UNIVERSITI PUTRA MALAYSIA

GENE EXPRESSION AND PROMOTER CHARACTERISATION OF STEAROYL-ACP DESATURASE AND ACYL CARRIER PROTEIN GENES FROM OIL PALM (*ELAEIS GUINEENSIS* JACQ.) FRUITS

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By

ASHIDA OTHMAN

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

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Chairman: Assoc Prof Datin Siti Nor Akmar Abdullah, PhD

Faculty: Faculty of Agriculture

In this study, the expression profiles of the genes encoding two key proteins, stearoyl-ACP desaturase (*SAD1*) and acyl carrier protein (*pACP3*) which are involved in plant fatty acid biosynthesis were analysed and their promoter sequences characterized with the aim of obtaining a better understanding of the regulation of storage oil production in oil palm fruits. Real-time PCR was carried out using RNA extracted from different oil palm tissues including mesocarp at 7, 10, 12, 15, 17 and 19 weeks after anthesis (w.a.a.), kernel (12 and 15 w.a.a.), spear and mature leaves as well as abscisic acid and ethylene treated spear leaves. In the mesocarp, *SAD1* showed a sharp increase in expression levels between 7 to 12 w.a.a. reaching a peak at 15 w.a.a. followed by small decline at 19 w.a.a.. Meanwhile *pACP3* expression levels increased 45-fold between 7 to 10 w.a.a. remained high until 19 w.a.a. The expression levels of *pACP3* were slightly higher than *SAD1* between 12 – 19 w.a.a. In kernel, *SAD1* and *pACP3* expression levels were higher at 12 w.a.a. than 15 w.a.a. and *pACP3* was expressed at higher levels than *SAD1* in both tissues. Both genes were expressed at much lower levels in leaves and *SAD1* was shown

to be expressed at higher level than *pACP3* in spear leaves. For both genes, ABA and ethylene treatments of spear leaves resulted increases in expression levels. For *SAD1* and *pACP3* promoter isolation, genomic DNA from oil palm spear leaves was isolated and digested with seven different blunt end restriction enzymes: *Dra*I, *Eco*RV, *Pvu*II, *Sca*I, *Stu*I, *Mss*I, and *Smi*I, to produce seven different GenomeWalker libraries. One gene-specific primer and one gene-specific nested primer were designed based on the 5' –untranslated region (UTR) of both genes and used for genome walking in isolating the promoter sequences. Sequence alignment of the isolated promoters with their respective cDNAs showed 100% sequence identity. The transcription start sites (TSS) of both genes were determined using 5'-rapid amplification of cDNA ends (5'-RACE) strategy. Based on the results, both *SAD1* and *pACP3* TSS have an adenine base (A) as their transcription initiation sites. Relative to the TSS, the isolated *SAD1* and *pACP3* promoter sequences were determined to be 1022 bp and 1227 bp in length, respectively. Identification of *cis*-regulatory elements using Softberry, PLACE and TRANSFAC softwares showed many motifs in common for both promoters. These include proximal regulatory elements (TATA and CAAT boxes), phytohormone responsive [abscisic acid (Athb1, ACGTATERD1 MYB1AT), gibberillic acid (GAmyb), ethylene (ERE)], light responsive (CIACADIANLELHC, Dof1, Dof2, -10PEHVPSBD, EBOXBNNAPA, GATABOX, REALPHALGLHCB2), abiotic factors/wounding responsive (OSE2ROOTNODULE, WBOXATNPR1, WBOXHVISO1, WBOXNTERF3, WRKY71OS), cold or drought responsive (MYCCONSENSUSAT) and endosperm specificity (PBF, DOFCOREEZM, RYREPEATBNNAPA). Using these two genes as models, a better insight on the transcriptional control of fatty-acid biosynthetic genes during period of oil synthesis in oil palm fruits has been achieved in this study.

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

PENGEKSPRESAN DAN PENCIRIAN PROMOTER GEN STEAROYL-ACP DESATURASE & ACYL CARRIER PROTEIN DALAM BUAH KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

