UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF MSP1 PROMOTER AND IDENTIFICATION OF TRANSCRIPTION FACTORS INVOLVED IN REGULATION OF ABSCISIC ACID- AND ETHYLENE-RESPONSIVE GENE EXPRESSION IN OIL PALM

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By

VAHID OMIDVAR

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

November 2011

Specially Dedicated

To my

Father

Mother

Wife

Sister

For their Love & Supports

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

CHARACTERIZATION OF MSP1 PROMOTER AND IDENTIFICATION OF TRANSCRIPTION FACTORS INVOLVED IN REGULATION OF ABSCISIC ACID- AND ETHYLENE-RESPONSIVE GENE EXPRESSION IN OIL PALM

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Chairman: Associate Professor Datin Siti Nor Akmar Abdullah, PhD

Institute : Tropical Agriculture

The 1,053-bp promoter of the oil palm metallothionein gene (so-called MSP1) and its 5´ deletions were analyzed in transiently transformed oil palm tissues. The full length promoter showed 7-fold higher activity in the mesocarp than in leaves and 1.5-fold more activity than the CaMV35S promoter in the mesocarp. Two positive regulatory regions at nucleotides (nt) -953 to -619 and -420 to -256 regions and a negative regulatory region at -619 to -420 nt region were identified within the promoter sequence. Fine-tune deletion analysis of the -619 to -420 nt region led to the identification of a novel negative regulatory AGTTAGG core-sequence, responsible for controlling the fruit-specific activity of the MSP1promoter. Abscisic acid (ABA) and copper (Cu^{2+}) induced the activity of the promoter and its 5´ deletions more effectively than methyl jasmonate (MJ) and ethylene. Regulatory DNA motifs responsive to ABA, copper, MJ, and ethylene were identified within the promoter sequence. These results suggest that the MSP1 promoter and its regulatory

regions are potentially useful for engineering fruit-specific and inducible gene expression in oil palm. ABF family of transcription factors (TFs) play important regulatory roles in plant response to abiotic stresses as well as plant growth and development. Two ABA-responsive cDNA clones, named EABF and EABF1 were isolated from oil palm fruits using yeast one-hybrid system. EABF had a conserved AP2/EREBP DNA-binding domain (DNA-BD) and a potential nuclear localization sequence (NLS). No previously known DNA-BD was identified from the EABF1 sequence. EABF and EABF1 proteins were classified as DREB/CBF and bZIP family members based on the multiple sequence alignment and phylogenetic analysis. Both proteins showed ABRE-binding and transcriptional activation properties in yeast. Furthermore, both proteins were able to trans-activate the down-stream expression of the *Lac*Z reporter gene in yeast. EABF was induced in response to ABA in oil palm fruits and leaves, but not in roots, while expression of EABF1 was constitutively induced in all tissues. The expression of both genes were strongly induced in fruits in response to ABA, ethylene, MJ, drought, cold and high-salinity treatments, indicating that EABF and EABF1 might act as connectors among different signal transduction pathways. Our current results suggest that EABF and EABF1 are involved in abiotic stress response and ABA signaling in oil palm. AP2/ERF family of TFs are involved in plant response to various biotic and abiotic stresses and also regulate many aspects of plant growth and development, such as flowering control and fruit ripening. Two ethylene-responsive cDNA clones designated EgEREBP and EgAP2-1were isolated from oil palm fruits. EgEREBP had a conserved AP2/EREBP DNA-BD and a potential NLS and was similar to DREB/CBF subfamily of AP2/ERFs. EgAP2-1 contained two AP2/EREBP domains and classified as AP2 subfamily member of AP2/ERFs. Both proteins showed ERE-

binding, transcriptional activation, and transactivation properties in yeast and *in vitro*. EgEREBP had a very basal expression in fruits, leaves and roots, while the expression of EgAP2-1 was found to be developmentally-regulated in ripening fruits, but not in leaves and roots. EgEREBP was induced in response to a range of hormone treatments and abiotic stresses, while EgAP2-1 was only induced in response to ethylene and ABA, but not other hormones and not to abiotic stresses as well. These observations imply the regulatory function of EgEREBP in plant response to biotic and abiotic stresses, while EgAP2-1 is involved in ethylene and/or ABA signaling pathways and perhaps might have a regulatory function more towards fruit-ripening control rather than the stress response in plant. Taken together, our studies have provided valuable information on the transcriptional regulatory mechanisms in oil palm for specific and inducible expression in fruits and stress responses involving specific interaction of transcription factors and regulatory motifs which are essential for future genetic improvement efforts.

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

PENCIRIAN PROMOTER MSP1 DAN PENGENALPASTIAN FAKTOR TRANSKRIPSI TERLIBAT DALAM PENGAWALATURAN PENGEKSPRESAN RESPONSIF ABSCISIC ACID DAN ETILENA DALAM KELAPA SAWIT

Oleh

VAHID OMIDVAR

November 2011

Pengerusi: Professor Madya Datin Siti Nor Akmar Abdullah, PhD

Institut : Pertanian Tropika

Promoter bersaiz 1053 pasangan bes (pb) gen metalotionin (MSP1) dan delesi 5'-nya dianalis secara transien dalam tisu kelapa sawit. Promoter penuh menunjukkan aktiviti 7 kali ganda lebih tinggi dalam mesokarpa berbanding dalam daun dan 1.5 kali ganda lebih tinggi berbanding promoter CaMV35S dalam mesokarpa. Dua kawasan pengawalaturan positif pada kedudukan nukelotida (nt) -953 hingga -619 dan -420 hingga -256 serta kawasan pengawalaturan negatif pada -691 hingga -420 nt telah dikenalpasti dalam kawasan promoter. Analisis delesi secara terperinci pada kedudukan -619 hingga -420 nt telah membawa kepada penemuanbaru kawasan pengawalaturan negatif berjujukan teras AGTTAGG, yang bertanggungjawab untuk mengawal aktiviti spesifik buah oleh promoter MSP1. Asid absisik (ABA) dan kuprum (Cu^{2+}) mengaruhkan aktiviti promoter juga delesi 5' promoter tersebut secara lebih berkesan berbanding metil jasmonat (MJ) dan etilena. Motif DNA pegawalatur responsif terhadap ABA, kuprum, MJ dan etilena telah dikenalpasti dalam jujukan

