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FUNCTIONAL CHARACTERISATION OF TRANSCRIPTION FACTORS WITH POTENTIAL AS KEY REGULATORS OF PALM OIL PRODUCTION

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

FUNCTIONAL CHARACTERISATION OF TRANSCRIPTION FACTORS WITH POTENTIAL AS KEY REGULATORS OF PALM OIL PRODUCTION

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June 2016

Chairman : Professor Datin Siti Nor Akmar Abdullah, PhD
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Transcription factors play important and diverse roles in regulating gene expression. Each transcription factor binds to a specific DNA sequence (cis-elements) in the promoter region and can either activate or suppress the downstream gene expression. They have been known to coordinate expression of genes involved in the same biosynthetic pathway or physiological process. Three transcription factors EgNFYB3,EgLEC2 and EgWRI1-ANT were the target for studying transcriptional regulation of fatty acid biosynthesis in the oil palm. The sequences of the open reading frame were verified based on homology with known transcription factor sequences from other plant species. Recombinant vector constructs, each harbouring one of the selected transcription factors were constructed via Gateway cloning system. The oil palm genes driven by the cauliflower mosaic virus 35S constitutive promoter were introduced separately into 12 WAA (weeks after anthesis) oil palm mesocarp tissue slices, which is the period preceding oil synthesis through particle bombardment system. Each one was co-bombarded with a green fluorescent protein (GFP) reporter vector. Mesocarp tissue slices which expressed GFP protein at 48 hours post-bombardment were selected for total RNA extraction. High throughput sequencing (RNA-Seq) and transcript profiling of transiently transformed mesocarp slices were performed to determine genes whose expression is affected by transient over-expression of each transcription factor. RNA-Seq results were analysed for differentially expressed genes that are involved in fatty acid biosynthesis, glycolysis and TAG assembly pathways. EgNFYB3 bombarded tissues upregulated transcripts encoding enzymes for glycolysis, EgWRI1-ANT bombarded tissues upregulated transcripts encoding enzymes in fatty acid biosynthesis and glycolysis, while EgLEC2 bombarded tissues upregulated a transcript encoding an enzyme from fatty acid biosynthesis pathway in both biological replicates. RT-qPCR validation of the differentially expressed genes were performed and the results showed correlation to the RNA-Seq data. EgWRI1-ANT was chosen for subsequent DNA-protein interaction in vivo with yeast one-hybrid assay and EMSA for in vitro study. RNA-Seq results reveal the role of EgWRI1-ANT transcription factor in regulating genes related to glycolysis and fatty acid biosynthesis. Yeast one-hybrid assay confirmed that EgWRI1-ANT

transcription factor binds to the AW-box 1*cis*-element to activate transcription of the genes it upregulated. These results demonstrate that oil palm transcription factors EgNFYB3, EgLEC2 and EgWRI1-ANT are important transcriptional regulator of carbohydrate metabolism and fatty acid biosynthesis in oil palm mesocarp, a non-seed oil-rich tissue, thus making them candidates for future development of molecular tools for genetic improvement of the oil palm.

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

PENCIRIAN BERFUNGSI FAKTOR-FAKTOR TRANSKRIPSI YANG BERPOTENSI SEBAGAI PENGAWAL SELIA PROSES PENGELUARAN MINYAK KELAPA SAWIT

