh tumbesaran tumbuhan dan penggunaan medium yang bersesuaian. Keberkesanan kalogenesis berbeza dari satu tumbuhan ke tumbuhan yang lain, dan bergantung kepada kebolehan setiap satu pemain utama di dalam sel atau tisu tertentu untuk bertindak balas terhadap pengaruh induksi. Pemain utama dalam proses ini merupakan protein-protein berfungsi aktif yang diperlukan dan mestilah hadir pada waktu dan jumlah yang tepat bagi melengkapkan proses tersebut. Kajian ini memberi fokus kepada protein yang diekspres berbeza di dalam tisu kelapa sawit dan *A. thaliana* semasa kalogenesis. Mikroperambatan adalah diminati secara komersil untuk pengklonan dan penggandaan bahan tanaman berhasil tinggi dengan ciri-

ciri elit. Walaubagaimanapun, mikroperambatan kelapa sawit merupakan proses yang perlahan dan tidak efisyen. Kadar kalogenesis kelapa sawit rendah berbanding dengan tumbuhan lain. Dengan menggunakan *A. thaliana* sebagai tumbuhan model, matlamat kajian ini adalah untuk mengenalpasti calon protein aktif yang berpotensi mengaruh kalogenesis *in vitro*. Langkah pertama dalam kaedah kajian ini yang menggunakan pendekatan Proteinchip®-SELDI-TOF-MS adalah untuk membina profil protein yang lengkap daripada tisu kedua-dua tumbuhan semasa kalogenesis berdasarkan kepada perbezaan penampilan morfologi. Sepuluh ortet kelapa sawit telah disampel, empat replikasi daun umbut diambil daripada setiap ortet untuk induksi kalus. Dua ratus eksplan dikultur dari setiap daun, memberi jumlah keseluruhan 800 eksplan daripada setiap ortet. Ekstraksi dan pemprofilan protein secara masa-berjadual dijalankan pada setiap bulan, iaitu bulan 0 (sebelum rawatan media), bulan pertama, kedua dan ketiga sehingga kalus terhasil pada bulan keempat. Sekurang-kurangnya 37 puncak protein 'up-regulated' apabila kebanyakan eksplan bertukar kepada kalus di bulan keempat. Daripada 37 protein tersebut, 14 protein telah diekspres berbeza hanya di peringkat kalus sahaja. Sepuluh replikasi *A. thaliana* telah dikultur. Perkembangan eksplan and induksi kalus *A. thaliana* diperhatikan sehingga kalus terhasil pada hari ke-20. Pemprofilan protein dijalankan pada 5 peringkat perkembangan yang berbeza, iaitu semasa hari 0 (sebelum rawatan media), hari ke-5, hari ke-10, hari ke-15 dan hari ke-20 (selepas rawatan media). Enam protein 'up-regulated' berpotensi dikenalpasti

hadir pada peringkat kritikal semasa kalogenesis *A. thaliana*. Protein yang diekspres berbeza daripada kelapa sawit dan *A. thaliana* telah dibandingkan. Sebanyak enam calon protein kalogenesis berpotensi telah dikenalpasti. Protein-protein ini tidak hadir di dalam daun eksplan, lantas mencadangkan bahawa mereka diaruh daripada induksi kalus *in vitro*.

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APPROVAL I

I certify that a Thesis Examination Committee has met on 16th July 2010 to conduct the final examination of **Rozita Binti Zamri** on her degree thesis entitled "Differential expressions of proteomes of oil palm (*Elaeis guineensis* Jacq.) and *Arabidopsis thaliana* tissues during callogenesis" 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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Date: December 2010

DECLARATION

I declare that the thesis is my original work except for quotations 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 any other institution.

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ROZITA ZAMRI

Date: 16 July 2010

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