

## **IDENTIFICATION OF REGULATORY MOTIF FOR ENHANCING EXPRESSION OF OIL PALM FATTY ACID BIOSYNTHETIC GENES**

Farah Hanan Abu Hanifah<sup>1</sup> and Siti NorAkmar Abdullah<sup>2</sup>

<sup>1</sup>Laboratory of Agrobiotechnology, Faculty of Agriculture, University Putra Malaysia, 43400 Serdang, Selangor

farah.hanan298@gmail.com

<sup>2</sup>Institute of Plantation Studies, University Putra Malaysia, 43400 Serdang, Selangor  
snaa@upm.edu.my

**Abstract:** Stearoyl-ACP desaturase (*SAD*) plays a central role in regulating the levels of unsaturated fatty acids in plant storage lipid and is a potential candidate gene for improvement of oil traits in oil palm. This study aims to discover a new regulatory motif that can enhance or suppress the activity of the oil palm *SAD1* promoter. The *SAD1* promoter of 1111 bp in size was isolated and its 5' deletion fragments of 698bp (D1), 643bp (D2), 594bp (D3), 516bp (D4), 444bp (D5) and 413bp (D6) were generated and cloned into a pBGWFS7.0 vector harbouring both the  $\beta$ -glucuronidase (*GUS*) and green fluorescent protein (*GFP*) reporter genes. The recombinant plasmids were bombarded into oil palm mesocarp tissues at the start of oil synthesis and transient expression assay of both reporter genes were performed. *GUS* activity in the D3 deletion construct (-486 to +108) was significantly increase while the D2 (-535 to +108) deletion construct directed the lowest expression of *GUS* reporter gene. In order to determine the presence of a negative *cis*-acting regulatory element(s) whose removal led to the increase *GUS* expression in the deleted -535 to -486 (49bp), an electrophoretic mobility shift assay (EMSA) was carried out. The 49bp region interacted with the nuclear protein extract from mesocarp but not to the extract from leaves. Fine-tuned analysis of this 49 bp region using truncated DNA and nucleotide mutations led to the identification of a novel GCTTCA motif. The presence of LECPLEACS2 (TAAAAT) is essential for effective competition by GCTTCA in binding to mesocarp nuclear protein extract. GCTTCA with one variant nucleotide is also found in the promoter sequence of acyl-carrier protein (*ACP3*), an important co-factor for plant fatty acid biosynthesis supporting the important role of this new found motif in regulating gene expression in the mesocarp tissues.

**Keywords:** oil palm fatty acid, stearoyl-acyl desaturase gene, *cis*-acting regulatory element

### **INTRODUCTION**

Oil palm (*E. guineensis*) is a monocotyledonous plant which comes from the family Arecaceae. It is an important plant that produces oil which significantly contributes to Malaysian economy. The isolation of fatty acid biosynthetic genes in oil palm is important in genetic improvement through genetic manipulation. Stearoyl-ACP desaturase (*SAD*) is a plastid localized enzyme which was reported to be one of the first enzymes purified from fatty acid biosynthesis pathway (Shaklin and Sommerville, 1991). *SAD* that catalyses the conversion of saturated stearoyl-ACP to mono-unsaturated oleoyl-ACP plays a central role in regulating the levels of unsaturated fatty acids in plant storage lipid. Sequence of *SAD* promoter contains an array of *cis*-acting regulatory elements that will interact with transcription factor to

regulate or modify the expression of the gene (Juven-Gershon and Kadonaga, 2010). Even though studies on specific oil palm promoters have been reported, there is no study in identifying the specific motif that may be useful in regulating expression of fatty acid biosynthetic genes in oil palm. Thus, the objectives of this study are; (1) To prepare a series of 5' deletion constructs of *SAD1* gene promoter from oil palm. (2) To identify regulatory region based on promoter deletion analysis using reporter gene of bombardment mesocarp tissue slices. (3) To identify a specific regulatory motif involved in regulating *SAD1* expression using Electrophoretic Mobility Shift Assay (EMSA) method.

## **MATERIAL AND METHODS**

### **Construction of 5' deletion of the SAD promoter**

The 5' deletions of the *SAD1* promoter containing the 5'-UTR were generated by PCR amplification. The primers were designed using primer3 tools from SDSC Biology Workbench. All six deletion constructs were cloned in pBGWFS7.0 vector using Gateway Cloning Technology.