Oleh

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Faktor-faktor transkripsi memainkan peranan yang penting dan pelbagai dalam pengawalan selia ekspresi gen. Setiap faktor transkripsi mengikat kepada jujukan DNA spesifik (cis-elemen) di rantau penganjur dan boleh mengaktifkan atau menyekat ungkapan gen hiliran. Faktor-faktor transkripsi dikenal dalam menyelaraskan ekspresi gen yang terlibat dalam laluan biosintetik yang sama atau proses fisiologi yang sama. Tiga faktor transkripsi iaitu EgNFYB3, EgLEC2 dan EgWRI1-ANTadalah sasaran untuk mengkaji peraturan transkripsi biosintesis asid lemak dalam pokok kelapa sawit. Urutan rangka bacaan terbuka telah disahkan berdasarkan homologi dengan urutan faktor transkripsi diketahui dari spesis tumbuhan yang lain melalui penjajaran jujukan berbilang dan analisis filogenetik. Konstruk vektor rekombinan, setiap salah satu daripadanya mengandungi faktor-faktor transkripsi terpilih dari kelapa sawit dibina melalui sistem pengklonan Gateway. Gen faktor transkripsi kelapa sawit dibawah kawalan promoter constitutive cauliflower mosaic virus 35S diperkenalkan secara berasingan ke dalam tisu hirisan mesokarp kelapa sawit berusia 12 WAA (minggu selepas berbunga), iaitu tempoh sebelumnya sintesis minyak melalui sistem pembedilan zarah. Masing-masing telah dibedil bersama vektor Green fluorescent protein (GFP) yang merupakan gen pelapor. Hirisan tisu mesocarp yang mengekspresi protein GFP pada masa 48 jam selepas pembedilan telah dipilih untuk pengekstrakan RNA. Penjujukan kendalian tinggi (Penjujukan-RNA) dan pemprofilan transkrip hirisan mesokarp yang telah ditransformasi secara transient dijalankan untuk menentukan ungkapan gen yang terjejas hasil daripada ungkapan transien setiap faktor transkripsi. Keputusan RNA-Seq telah dianalisa bagi menentukan gen yang mengalami pembezaan pengeskpresan dan juga terlibat dalam biosintesis asid lemak, glikolisis dan laluan pemasangan TAG. Didapati bahawa faktor transkripsi EgNYFB3 menyebabkan peningkatan pengekspresan tiga transkrip yang mengekod enzim untuk glikolisis. EgWRI1-ANT menyebabkan peningkatan pengekspresan tiga transkrip yang mengekod enzim untuk biosintesis asid lemak dan glikolisis manakala EgLEC2menyebabkan peningkatan pengekspresan transkrip yang mengekod enzim untuk biosintesis asid lemak dalam kedua-dua replikat biologi. Pengesahan RT-qPCR

ke atas gen yang mengalami pembezaan pengeskpresan telah dijalankan dan hasil kajian menunjukkan korelasi dengan data RNA-Seq. Faktor transkripsi EgWRI1-ANT telah dipilih untuk kajian seterusnya iaitu interaksi DNA-protein secara in vivo dengan asai yis-satu hibrid dan EMSA secara in vitro. Keputusan RNA-Seq mendedahkan peranan faktor transkripsi EgWRI1-ANT dalam mengawal selia gen-gen yang berkaitan dengan glikolisis dan biosintesis asid lemak. Asai yis-satu hibrid mengesahkan bahawa faktor transkripsi EgWRI1-ANT mengikat kepada jujukan DNA-spesifik AW box-1 untuk mengaktifkan transkripsi gen yang tahap pengekspresannya meningkat. Keputusan ini menunjukkan bahawa faktor transkripsi kelapa sawit EgNFYB3, EgLEC2 dan EgWRI1-ANT adalah pengawal-selia transkripsi yang penting terhadap karbohidrat metabolisme dan biosintesis asid lemak dalam mesocarp kelapa sawit, salah tisu kaya minyak bukan biji. Faktor-faktor transkripsi kelapa sawit ini adalah calon-calon untuk pembangunan alat molekul masa hadapan bagi usaha pembaikan genetik kelapa sawit.

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I certify that a Thesis Examination Committee has met on 8 June 2016 to conduct the final examination of Chin Mei - Yee on her thesis entitled "Functional Characterisation of Transcription Factors with Potential as Key Regulators of Palm Oil Production" 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.

