Cloning and Characterization of stearoyl–ACP desaturase gene (*SAD*) in oil palm (*Elaeis guineensis* Jacq.)

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ABSTRACT

The Oil palm (*Elaeis guineensis* Jacq.) is the highest producing oil seed crop on a per hectare basis in the world. Current estimates are that within the next decade, palm oil will become the largest single oil traded globally. In this study, the stearyl–ACP desaturase gene (*SAD*) was cloned and thoroughly characterized in oil palm. Mesocarp tissue of oil palm fruit at different developmental stages including 0, 30, 60, 90, 120, 150, and 180 days after pollination (DAP) was used for expression pattern analysis. The results showed that *SAD* gene was highly expressed at 90 DAP. *SAD* gene is 1,179 base pairs in length, comprised of 3 exons and 2 introns, and translated into 393 amino acids. The phylogenetic analysis was constructed using nucleotide sequences, showing that it is closely related to *SAD* gene from *Elaeis oleifera.*

Keyword: oil palm, fatty acid, *SAD* (Stearyl-ACP desaturase)

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a versatile oil of great economic value for the food production, non-food, derivatives, oleochemical and biofuel industries. Therefore, cultivation of oil palm has expanded enormously in recent years, with worldwide yearly increase in demand for diet and biofuel (ThuZar *et al*., 2011).

Stearoyl–acyl carrier protein (ACP) desaturase is an important enzyme of fatty acid synthetic metabolism in higher plants. Located in plastid stroma, *SAD* catalyzes the desaturation of stearoyl-ACP to oleoyl-ACP. *SAD* plays a key role in determining the ratio of saturated fatty acids to unsaturated fatty acids in plants. Many *SAD* genes have been cloned from different plants and the structures and functions of several *SAD* have been studied (Lindqvist *et al*., 1996). The objectives of this study were to clone and to study gene expression of *SAD* gene in oil palm during oil palm fruit development.

MATERIALS AND METHODS

Plant materials

Oil palm tree cultivar ‘‘Tenera’’ from Golden Tenera Co. was used in this study and fruits were harvested at various developmental stages of the immature oil palm fruits at 0, 30, 60, 90, 120, 150, and 180 days after pollination (DAP).

RNA isolation and cDNA synthesis

Approximately 1 gram of mesocarp tissue from oil palm fruit samples were ground in liquid nitrogen using a pre-cooled sterile mortar and pestle. Total RNA was isolated using RNA extraction buffer 2% (w/v) CTAB (modified from Wang and Zhang, 2005). RNA quality was measured using NANODROP 2000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Treatment of total RNA was performed with DNase (PROMAGA), following the manufacturer’s instructions. First strand cDNA was synthesized using Verso kit (Thermo), following the manufacturer’s instructions.

Expression pattern analysis of *SAD* gene in oil palm during fruit developmental stages by semi-quantitative PCR

Semi-Quantitative PCR examination was used to examine the expression of *SAD* gene with forward primer (5'-CACCGCCAGAT CTCCGAGGGTTTCC-3') and reverse primer (5'-CTTCCCTGAGCTCTTTCTTC-3'). The oil palm elongation factor gene (EF) was used as an internal control for cDNA normalization using forward primer (5'-GGTGTGAAGCAGATGATT TGC-3') and EF reverse primer (5'-CCTGG ATCATGTCAAGAGCC-3'). The amplification program was as follows: an initial denature step for 3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 58°C, 60 s at 72°C and the final extension step for 9 min at 72°C. The relative expression of *SAD* gene in oil palm during developmental stages was examined using 1% agarose gel electrophoresis.

Multiple alignments and bioinformatic analyses

The 25 *SAD* gene sequences from different organisms were downloaded from NCBI (http://www.ncbi.nlm.nih.gov). Multiple sequence alignments and analysis were conducted by Muscle. The phylogenetic relationship from nucleotide sequences were constructed by UPGMA method using the MAGA version 5.05 software program.

RESULTS AND DISCUSSION

Expression pattern of *SAD* gene

Semi-Quantitative PCR analysis of *SAD* gene of immature oil palm fruits at 0, 30, 60, 90, 120, 150, and 180 days after pollination (DAP) revealed that the highest expression of *SAD* was at 90 DAP, the expression was lower at 120, 150 and 180 DAP and was lowest at 0 and 60 DAP (Fig. 1). The expression pattern corresponded to the time that oil starts to accumulate in oil palm fruit (Fig. 2) (Tranbarger *et al*., 2011).

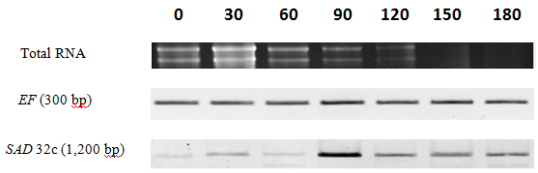

Figure 1 Amplification of the *SAD* gene transcripts from the immature oil palm fruits at 0, 30, 60, 90, 120, 150, and 180 days after pollination (DAP). Top panel shows total RNA in each developmental stage. Middle panel shows *Elongation Factor* (*EF*) expression, an internal control. Bottom panel shows *SAD* gene expression pattern.