### **Biolistic transformation, real time PCR (qPCR) and GUS fluorometric assays**

The oil palm 12 weeks postanthesis fruits were obtained from Taman Pertanian Universiti (TPU, UPM). Biolistic transformation was carried out using the PDS-1000/He Biolistic® Particle Delivery System by bombarding each 5' deletion plasmid construct into mesocarp tissues slices in Murashige and Skoog medium. The qPCR was conducted for normalization by using the DNA of bombarded mesocarp tissues in 10 fold serial dilution of *SAD* gene promoter. The GUS activity in transiently transformed mesocarp tissues was carried out according to (Jefferson, 1987). The fluorescence generated from each sample was measured based on 4-methylumbelliferone (4-MU) standard curve, using the Bio Tek Synergy Microplate Reader. Protein concentration was determined in each sample using the Quick Start™ Bradford Protein Assays Kit. The GUS activity was calculated in pmol MU min<sup>-1</sup> mg protein<sup>-1</sup>.

### **Electrophoretic Mobility Shift Assay (EMSA)**

EMSA was conducted on the specific region in *SAD1* promoter by using extracted nuclear protein of mesocarp tissue. The nuclear protein was extracted using NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Scientific). The DNA probes was labelled using Biotin 3' End Labeling Kit by Thermo Scientific and the LightShift Chemiluminescent EMSA Kit (Thermo Scientific) was used in identifying 49bp oligonucleotide at nt -535 to -486. The biotin labelled detection was done using The Chemiluminescent Nucleic Acid Detection Kit from Thermo Scientific.

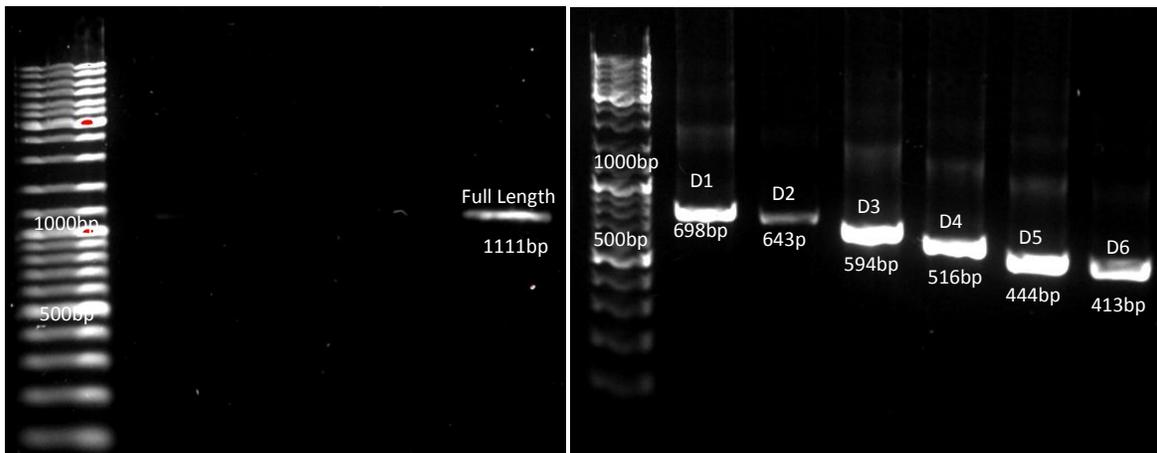
### **Experimental design and data analysis**

For particle bombardment experiments, the samples were arranged in a completely randomized design with five replicates for each fragment. The data obtained were subjected to one-way ANOVA. The mean comparisons for the experiments were carried out using Duncan New Multiple Range Test (DNMRT) by using the software of SAS from version 9.4. For normalization of the differences in each bombardment efficiency on GUS activity, the expression data for 5' deletion constructs were reported as of GUS to copy numbers values.

## **RESULTS AND DISCUSSION**

The known *SAD* promoter was amplified by PCR where the 1111bp DNA fragment obtained was isolated from the oil palm genomic DNA. Six deletion fragments of 698bp (D1), 643bp (D2), 594bp (D3), 516bp