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LIST OF ABBREVIATIONS

AP2/EREBP	APETALA2 Ethylene Response Element Binding Protein
RAV	Related to ABI3/VP1
OPTF	Oil Palm Transcription Factor
NFYB3	Nuclear Factor Y Subunit B3
LEC2	LEAFY COTYLEDON2
WRI1-ANT	APETALA2 Ethylene Response Element Binding Protein-ANT
CDS	Coding Region
ORF	Open Reading Frame
cDNA	Complementary Deoxyribonucleic Acid
LiCl	Lithium Chloride
EMSA	Electrophoretic Mobility Shift Assay
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RT-qPCR	Reverse transcription quantitative Real-Time PCR
NCBI	National Center for Biotechnology Information
LiAc	Lithium Acetate
PEG	Polyethylene Glycol
mRNA	Messenger RNA
CaMV	Cauliflower Mosaic Virus
EVC	Empty Vector Control
DNase	Deoxyribonuclease
RPM	Revolutions per minute
OD	Optical Density
WAA	Weeks After Anthesis
ALDO-P	fructose-bisphosphate aldolase, chloroplast precursor, putative
GAPDH-A	glyceraldehyde-3-phosphatedehydrogenase A, chloroplast precursor,putative
GAPDH-B	glyceraldehyde-3-phosphatedehydrogenase B chloroplast precursor,putative
FatB	acyl-ACP thioesterase, chloroplast precursor, putative
HK2	Hexokinase-2
Fad7	omega-3 fatty acid desaturase,chloroplast precursor, putative
GFP	Green Fluorescent Protein
MCS	Multiple Cloning Site
TAG	Triacylglycerol

CHAPTER 1

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is an economically important oil crop originating from central and West Africa (Corley and Tinker, 2003). It is the most productive amongst all oil crops, producing an average yield of about 4 t ha^{-1} $year^{-1}$ of palm oil. The oil palm produces two different types of oils. Crude palm oil, also known as mesocarp oil, is extracted from the mesocarp and accounts for 95% of the total oil of the fruit, whereas the other 5% of crude palm kernel oil (CPKO), which is a non-edible oil, comes from the kernel. As global demand for palm oil continues to increase, from 17.7 million tonnes in 1997 to 61.1 million tonnes in 2015 (Oil World 2015), it is thus critical to meet this demand by increasing the oil palm yield. To meet this demand, the yield per unit area needs to be increased in the efforts to raise productivity without jeopardizing the environment by opening more lands for agriculture (Parveez et al., 2015).

Transcription factors are known to coordinate the expression of genes involved in the same biosynthetic pathway or physiological process, thus providing an interesting alternative to single-enzyme based solutions to improve complex traits such as oil accumulation in crops (Broun et al., 2004; Grotewold et al., 2008). However, the mechanism of regulation by these transcription factors are complex and may involve a network of different transcription factors interacting with different cis-acting elements working in synergy to produce the desired output.

Nuclear factor Y (NF-Y) is a heterotrimeric complex composed of NF-YA, NF-YB (HAP3), and NF-YC subunits which can bind to the CCAAT promoter elements (Dolfini et al., 2012) Based on sequence similarity of the conserved B-domain region, HAP3 transcription factors are divided into LEAFY COTYLEDON1 (LEC1) type and Non-LEC1 type (Kwong et al., 2003; Lee et al., 2003). Functional studies in Arabidopsis showed that LEC1-type are mostly expressed specifically in developing seeds, while most non-LEC1 type subunits are expressed in various other tissues (Zimmerman et al., 2004).

LEAFY COTYLEDON2 (LEC2) belongs to the B3 domain superfamily of plant specific transcription factors (Suzuki and McCarty, 2008). It is part of the closely related AFL clade which comprises ABSCISIC ACID INSENSITIVE3 (ABI3), FUSCA3 (FUS3), and LEC2, playing important role in seed development and maturation (Stone et al., 2001). The conserved structure of the B3 domain allows LEC2 to regulate the expression of downstream genes possessing the specific RY recognition motif "CATGCA", enriched in the promoter region of seed-specific genes (Stone et al., 2008). Due to partial functional redundancy and the overlapping expression patterns of the B3 AFL genes, the specific function of each AFL is hard to be inferred from genetic analyses (Roscoe et al., 2015).

