UNIVERSITI PUTRA MALAYSIA

PROTEIN AND HORMONE PROFILING OF HORMONE-TREATED OR UNTREATED EXCISED OIL PALM (Elaeis guineensis Jacq.) SPEAR LEAVES

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PROTEIN AND HORMONE PROFILING OF HORMONE-TREATED OR UNTREATED EXCISED OIL PALM (*Elaeis guineensis* Jacq.) SPEAR LEAVES

By

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

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Chair: **Associate Professor Mohd Puad Abdullah, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Clonal propagation of elite oil palm hybrids via tissue culture has been developed over the past twenty years, but still not perfect. In the procedure, an initial callus phase is required to establish primary cultures. However, the initiation and proliferation of callus in oil palm is a slow process with a low success rate ranging between 20 and 50%. Furthermore, the time required for the initiation and the percentage of callusing varies by genotype and origin of the palm materials. This setback may be attributed by the inappropriate combinations and levels of plant growth regulator (PGR) used in the media.

This study was undertaken to evaluate the effect of clonal material and PGR concentration on callus induction, to determine the existance of exogenous hormones and also to determine the expression of proteins associated with toxicity levels of 2, 4-diclorophenoxyacetic acid (2,4-D) by SELDI-TOF profiling. Excised leaves of Tenera population were exposed exogenously to 2, 4-diclorophenoxyacetic acid (2, 4-D), benyladenine (BA) and kinetin (K) at 0, 0.1, 1.0, 2.5, 5.0, 10.0 and 15.0 mg/L concentrations, alone and in combinations. The range of 2,4-D concentrations for oil palm callus formation was between 0.1 mg/L and 2.5 mg/ as evaluated after 4 months of incubation. However, at high concentrations of 2,4-D i.e. 10 -15 mg/l, the explants turned brown and some were dried up. Morphological changes on these explants indicated that 2,4-D at 0.1 mg/L was the most appropriate for callus induction.

Hormonal profiling was conducted to determine the existence of the hormones in the samples exposed exogenously to 2,4-D, BA and kinetin (K). The experiment revealed that 2,4-D was undetectable by HPLC, and very low amounts of BA and kinetin (K) were traced. In conjunction with the influence of hormone especially 2,4-D on callogensis and exogenous hormone profiling, the study has been extended to evaluate the influence of 2,4-D on protein expressions by using SELDI-TOF MS (Surface-Enhanced Laser Desorption/Ionization-Time of Flight Mass Spectrometry). Expressed proteins that are associated with toxicity levels of 2,4-D in oil palm spear leaves were profiled and analysed. A set of up-regulated and down-regulated proteins was expressed at different concentrations of 2,4-D with some were only

present at 4.0 mg/L 2,4-D (15.94 kDa, 19.91 kDa, 20.8 kDa and 24.7 kDa). The presence of these proteins in cultured explants may reflect a specific biochemical event that appears in response to high or toxic concentrations of 2,4-D. Subject to further validation, these proteins may potentially be used as biochemical markers for indicating the suitability of 2,4-D concentrations in oil palm tissue culture media.

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

PROFIL PROTIN DAN HORMON POTONGAN DAUN UMBUT KELAPA SAWIT (*Elaeis guineensis* Jacq.) YANG DIRAWAT ATAU TIDAK DIRAWAT DENGAN HORMON

Oleh

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Pengerusi: **Profesor Madya Mohd Puad Abdullah, PhD**

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Pembiak bakaan hibrid kelapa sawit elit melalui kultur tisu telah dilakukan selama lebih 20 tahun, akan tetapi masih lagi belum sempurna. Dalam prosedur berkenaan, fasa kalus diperlukan untuk menghasilkan kultur permulaan. Bagaimanapun, proses permulaan and proliferasi kalus kelapa sawit memakan masa yang sangat lama dengan peratusan kejayaan yang rendah di antara 20% dan 50%. Selain itu, masa yang diperlukan untuk pertumbuhan dan peratusan penghasilan kalus berbeza mengikut bahan genetik dan asal usul kelapa sawit tersebut. Kelemahan ini mungkin disebabkan oleh ketidaksesuaian kombinasi dan tahap pangawal-atur tumbuhan (PGR) yang digunakan di dalam media.

Kajian ini dijalankan untuk mengkaji kesan klon yang berbeza dan hormon kepada peratus induksi kalus, menentukan kewujudan eksogenus hormon di dalam eksplan kelapa sawit, dan untuk menentukan profil protein yang berkaitan dengan tahap toksik 2, 4-diclorophenoxyacetic acid (2,4-D) di dalam eksplan. Potongan daun daripada populasi Tenera direndam dalam 2,4-D, benyladenine (BA) dan kinetin (K), secara tunggal dan kombinasi, pada kepekatan 0, 0.1, 1.0, 2.5, 5.0, 10.0 and 15.0 mg/L. Julat kepekatan 2,4-D yang berjaya menghasilkan kalus adalah di antara 0.1 mg/L dan 2.5 mg/L, dinilai selepas 4 bulan pengeraman. Bagaimanapun, pada kepekatan 2,4-D yang tinggi iaitu 10 – 15 mg/L, didapati eksplan bertukar menjadi coklat dan sebahagiannya mengering. Perubahan morfologi yang berlaku ke atas eksplan menunjukkan bahawa kepekatan 2,4-D yang sesuai untuk menghasilkan kalus ialah 0.1mg/L.

Pemprofilan hormon telah dijalankan untuk menentukan hormon yang wujud di dalam sampel selepas dirawat secara eksogenus dengan hormon 2,4-D, benyladenine (BA) dan kinetin (K). Eksperimen ini menunjukkan 2,4-D tidak dapat dikesan melalui HPLC, dan hanya sedikit hormon benyladenine (BA) dan kinetin (K) yang berjaya dikesan. Lanjutan dari kajian kesan 2,4-D keatas morfologi kultur dan profil hormon, kajian telah dikembangkan untuk menilai kesan 2,4-D ke atas ekspresi protein dengan menggunakan teknologi SELDI-TOF MS (Surface-Enhanced Laser Desorption/Ionization-Time of Flight Mass Spectrometry). Protein yang diekspres pada tahap 2,4-D yang toksik dalam eksplan kelapa sawit telah diprofil dan dianalisis. Satu set protein ’up-regulated’ dan ’down-regulated’ telah dikesan pada kepekatan

2,4-D yang berbeza dengan sesetengah protein hanya wujud pada kepekatan 4.0 mg/L 2,4-D (15.94 kDa, 19.91 kDa, 20.8 kDa dan 24.7 kDa). Kehadiran protein-protein ini menggambarkan satu proses biokimia yang terjadi sebagai tindakbalas ke atas kepekatan 2,4-D yang toksik. Tertakluk kepada pengesahan lanjut, protein-protein ini berpotensi untuk dijadikan penanda biologi bagi menunjukkan kesesuaian kepekatan 2,4-D yang digunakan di dalam media kultur tisu kelapa sawit.

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I certify that a Thesis Examination Committee has met on **9th August, 2010** to conduct the final examination of Halina Binti Mohamed Ramly on her thesis entitled **"Proteins and hormones profiling of hormonal treated or untreated excised oil palm (*Elaeis guineensis* Jacq.) spear leaves"** 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.

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

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HALINA MOHAMED RAMLY

Date: 9th August 2010

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