(D4), 444bp (D5) and 413bp (D6) (Figure 1) were isolated and all deletion fragments including the full promoter sequences were cloned into the expression vector and bombarded into 12 w.a.a. oil palm mesocarp. The gene expression was analysed by using GUS transient assay. The result (Figure 2) showed that the D3 deletion construct (-486/+108) showed significant increase in GUS expression and which was higher than the expression in the full length promoter. Meanwhile, the D2 (-535/+108) deletion construct was found to direct the least expression of the GUS reporter gene. Thus, this observation suggests the presence of a negative *cis*-acting regulatory element(s) in the deleted -535 to -486 of 49bp in length. The 49bp of regulatory elements was subjected to EMSA to identify the motif which might contribute in enhancing the expression in oil palm mesocarp tissues. The result found that the 49bp region did not bind to nuclear protein from leaf but it binds to the nuclear protein from the mesocarp. Fine-tuned analysis was done subsequently using truncated DNA and the result showed that the mesocarp nuclear protein extract bind with GCTTCA and TAAAAT. TAAAAT is a known motif referred to as LECPLEACS2 motif while GCTTCA is an unknown motif. GCTTCA (Figure 3) was further analyzed by nucleotide mutation and the result found that all six positions in the GCTTCA nucleotides are potentially important and possibly served as the core sequences of the motif. Thus the GCTTCA sequence was considered as the actual motif involved in regulating the promoter activity of the important fatty acid gene, *SAD1* involved in palm oil biosynthesis. The results also suggest that both motifs, GCTTCA and TAAAAT are required in binding to the nuclear protein extract as the 49-bp sequence lost the competition with the excess competitors when one of them are deleted. The GCTTCA was found in the promoter of acyl-carrier protein (ACP3) gene, an important co-factor of plant fatty acid biosynthesis with one nucleotide altered in the GCTTCA (GCTTGA) and this further supports the importance of this motif in regulating the expression of fatty acid biosynthetic genes in oil palm mesocarp.



**Figure 1: Agarose gel electrophoresis analysis of the purified PCR products for isolating full length and six deletion fragments from *SAD* promoter.**

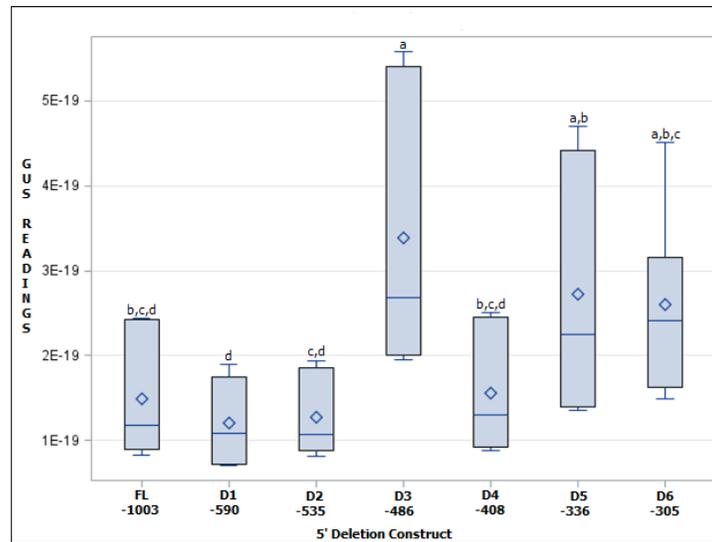


Figure 2: Transient expression of GUS driven by *SAD1* promoter and its 5' deletion fragments in oil palm mesocarp tissues.

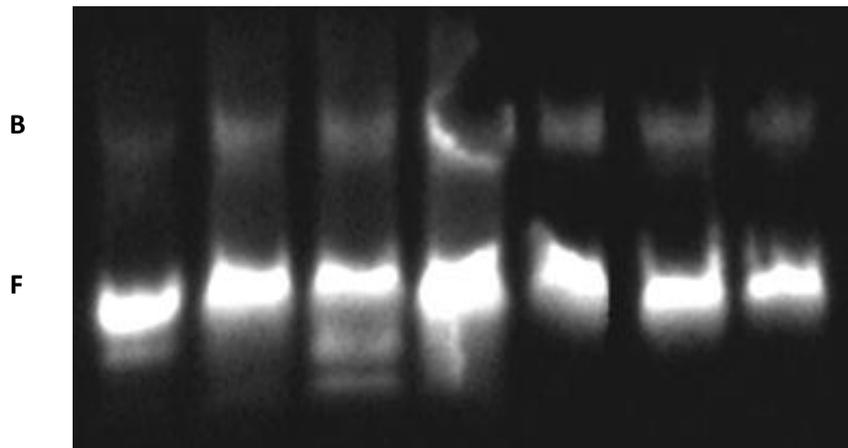


Figure 3: The competition EMSAs showing the interaction between 49-bp regulatory region (NR) with its mutated derivatives with mesocarp nuclear protein extract. Lanes 1-6 shows the interaction of the mutated M1 to M6 probes with nuclear protein extract from mesocarp in competition with NR probe. The mutated nucleotide is shown in red color. F, free probe and B, bound complex.

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