AP2-like ethylene-responsive binding protein (AP2/EREBP) is one of the largest families of plant specific transcription factors, with roles in the control of primary and secondary metabolism, plant growth and development and responses to biotic and abiotic stresses (Licausi et al., 2013). Members of the AP2 subfamily contain conserved double AP2/ EREBP domains composed of 60-70 amino acid residues joined by a linker sequence of about 25 amino acids (Riechmann and Meyerowitz, 1998). Based on phylogenetic analyses, AP2 subfamily is further divided into two clades, APETALA2-like (AP2-like) and AINTEGUMENTA-like (ANT-like) groups (Shigyo & Ito, 2004). The ANT group is characterized by lineage-specific motifs or amino acid insertions in the two AP2 domains (Kim et al., 2006) with functions in lateral organ development by controlling cell number and growth (Elliot et al., 1996). Another member of the AP2 subfamily, WRINKLED1 (WRI1), regulates oil accumulation in seed tissues and its direct targets are late glycolysis and fatty acid biosynthesis genes (Cernac and Benning, 2004; Baud et al., 2009). Sequence analysis of its putative target gene promoters led to the identification of the AW-box DNA-binding site with the consensus sequence "[CnTnG](n)7[CG)]" (Maeo et al., 2009).

Recent studies have found that genes involved in de novo fatty acid biosynthetic pathway share the same temporal transcription pattern. Before the start of seed maturation in Arabidopsis thaliana, coordinated upregulation of genes encoding different enzymes were observed, suggesting that the pathway may undergo a system of global transcriptional regulation. This was further supported by the discovery of master regulators of seed maturation such as LEC2 and WRI1 transcription factors, which have been shown to be involved in a regulatory cascade determining the induction of fatty acid biosynthetic genes before the start of seed maturation (Baud and Lepiniec, 2010). LEC2 induction was shown to correlate with the onset of oil accumulation in maturing seeds while its ectopic expression triggered the TAG accumulation in rosette leaves (Stone et al., 2001; Santos-Mendoza et al., 2005). Furthermore, it has been shown that in *Arabidopsis thaliana*, LEC1 positively regulates WRI1 expression and various genes from fatty acid biosynthetic pathway (Mu et al., 2008). WRI1 is well studied in oil synthesis but the other members of AP2 subfamily including ANT, which have a common function related to flowering (Li et al., 2015) and fruit development (Omidvar et al., 2013) is unknown in relation to oil synthesis. Flower development is important for fruit growth and proper development of oil palm fruit is crucial as the source of palm oil is from the mesocarp and kernel of the fruit (Sarpan et al., 2015).

Extensive research on oil biosynthesis has been done on seeds in which triacylglycerols (TAGs) accumulated during maturation (Baud et al., 2010) but the molecular mechanisms of lipid metabolism in the mesocarp of oil palm, which accumulate high levels of TAG, and the mesocarp of similar fleshy fruits such as olive and avocado are still unclear. An understanding of the transcriptional control of oil synthesis in mesocarp tissues of the oil palm is crucial in the efforts to produce high yielding oil palm genotypes. Therefore, in this study, we are interested to determine if OPTF members from NF-YB family (EgNFYB3), B3 domain family (EgLEC2) and AP2 subfamily (EgWRI1-ANT) play a role in the regulation of gene expression of glycolysis, fatty acid biosynthesis and TAG assembly pathways through identification of their putative target genes.

1.1 Research Objectives

Below are the objectives of this research project:

1) To produce recombinant vector constructs, each harbouring one of the selected transcription factors (NFYB3, LEC2 and WRI1-ANT) from oil palm and to introduce into oil palm mesocarp tissue slices

2) To determine genes whose expression are affected by transient over expression of each transcription factor in the mesocarp tissue slices by high throughput sequencing and transcript profiling method.

3) To validate the interaction with corresponding cis-acting elements of a selected transcription factor which may have a role in regulating oil production, using yeast one-hybrid assay and electrophoretic mobility shift assay (EMSA).

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