

UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF REGULATORY MOTIF FOR ENHANCING EXPRESSION OF OIL PALM (Elaeis guineensis Jacq.) STEAROYL-ACP DESATURASE 1

FARAH HANAN BINTI ABU HANIFIAH

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By

FARAH HANAN BINTI ABU HANIFIAH

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

January 2018

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This thesis is dedicated to my love ones who always beside me through good and bad times during my study which are my husband and my daughter and also the blessings from my parents and all. Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

IDENTIFICATION OF REGULATORY MOTIF FOR ENHANCING EXPRESSION OF OIL PALM (*Elaeis guineensis* Jacq.) STEAROYL-ACP DESATURASE 1

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January 2018

Chair: Professor Datin Siti Nor Akmar Abdullah, PhD Faculty: Institute of Tropical Agriculture and Food Security

Understanding the regulation of fatty acid biosynthesis is important for genetic improvement of oil traits especially palm oil yield and composition. Stearoyl-ACP desaturase (SAD) plays a central role in regulating the levels of unsaturated fatty acids in plant storage lipid as the fatty acid composition is known to change during fruit maturity. The sequence of the oil palm SAD promoter contains an array of *cis*-acting regulatory elements such as phytohormone and light responsive regulatory elements and tissue-responsive regulatory elements that interact with transcription factors to regulate or modify the expression of this gene. This study was undertaken to identify or prove the specific regulatory motif that can enhance or suppress the activity of the oil palm SAD1 promoter and its potential role in regulating fatty acid biosynthesis. The SAD1 promoter of 1111 bp in size was isolated using polymerase chain reaction (PCR). A total of six 5' deletion fragments of the SAD promoter which are 698 bp (D1), 643 bp (D2), 594 bp (D3), 516 bp (D4), 444 bp (D5) and 413 bp (D6) were isolated by PCR and all the deletion fragments including the full promoter sequence were cloned into a pBGWFS7.0 vector containing both the β-glucuronidase (GUS) and green fluorescent protein (GFP) reporter genes. The recombinant plasmids were bombarded into 12 week after anthesis (WAA) oil palm mesocarp tissues and the gene expression in GFP positive tissues were analyzed by transient GUS assay. The results of the quantitative fluorometric GUS assay showed that GUS activity in the D3 deletion construct (-486 to +108) was significantly increased and was higher than that of the full length promoter. In addition, the D2 (-535 to +108) deletion construct was found to direct the least expression of the GUS reporter gene. This observation suggests the presence of negative *cis*-acting regulatory element(s) in the deleted -535 to -486 (49 bp).

Electrophoretic mobility shift assay (EMSA) was done to identify the specific regulatory element responsible for the altered gene expression in the mesocarp tissues. It was found that the 49 bp region bind to the nuclear protein extract from mesocarp and did not bind to the extract from leaves. Further fine-tuned analysis of this 49 bp region by EMSA using truncated DNA and nucleotide mutations led to the identification of GCTTCA as a novel motif in the *SAD* promoter. The presence of another known motif LECPLEACS2 (TAAAT) are required for effective competition by GCTTCA in binding to mesocarp nuclear protein extract. GCTTCA with one variant nucleotide is also found in another oil palm fatty acid biosynthetic gene, acylcarrier protein (*ACP3*) suggesting its potential role in regulating expression in the mesocarp tissues.



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

IDENTIFIKASI MOTIF PENGAWALSELIAAN BAGI MENINGKATKAN EKSPRESI STEAROYL-ACP DESATURASE 1 KELAPA SAWIT (*Elaeis* guineensis Jacq.)

Oleh

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Januari 2018

Pengerusi: Profesor Datin Siti Nor Akmar Abdullah, PhD Fakulti: Institut Pertanian Tropika dan Sekuriti Makanan