ASHIDA OTHMAN

Oktober 2010

Pengerusi: Prof Madya Datin Dr. Siti Nor Akmar Abdullah, PhD

Fakulti: Fakulti Pertanian

Dalam kajian ini, profil pengekspresan bagi dua gen yang mengkodkan stearoyl-ACP desaturase (*SAD1*) dan acyl carrier protein (*pACP3*) iaitu dua protein yang penting dalam biosintesis asid lemak telah dianalisis dan jujukan promoter kedua-dua gen tersebut telah dicirikan demi memahami regulasi penghasilan simpanan minyak di dalam buah kelapa sawit. Real-time PCR telah dilakukan menggunakan RNA yang telah diekstrak daripada pelbagai jenis tisu kelapa sawit termasuk mesokarp 7, 10, 12, 15 dan 17 minggu selepas antesis (m.s.a.), kernel (12 dan 15 m.s.a.), pucuk daun, daun matang dan juga daun yang telah dirawat dengan etilena dan asid absisik. *SAD1* menunjukkan peningkatan pengekspresan yang mendadak di dalam tisu mesokarp di antara 7 hingga 12 m.s.a. dengan tahap yang tertinggi pada 15 m.s.a. dan disusuli dengan sedikit penurunan pada 19 m.s.a. Sementara itu, tahap pengekspresan *pACP3* meningkat sebanyak 45 kali ganda di antara 7 hingga 10 m.s.a. dan kekal tinggi sehingga 19 m.s.a. Tahap pengekspresan *pACP3* adalah lebih tinggi berbanding *SAD1* di antara 12 hingga 19 m.s.a. Tahap pengekspresan *SAD1* dan *pACP3* adalah tinggi pada 12 m.s.a berbanding 15 m.s.a pada tisu kernel dengan *pACP3* diekspreskan lebih tinggi

berbanding *SAD1* pada kedua-dua tisu. Kedua-dua gen diekspreskan pada tahap yang sangat rendah di dalam daun dan *SAD1* telah menunjukkan pengekspresan yang tinggi daripada *pACP3* dalam pucuk daun. Rawatan pucuk daun dengan etilena dan asid absisik menunjukkan peningkatan yang nyata dalam tahap pengekspresan pada kedua-dua gen. Bagi kajian pemencilan promoter *SAD1* dan *pACP3*, DNA genomik telah dipencilkan daripada pucuk daun sawit dan dicernakan dengan tujuh enzim penyekat berhujung tumpul yang berbeza iaitu *Dra*I, *Eco*RV, *Pvu*II, *Sca*I, *Stu*I, *Mss*I, dan *Smi*I untuk menghasilkan tujuh perpustakaan GenomeWalker yang berlainan. Satu pencetus spesifik-gen dan satu pencetus tersarang kedua spesifik-gen telah direka berdasarkan kawasan 5'-tak tertranslasi (5'-UTR) kedua-dua gen dan digunakan untuk memencilkan jujukan promoter menggunakan teknik 'genome walking'. Pensejajaran jujukan promoter-promoter yang dipencilkan bersama cDNA masing-masing menunjukkan 100% jujukan identiti. Tapak permulaan transkripsi (TPT) kedua-dua gen telah ditetapkan menggunakan strategi 5'-RACE (Amplifikasi Pantas 5' untuk Hujung cDNA). Berdasarkan hasil kajian, kedua-dua TPT bagi gen *SAD1* dan *pACP3* dimulakan dengan bes adenin (A). Sejajar dengan TPT masing-masing, saiz jujukan promoter *SAD1* dan *pACP3* adalah 1022 bp dan 1227 bp. Pengenalpastian elemen pengawalatur-cis menggunakan perisian Softberry, PLACE dan TRANSFAC menunjukkan banyak motif yang sama ditemui pada kedua-dua promoter. Motif ini termasuk elemen pengawalaturan proksimal (kotak TATA dan CAAT), tindak balas terhadap fitohormon [asid absisik (Athb1, ACGTATERD1, MYB1AT), asid giberelik (GAmyb), etilena (ERE)], cahaya (CIACADIANLELHC, Dof1, Dof2, -10PEHVPSBD, EBOXBNNAPA, GATABOX, REALPHALGLHCB2), faktor abiotik / kecederaan (OSE2ROOTNODULE, WBOXATNPR1, WBOXHVISO1, WBOXNTERF3,

WRKY71OS), sejuk atau kemarau (MYCCONSENSUSAT) dan endosperm spesifik (PBF, DOFCOREEZM, RYREPEATBNNAPA). Kebanyakan elemen-elemen ini termasuk etilena, asid absisik, kecederaan dan responsif terhadap cahaya telah ditemui dalam gen kemasakan buah yang lain, manakala asid absisik telah terbabit dalam mengawalatur pengumpulan gen dalam biosintetis asid lemak bagi tanaman biji minyak. Elemen endosperm-spesifik juga telah ditemui dalam promoter gen protein penyimpanan spesifik-biji. Dengan menggunakan kedua-dua gen ini sebagai model, kefahaman dalam pengawalan transkripsi gen biosintesis asid lemak semasa tempoh penghasilan minyak dalam buah kelapa sawit telah ditingkatkan dalam kajian ini.

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APPROVAL

I certify that a Thesis Examination Committee has met on to conduct the final examination of Ashida binti Othman on her thesis entitled "Gene Expression And Promoter Characterisation Of Stearoyl-Acp Desaturase And Acyl Carrier Protein Genes From Oil Palm (*Elaeis Guineensis* Jacq.) Fruits" 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 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 fulfillment 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 this 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 at any other institution.

ASHIDA OTHMAN

Date: 29 October 2010

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