UNIVERSITI PUTRA MALAYSIA

DIFFERENTIAL GENE EXPRESSION OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.) FRUITS DURING LATE RIPENING STAGES AND MOLECULAR CHARACTERISATION OF METALLOTHIONEIN-LIKE TRANSCRIPTS

AHMED BAKHIT SALIM AL-SHANFARI

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AHMED BAKHIT SALIM AL-SHANFARI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2012

DEDICATIONS

This thesis is dedicated to my wonderful parents, who have raised me to be the person I am today. To my wife, sisters, and children, you have been with me every step of the way, through good times and bad. Thank you for all the unconditional love, guidance, and support that you have always given me, helping me to succeed and instilling in me the confidence that I am capable of doing anything I put my mind to. Thank you for everything. I love you!

Finally, this thesis is dedicated to all those who believe in the richness of learning.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DIFFERENTIAL GENE EXPRESSION OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.) FRUITS DURING LATE RIPENING STAGES AND MOLECULAR CHARACTERISATION OF METALLOTHIONEIN-LIKE TRANSCRIPTS

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Chairman: Associate Professor Datin Siti Nor Akmar binti Abdullah, PhD

Faculty: Agriculture

Oil palm (*Elaeis guineensis* Jacques) is a major oil crop of economic importance in the world. Different biological and physiological mechanisms occur during oil palm fruit ripening. These mechanisms are regulated by the genes expressed during fruit ripening processes. Therefore, determining the structure and function of the expressed genes involved in these mechanisms will help to improve the important characters for enhancing palm fruit oil yield and quality. A forward suppression subtractive hybridization (SSH) library was constructed to identify genes involved in fruit ripening in oil palm. Oil deposition, as an oil palm fruit ripening indicator, will start at approximately 12 weeks after anthesis (w.a.a.) with an active period of oil synthesis at 15-16 w.a.a. until fruit maturity at about 20 w.a.a. in the mesocarp. Hence, the suppression was performed with cDNA extracted from fruits at 12 w.a.a. as “driver” and fruits at 17 w.a.a. as “tester”. A single-pass sequencing from the 5′-end of 2,112 randomly selected cDNA clones resulted in 2,019 high-quality expressed sequence tags (ESTs). Clustering of the 2,019 EST sequences showed 20 unigenes consisting of 9 contigs and 11 singletons. Among the edited EST sequences, 1,109 (14 unigenes) had

significant matches with protein sequences in the public databases. The matched ESTs were further classified into six putative cellular functions including unclassified proteins (38.0%), cell rescue and defense (33.8%), proteins with binding functions (27.7%), biogenesis of cellular component (0.3%), metabolism (0.1%), and cell cycle (0.1%). At 17 w.a.a., the 20 unigenes were expressed at higher abundance compared to 12 w.a.a.

Two of the abundant transcripts encoding type 2 metallothionein-like proteins designated as *MET2a* and *MET2b* were selected for further study due to their high abundance (16.05%) in the SSH library and their involvement in biological processes in fruit development and maturation. The second part of the present study involved the isolation of the full-length cDNA encoding *MET2a* and *MET2b* from the ripening oil palm fruit mesocarp at 17 w.a.a. and examing their expression patterns compared to the other two previously reported type-3 MT members (*MT3-A* and *MT3-B*) in various oil palm organs including different vegetative and reproductive tissues. The full-length cDNA sequence of *MET2a* and *MET2b* were 571 and 553 bp designated as *EgMT2a* and *EgMT2b*, respectively. Their sequences were homologous (67–77%) with several type-2 MTs in plants. All four genes were differentially expressed in the ripening oil palm mesocarp tissues but undetectable in the vegetative tissues examined. All the MT genes examined were significantly up-regulated in the mature developmental stages of oil palm fruit mesocarp except for *EgMT2b* which was expressed only at 17 w.a.a. but at a much lower level. The type 2 MT-like proteins are more in relation with late fruit ripening stage than the type 3 MT-like proteins consistent with their reported functions in the homeostasis or detoxification. The findings in the present study will contribute to a better understanding of the molecular mechanisms involved in fruit ripening in oil palm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBEZAAN PENGEKSPRESAN GEN SEMASA TEMPOH AKHIR KEMATANGAN BUAH KELAPA SAWIT (*Elaeis guineensis* Jacq.) DAN PENCIRIAN MOLEKULAR TRANSKRIP-TRANSKRIP BAK-METALLOTIONIEN YANG TELAH DIKENALPASTI