Pengetahuan mengenai pengawalan biosintesis asid lemak adalah penting untuk penambahbaikan genetik pada ciri-ciri minyak terutamanya hasil dan komposisi. Stearoyl-ACP desaturase (SAD) memainkan peranan penting dalam mengawal selia tahap asid lemak tak tepu dalam lemak simpanan tumbuhan kerana komposisi asid lemak difahamkan berubah semasa kematangan buah. Jujukan promoter SAD kelapa sawit mengandungi pelbagai cis-elemen pengawalseliaan seperti pengawalseliaan tindak balas fitohormon dan cahaya dan pengawalseliaan tisu-responsif yang berinteraksi dengan faktor transkripsi untuk mengawal selia atau mengubah pengekspresan gen ini. Kajian ini telah dijalankan untuk mengenalpasti atau membuktikan motif pengawalseliaan yang khusus yang dapat meningkatkan atau mengurangkan aktiviti promoter SAD1 kelapa sawit dan potensinya dalam mengawal seliaan biosintesis asid lemak. Promoter SAD1 ini yang bersaiz 1111 bp telah diasingkan menggunakan tindak balas rantai polimerase (PCR). Sejumlah enam keratan fragmen 5' dari promoter SAD iaitu 698 bp (D1), 643 bp (D2), 594 bp (D3), 516 bp (D4), 444 bp (D5) dan 413 bp (D6) telah diasingkan dengan PCR dan semua potongan fragmen termasuk jujukan promoter yang penuh telah diklon ke vektor pBGWFS7.0 yang mengandungi gen pelapor β-glucuronidase (GUS) dan protein fluoresen hijau (GFP). Plasmid rekombinan telah dibedil ke dalam tisu mesokarp kelapa sawit 12 minggu selepas pendebungaan (WAA) dan ekspresi gen GUS telah di analisa dalam tisu yang positif GFP. Hasil daripada ujian esei GUS fluorometrik kuantitatif menunjukkan aktiviti GUS dalam konstrak potongan D3 (-486 hingga +108) telah meningkat dengan ketara dan lebih tinggi daripada jujukan promoter penuh. Di samping itu, konstrak potongan D2 (-535 hingga +108) telah didapati mengarahkan ekspresi terendah gen pelapor GUS. Pemantauan ini mencadangkan kehadiran beberapa cis-elemen pengawalseliaan negatif pada pemotongan di -535 ke -486 (49 bp). Penganjakan pergerakan elektroforetik (EMSA) telah dilakukan untuk mengenal pasti elemen pengawalseliaan khusus yang bertanggungjawab kepada perubahan ekspresi gen dalam tisu mesokarpa. Telah didapati bahawa bahagian 49 bp mengikat ekstrak protein nuklear daripada mesokarpa dan tidak mengikat ekstrak dari daun. Analisis terperinci bahagian 49 bp ini dengan EMSA menggunakan DNA yang dipotong dan nukeotida yang dimutasikan menghasilkan penemuan GCTTCA sebagai motif yang baharu dalam promoter SAD. Kehadiran motif lain yang telah diketahui iaitu LECPLEACS2 (TAAAT) diperlukan untuk persaingan yang berkesan oleh GCTTCA untuk mengikat ekstrak protein nuklear mesokarpa. GCTTCA dengan satu varian nukleotida juga dijumpai dalam biosintetik asid lemak kelapa sawit yang lain, acylprotein yang mencadangkan peranan potensinya dalam carrier (*ACP3*) mengawalseliaan ekspresi dalam tisu mesokarpa.



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LIST OF ABBREVIATIONS

4-MU	4-methylumbelliferone	
4-MUG	4-methylumbelliferyl-β-d-glucuronide	
ACCase	acetyl-coa carboxylase	
CH ₃	methyl group	
CaCl ₂	calcium chloride	
CaMV	cauliflower mosaic virus	
COOH	carboxyl group	
DIECA	diethylcarbamic acid	
DNA	deoxyribonucleic acid	
DNMRT	duncan new multiple range test	
EDTA	ethylenediaminetetra acetic acid	
EMSA	electrophoretic mobility shift assay	
ER	endoplasmic reticulum	
FAS	fatty acid synthase	
FFA	free fatty acids	
G3P	glycerol-3-phosphate	
GFP	green fluorescent protein	
GTF	general transcription factor	
GUS	β-glucuronidase	
KAS	β-ketoacyl-acp synthases	
LB	luria bertani	
LACS	long-chain acyl-CoA synthetases	
MAS	marker assisted selection	
MgCl ₂	magnesium chloride	
NaCl	sodium chloride	
NR	nuclear probe	
PBS	phosphate-buffered saline	
PCR	polymerase chain reaction	
PVP	Polyvinylpyrrolidone	
RNA	ribonucleic acid	
RT qPCR	real-time quantitative pcr	
SDS	sodium dodecyl sulfate	
TAE	tris-acetate edta	
TAGs	Triacyglycerols	
TdT	terminal deoxynucleotidyl transferase	
TE	tris-edta	
TFIID	transcription binding site	
TFs	transcription factors	
Tris-HCl	tris (hydroxymethyl) aminomethane hydrochloride	
TSS	transcription start site	
WAA	week after anthesis	

