ISOLATION OF ETHYLENE RESPONSE SENSOR GENE AND GENERATION OF EXPRESSED SEQUENCE TAGS FROM THE OIL PALM (*ELAEIS GUINEENSIS* JACQ.) MESOCARP

By

NURNIWALIS ABDUL WAHAB

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

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DEDICATIONS

To my family especially my dearest husband, son and daughter, who have always been patient and understanding as I added the roles of wife and mother to the competing demands of work, study, personal and family development. Without your love and support, the road would have been a lot rockier. I thank you from the bottom of my heart.

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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Chairman: Associate Professor Suhaimi Napis, PhD

Faculty: Biotechnology and Biomolecular Sciences

In this study, RT-PCR and partial sequencing of randomly selected cDNA clones were carried out to identify and hence lead to the isolation of a candidate mesocarp-specific gene from the oil palm. A 726 bp partial cDNA encoding an ethylene response sensor (ERS)-type ethylene receptor was first isolated by RT-PCR. Preliminary analysis *via* dot blot indicated that the partial cDNA showed very high expression in the oil palm mesocarp tissues with very low expression in the other tissues compared. Thus, ~ 1.1 kb partial cDNA (pER3RC A4) representing the 3' end of the clone was isolated *via* 3' RACE. Subsequently, three 17- week mesocarp cDNA libraries (GM17-1, GM17-5 and GM17-9) were successfully constructed. Based on the titer and average insert size, library GM17-5 was chosen for the generation of the ESTs. STACKpack clustering analysis generated 1011 unique transcripts comprising of 841 singletons and 170 consensus sequence representing 622 clones. Sequence homology searches against the non-redundant sequences in GenBank database revealed that approximately 48.0% of the clones had significant hits to other organisms (score > 50 and/or E value < 10^{-5}). At least 10.2% of the ESTs have low similarity score whereas the remaining 41.8% had no

match to other organisms in the public databases. The clones were found to have high sequence similarities to plant genes especially rice (34.9%) and *Arabidopsis* (20.3%). Majority (34.4%) of the clones were unable to be classified whereas 15.4%, 12.9% and 12.0% of the clones were categorized under cell rescue, defence and virulence, metabolism and protein synthesis, respectively. Two clones coding for a lipase class 3 family protein and an ethylene receptor (Q78EST) selected from the GM17-5 cDNA library were also found to show high differential gene expression in the mesocarp tissues *via* dot blot analysis. Alignment between Q78EST and pER3RC A4 indicated that they are highly similar to one another with 98% identity and with this information thus leads to the isolation and characterization of the full-length ethylene receptor gene. The full-length cDNA designated as EREG D3 is 2225 kb long and encodes a polypeptide of 629 amino acid residues. Northern and Southern analyses revealed that it is expressed highly in the mesocarp tissues as compared to the other tested tissues and that this gene exists as multi copy in the oil palm genome. Sequence analysis showed that EREG D3 has a structure similar to the bacterial two-component histidine kinase transduction system. These finding suggest that this gene may play an important role in plant signal transduction.

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

PEMENCILAN GEN TINDAKBALAS PENGESAN ETELIN DAN PENJANAAN PENANDA JUJUKAN TERUNGKAP DARI TISU MESOKARPA SAWIT (*ELAIES GUINEENSIS* JACQ.)

Oleh

NURNIWALIS BT ABDUL WAHAB

Julai 2006

Pengerusi: Profesor Madya Suhaimi Napis, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Di dalam kajian ini, RT-PCR and penjujukan separa cDNA yang dipilih secara rawak telah dijalankan untuk mengenalpasti serta memencilkan calon gen yang mesokarpa-spesifik dari pokok sawit. Melalui kaedah RT-PCR, gen yang bersaiz 726 pb dan mengekod reseptor etelin jenis-ERS telah berjaya dipencilkan. Analisis awal menggunakan kaedah pemblotan titik menunjukkan bahawa gen ini diekspres dengan kadar yang tinggi di dalam tisu mesokarpa dan sebaliknya di dalam tisu-tisu lain yang dibandingkan. Oleh itu, ~ 1.1 kb klon cDNA (pER3RC A4) yang mewakili hujung 3' klon ini telah dipencilkan menggunakan kaedah 3' RACE. Di samping itu, tiga perpustakaan cDNA mesokarpa 17 minggu (GM17-1, GM17-5 and GM17-9) telah berjaya dibentuk. Berdasarkan kepada nilai titer dan purata saiz klon, perpustakaan GM17-5 telah dipilih untuk generasi Penanda Jujukan Terungkap (EST). Analisis pengkelompokan StackPACK telah menghasilkan 1011 transkrip unik yang terdiri daripada 841 jujukan tunggal dan 170 jujukan konsensus yang mewakili 622 klon. Pencarian jujukan homologi dengan jujukan tidak redundan dalam pengkalan data GenBank menunjukkan bahawa 48% daripada klon-klon ini mempunyai padanan yang

signifikan (padanan skor > 50 atau nilai E < 10^{-5}) dengan organisma lain. Sekurang-kurangnya 10% daripada klon-klon tersebut mempunyai padanan skor yang rendah manakala 42% didapati tidak mempunyai padanan dengan organisma lain. Klon-klon dengan padanan yang signifikan ini didapati mempunyai padanan jujukan yang tinggi dengan gen tumbuhan lain terutamanya padi (35%) dan *Arabidopsis* (20%). Majoriti (35%) daripada klon-klon ini tidak dapat diklasifikasikan ke dalam mana-mana kategori berfungsi manakala 15%, 13% dan 12% daripada klon-klon ini telah dikategorikan ke dalam kumpulan sel penyelamat, pertahanan dan virulen, metabolisma dan sintesis protein. Dua klon yang mengekod lipase kelas 3 protein keluarga dan reseptor etelin (Q78EST) telah didapati menunjukkan ekspresi yang tinggi di dalam tisu mesokarpa melalui kaedah pemblotan titik. Penjajaran antara jujukan Q78EST and pER3RC A4 menunjukkan padanan yang tinggi dengan 98% identiti dan dengan informasi ini telah mendorong kepada pemencilan dan pencirian jujukan lengkap gen ini. cDNA berjujukan lengkap ini (EREG D3) bersaiz 2225 kpb dan mengekod polypeptida yang mengandungi 629 residu asid amino. Pencirian EREG D3 menggunakan kaedah Northern and Southern menunjukkan bahawa ia diekspres dengan kadar yang tinggi di dalam tisu mesokarpa dan gen ini merupakan ahli daripada keluarga multigen di dalam genom pokok sawit. Analisa ke atas jujukan DNA ini juga telah menunjukkan bahawa is mempuyai struktur yang sama dengan sistem transduksi dua komponen histidin kinas bakteria. Penemuan ini mencadangkan bahawa gen ini berpotensi memainkan peranan di dalam transduksi isyarat tumbuhan.

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I certify that an Examination Committee has met on 21st July 2006 to conduct the final examination of Nurniwalis bte Abdul Wahab on her Master of Science thesis entitled "Isolation of Ethylene Response Sensor Gene and Generation of Expressed Sequence Tags from the Oil Palm (*Elaies guineensis* Jacq.) Mesocarp" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis 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 are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NURNIWALIS BTE ABDUL WAHAB

Date :

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