Oleh

AHMED BAKHIT SALIM AL-SHANFARI

September 2012

Pengerusi: Professor Madya Datin Siti Nor Akmar binti Abdullah, PhD

Fakulti: Pertanian

Kelapa sawit (*Elaeis guineensis* Jacques) adalah tanaman minyak utama sebagai penyumbang ekonomi dunia yang penting. Pembezaan mekanisma biologi dan fisiologi berlaku ketika pemasakan buah sawit. Mekanisma-mekanisma ini dikawalatur oleh gen terekspres ketika proses pemasakan buah. Oleh itu, penentuan struktur dan fungsi gen terekspres yang terlibat dalam mekanisma ini akan membantu meningkatkan ciri-ciri berkepentingan untuk meningkatkan hasil dan kualiti minyak kelapa sawit. Satu perpustakaan subtraktif hibridisasi (SSH) supresi kehadapan telah dibina untuk mengenalpasti gen-gen yang terlibat dengan kemasakan buah dalam kelapa sawit (*Elaeis guineensis* Jacq). Pengumpulan minyak sebagai petunjuk pemasakan buah sawit akan bermula kira-kira 12 minggu selepas antesis (m.s.a) dengan tempoh aktif ketika 15-16 m.s.a. sehingga buah masak pada 20 m.s.a. dalam mesokarp. Oleh itu, teknik supresi ini

telah dijalankan dengan menggunakan cDNA yang telah diekstrak daripada buah sawit pada 12 minggu selepas antesis (m.s.a) sebagai “pemacu” dan daripada 17 m.s.a sebagai “kajian”. Penjujukan sekali penghujung-5’ yang dijalankan keatas 2,112 klon-klon cDNA yang dipilih secara rawak telah menghasilkan 2,019 penanda jujukan terekspres (ESTs) berkualiti tinggi dengan purata panjang bacaan sebanyak 383 bp. Pengklusturan 2,019 jujukan EST menggunakan StackPACK menunjukkan 20 unigen yang terdiri dari 9 kontig dan 11 tunggalan. Di antara jujukan EST yang telah disunting, 1,109 (14 unigen) mempunyai padanan yang signifikan dengan jujukan protein di pangkalan data umum. EST yang mempunyai padanan seterusnya diklasifikasikan kepada enam fungsi selular putatif termasuk protein yang tidak diklasifikasikan (38.0%), penyelamat dan pertahanan sel (33.8%), protein dengan fungsi pengikat (27.7%), biogenesis komponen selular (0.3%), metabolisma (0.1%) dan kitaran sel (0.1%). Pada m.s.a ke 17, 20 unigen telah dikespreskan pada paras tertinggi berbanding pada m.s.a ke 12.

Dua transkrip paras tertinggi yang mengkodkan protein bak-metalotionin jenis ke-2 dilabelkan sebagai *MET2a* dan *MET2b* telah dipilih untuk kajian lanjut disebabkan ketinggian paras (16.05%) di dalam perpustakaan SSH dan penglibatannya di dalam proses biologi ketika pengembangan dan pematangan buah. Bahagian kedua kajian ini melibatkan pemencilan klon-klon cDNA lengkap *MET2a* dan *MET2b* daripada mesokarp buah kelapa sawit yang masak pada 17 m.s.a dan menguji corak pengekspresan yang dibandingkan dengan jenis ke-3 (*MT3-A* dan *MT3-B*) seperti yang dilaporkan terdahulu di dalam pelbagai organ-organ kelapa sawit termasuk tisu vegetatif dan reproduktif yang berbeza. Jujukan-jujukan cDNA *MET2a* and *MET2b* yang lengkap bersaiz 571 dan 553 bp yang dilabelkan sebagai *EgMT2a* dan *EgMT2b* mengikut

turutan. Kedua-dua jujukan tersebut mempunyai homologi (67-77%) dengan beberapa MT jenis ke-2 dalam tumbuh-tumbuhan. Keempat-empat gen tersebut telah diekspreskan secara berbeza pada tisu mesokarp buah kelapa sawit yang masak tetapi ianya tidak dapat dikesan pada tisu-tisu vegetative yang telah diuji. Kesemua gen MT yang telah diuji menunjukkan peningkatan ketara dalam regulasi semasa peringkat matang perkembangan mesokarpa buah kelapa sawit kecuali *EgMT2b* yang hanya diekspreskan pada 17 m.s.a pada paras yang rendah. Protein bak-MT jenis ke-2 mempunyai hubungkait yang tinggi dengan proses kemasakan buah pada tempoh akhir berbanding dengan protein bak-MT jenis ke-3. Ini adalah konsisten dengan fungsi yang telah dilaporkan dalam homeostasis atau detoksifikasi. Hasil kajian ini boleh menyumbang kepada pemahaman yang lebih meluas tentang mekanisma molekular yang terlibat dalam kemasakan buah kelapa sawit.

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Last but not least, I wish to express my sincere thanks to all those who have one way or another helped me in making this study a success.

I certify that a Thesis Examination Committee has met on 3 September 2012 to conduct the final examination of Ahmed Bakhit Salim Al-Shanfari on his thesis entitled "Differential Gene Expression of Oil Palm (*Elaeis guineensis* Jacq.) Fruits during Late Ripening Stages and Molecular Characterisation of Metallothionein-Like Transcripts" 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 PhD degree.

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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date:

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 at any other institution.

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AHMED BAKHIT SALIM AL-SHANFARI

Date: 3 September 2012

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