Phylogenetic tree

To investigate the evolutionary relationship of *SAD* gene in 26 plant and algae species, nucleotide sequences of CDS from GenBank were used to construct a phylogenetic tree using the UPGMA method. The result showed that the tree can be divided into two major groups: one group for monocotyledonary plants and the other group for dicotyledonary plants. The result showed that *SAD* gene of *E. guineensis* was grouped together with *SAD* gene from other monocot plants including *Zea mays*, *Oryza sativa,*

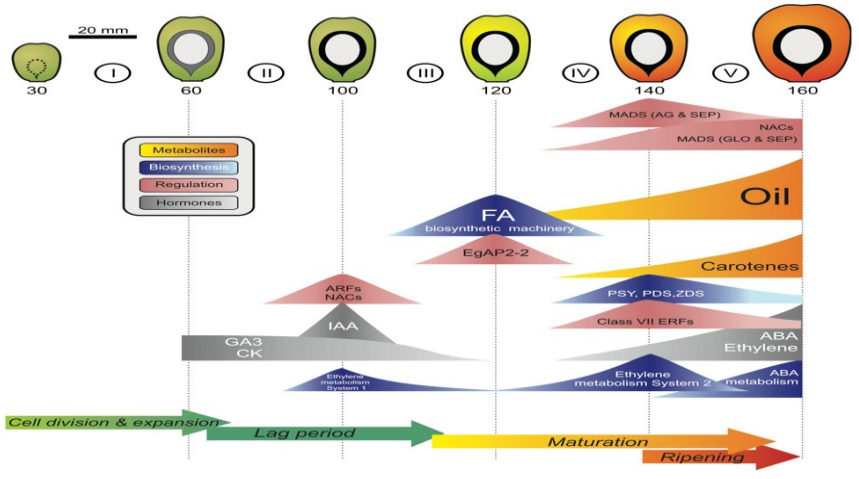

Figure 2 Schematic representation of the major events that occur during the phases of mesocarp development [3] and the time that oil starts to accumulate in oil palm fruits are between 100 to 140 DAP. See color figure on the website.

Triticum aestivum, *Hordeum vulgare* and it was close to *SAD* gene from *E. oleifera* (Fig 3).

Gene structure

The nucleotide sequences showed similarities between oil palm *SAD* and other plant *SAD* genes as revealed by a BLAST tool. Based on partial *SAD* sequence, the specific primers were designed and used to amplify a 5' RACE PCR product generating 881 nucleotides. These sequences were assembled into 1,179 bp CDS, 3 exons, 2 introns and 393 amino acids of *SAD* gene in *E. guineensis* (Fig. 4).

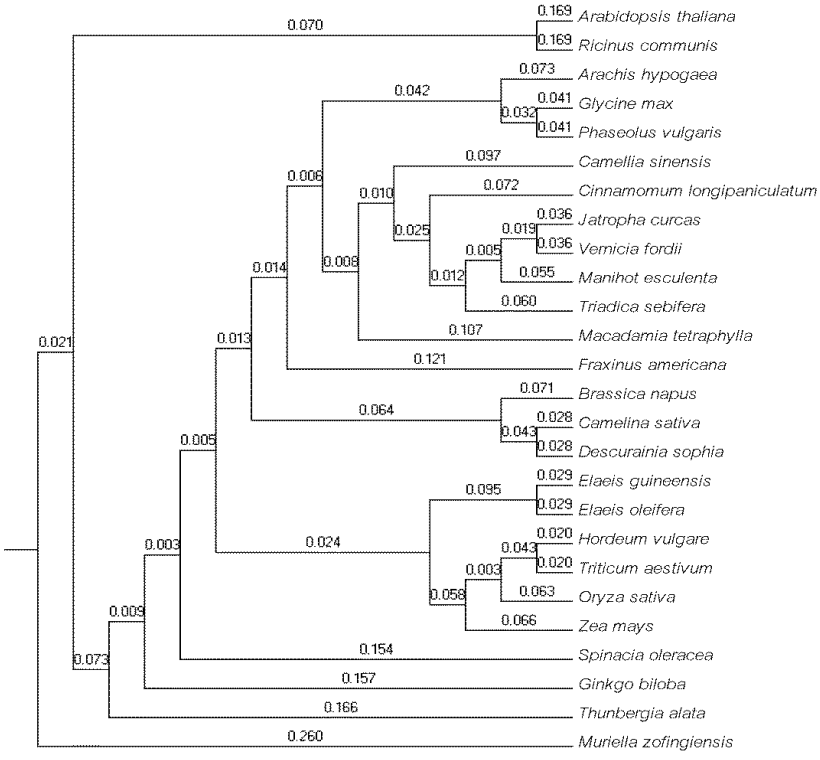

Figure 3 Phylogenetic tree of *SAD* gene using the UPGMA method. The optimal tree with the sum of branch length = 2.8688 is shown (above the branches). The distances were computed using the Maximum Composite Likelihood method. The analyses involved 26 nucleotide sequences and were conducted in MEGA 5 program.

Figure 4 Gene structure of the *SAD* gene in oil palm (*Elaeis guineensis*) with a 1,179 base-pair CDS segment. The orange rectangle indicates the size and location of coding regions of the gene and the grey denotes 5' - and 3' - untranslated regions (UTRs) of the mature *SAD* mRNA.

CONCLUSIONS

SAD gene from oil palm contains 3 exons and 2 introns. The CDS has 1,179 bp encoding 393 amino acids. The expression of the *SAD* gene was measured in immature oil palm fruits at 0, 30, 60, 90, 120, 150, and 180 days after pollination (DAP) and the result showed that *SAD* was highly expressed at 90 DAP during fruit development which corresponded to the time that oil start to accumulate in oil palm fruit. The phylogenetic analysis showed that *SAD* gene in *E. guineensis* was similar to that in other monocot plants and was closely related to *SAD* gene of *E. oleifera*. This research provides essential information for functional characterization of *SAD* gene in oil palm in future.

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