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis*) which is from the Arecaceae family is an important plantation crop in Malaysia since 1917, the year commercial planting started in the country. It has been extensively cultivated for the production of palm oil which is important in the economy of many developing countries in Southeast Asia, particularly Malaysia and Indonesia (Hayati et al., 2004). Now, Malaysia ranked second for world production of palm oil and the Malaysian palm oil industry is well established. Oils extracted from oil palm are classified into two classes which are palm oil from the mesocarp and palm kernel oil from the seeds. Palm oil is widely used for both edible and non-edible purposes while palm kernel oil is mainly used as feedstock for the oleochemical industry.

Improvement of the yield and quality of palm oil are priority areas for oil palm research in Malaysia. Due to limited land resources, we need to increase palm oil production in Malaysia through biotechnology approaches either through genetic engineering or marker assisted selection. However, there is limited study on the regulation of genes involved in palm oil production (Dussert et al., 2013). Researchers have discovered that the specific physical performance and nutritional attributes of edible oils like palm oil can be determined by their fatty acid composition (Guerin et al., 2016). Marker assisted selection could potentially be a reliable method in sustaining the production of palm oil based on association between DNA-based markers and specific oil traits (composition and content). It is crucial to understand the regulation of expression of key enzymes that are involved in palm oil fatty acid biosynthesis in order to improve the oil yield.

Fatty acid biosynthetic pathway in oil palm that occurs in the plastid involves a cascade of enzymatic reactions. The key enzymes of the pathway include acyl-carrier protein, β -ketoacyl-ACP synthase II, palmitoyl-ACP thioesterase and stearoyl-ACP desaturase. The genes encoding these enzymes were used in previous studies in oil palm genetic engineering programme to modify the composition of palm oil (Parveez et al., 2004). Stearoyl-ACP desaturase gene expression pattern was shown to correlate with oil synthesis in oil palm mesocarp tissue (Siti Nor Akmar et al., 1999).

Stearoyl-ACP desaturase (*SAD*) gene plays important role in plant by regulating the levels of unsaturated fatty acid through conversion of the main substrate stearoyl-ACP to oleoyl-ACP. It is an important enzyme that can determine the ratio of total saturated to unsaturated fatty acids in plant membranes and oil accumulating tissues (Shanklin and Somerville, 1991). *SAD* promoter may have an influence on the regulated process of storage lipid biosynthesis. Functional study of an oil palm *SAD1* was performed

using tomato as a model plant system. It was found that it drives fruit-specific gene expression in tomato fruit tissues (Leong et al., 2013).

Understanding the promoter function relates to the presence and positions of specific promoter regulatory motifs and expression profiles of the specific gene (John and Stewart, 2010). Previous efforts in oil palm genetic engineering have resulted in the isolation of *SAD1* promoter from oil palm and analysis of the promoter 5' deletion fragments in transgenic tomato tissues (Saed Taha et al., 2012). Based on their expression profiles, it can be suggested that *SAD1* can serve as a suitable tissue-specific promoter for modifying storage oil composition in oil palm. Alternatively, the regulatory motifs in this promoter can also provide the information on the mechanism of regulation of oil synthesis in oil palm.

Cis-regulatory elements play important role in controlling the development and physiology of a plant by regulating gene expression (Wittkoop and Kalay, 2012). The findings on regulatory elements have become a major challenge in genetic engineering these days. Many different approaches have been developed to detect *cis*-regulatory elements in various plant gene promoters. Researchers have found the core sequence which is functionally important in several promoters and respond to several stimuli such as light, jasmonic acid and hormones (Mehrotra et al., 2015). Unfortunately, there is ambiguous classification on the different roles of *cis*-regulatory elements such as tissue-specificity and inducibility which is not straight forward (Rombauts et al., 2003). Thus, the study aimed to identify the *cis*-regulatory elements involved in enhancing expression of oil palm fatty acid biosynthetic genes.

The specific objectives of the study were:

- 1. To prepare 5' deletion constructs of stearoyl-ACP desaturase (*SAD1*) gene promoter from oil palm
- 2. To identify regulatory region from promoter deletion analysis using reporter gene of bombarded mesocarp tissues slices
- 3. To identify a specific regulatory motif involved in regulating *SAD1* expression using Electrophoretic Mobility Shift Assay (EMSA) method

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