promoter. Hasil kajian ini mencadangkan bahawa promoter MSP1 dan kawasan pengawalaturannya adalah berpotensi dalam penjuruteraan spesifik buah dan pengaruhan ekspresi gen dalam kelapa sawit. Faktor transkripsi (TFs) dari famili ABF berperanan penting dalam pengawalaturan tindakbalas tumbuhan terhadap tekanan abiotik juga dalam tumbesaran dan perkembangan tumbuhan. Dua klon cDNA responsive terhadap ABA yang dinamakan EABF dan EABF1 telah dipencilkan dari buah kelapa sawit menggunakan sistem yis satu-hibrid. EAFB mempunyai domain AP2/EREBP pengikat-DNA (DNA-BD) yang terpelihara dan jujukan berpotensi untuk lokalisasi nuklear (NLS). Tiada rekod terdahulu mengenai DNA-BD telah dikenalpasti daripada jujukan EABF1.Protein EABF dan EABF1 dikelasifikasi sebagai DREB/CBF dan ahli famili BZIP berdasarkan penjajaran jujukan berkumpulan dan analisis filogeni. Kedua-dua protein menunjukkkan ciri-ciri pengikatan-ABRE dan pengaktifan transkripsi dalam yis. Selain itu, kedua-dua protein berupaya untuk pengaktifan-transekspresi gen pelapor *LacZ* hiliran dalam yis. EABF responsive terhadap ABA dalam buah kelapa sawit dan daun, tetapi tidak dalam akar, manakala EABF1 diekspres secara konstitutif dalam semua tisu. Ekspresi kedua-dua gen teraruhpada kadar tinggi dalam buah sebagai tindakbalas terhadap ABA, etilena, MJ, tekanan kemarau dan tekanan saliniti tinggi, menunjukkan bahawa EABF dan EABF1 mungkin bertindak sebagai penghubung antara tapak jalan transduksi isyarat yang berlainan. Keputusan terkini mencadangkan EABF dan EABF1 adalah terlibat dalam tindakbalas terhadap tekanan abiotik dan pengisyaratan ABA dalam kelapa sawit. TFs dari famili AP2/ERF terlibat dalam tindakbalas tumbuhan terhadap pelbagai tekanan biotik dan abiotik dan juga dalam pelbagai aspek pengawalaturan tumbesaran dan perkembangan tumbuhan, seperti pengawalan pembungaan dan pemasakan buah.

Dua klon cDNA responsif etilina yang dinamakan EgEREBP dan EgAP2-1 telah dipencilkan daripada buah kelapa sawit. EgEREBP mempunyai DNA-BD AP2/EREBP terpelihara dan NLS yang berpotensi serta mempunyai banyak persamaan dengan subfamili DREB/CBF dalam AP2/ERFs. EgAP2-1 mempunyai dua domain AP2/EREBP dan diklasifikasi sebagai ahlisubfamiliAP2 dalam AP2/ERFs. Kedua-dua protein menunjukkan ciri-ciri pengikatan-ERE, pengaktifan transkripsi, dan pengaktifan-trans dalamyis dan *in vitro*. EgEREBP terekspres pada kadar asas dalam buah, daun dan akar, manakala pengekspresan EgAP2-1 didapati lebih dikawalatur dalam buah yang sedang masak, tetapi tidak dalam daun dan akar. Ekspresi EgEREBP diaruhkan sebagai tindakbalas terhadap pelbagai hormon dan tekanan abiotik, manakala EgAP2-1 hanya diaruhkan sebagai tindakbalas terhadap etilina dan ABA, tetapi tidak responsif terhadaphormon lain dan tekanan abiotik. Pemerhatian ini mengandaikan bahawa fungsi pengawalaturan EgEREBP dalam tumbuhan bertindakbalas terhadap tekanan biotik dan abiotik, manakala EgAP2-1 terlibat dalam tindakbalas etilena dan/atau pengisyaratan tapak jalan ABA dan mungkin menpunyai fungsi pengawalatur yang cenderung kepada pengawalaturan pemasakan buah berbanding dengan tindakbalas terhadap tekanan dalam tumbuhan. Secara keseluruhan, kajian ini telah memberikan maklumat berguna mengenai mekanisma pengawalaturan transkripsi dalam kelapa sawit untuk pengekspresan khusus dan keboleh-aruhanan dalam buah dan tindakbalas terhadap tekanan yang melibatkan interaksi spesifik faktor transkripsi dan motif pengawalaturan yang penting bagi usaha pembaikan genetik pada masa hadapan.

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I hope anyone who is not mentioned by name may recognize my gratitude for their kindness, advice and moral support in completion of my PhD degree at University Putra Malaysia.

I certify that a Thesis Examination Committee has met on 31 January 2011 to conduct the final examination of Vahid Omidvar on his thesis entitled "**Characterization of the MSP1 Promoter and Identification of Transcription Factors involved in Regulation of ABA- and Ethylene-Responsive Gene Expression in Oil Palm**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Pertanian Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the relevant degree of Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of requirement for degree of Doctor of Philosophy. 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 other degree at Universiti Putra Malaysia or other institutions.

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VAHID OMIDVAR

Date: 15 November 2011

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