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GENETIC VARIATION IN THE *STEAROYL-ACYL-CARRIER-PROTEIN DESATURASE (SAD)* GENE AMONG AFRICAN AND AMERICAN OIL PALM SPECIES AND THEIR HYBRIDS

RISMAYANTI[1]*; SUDARSONO[2]; WAHYU PURBOWASITO[3];
NADIRMAN HASKA[4] and ANDI ROCHANDI[4]

ABSTRACT

*Stearoyl-acyl-carrier-protein desaturase (SAD) is crucial for oleic acid biosynthesis in oil palms. Reports show that American oil palm species (*Elaeis oleifera*) have higher oleic acid concentrations than African species (*Elaeis guineensis*). However, the impact of the* SAD *nucleotide sequence variation in various* SAD *gene structures on oleic acid biosynthesis remains unclear. This study aims to identify the* SAD *nucleotide sequence variations in diverse oil palm accessions as a basis for oleic acid biosynthesis research. Various SNP loci are identified in the* SAD *gene nucleotide sequences of five oil palms belonging to two species,* E. oleifera *and* E. guineensis *(i.e.,* Dura, Pisifera *and* Tenera *types and accessions having the* Virescens *characteristic) and the* E. oleifera – E. guineensis *hybrids (the O×G hybrid). The identified SNPs were used to design the Single Nucleotide Amplified Polymorphism (SNAP) primer sets and evaluated using genetically diverse 50 oil palm accessions. This study identified 15 SNPs in the* SAD *gene, five in intron, eight in exon and two loci in 3' UTR region. These SNP markers provide valuable insights into genetic diversity and offer promising further study to validate these markers using a larger breeding population with a specific target for oleic acid content.*

Keywords: *Elaeis guineensis*, *Elaeis oleifera*, O×G hybrids, oleic acid content, SNP.

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INTRODUCTION

The oil palm (*Elaeis* spp.) is a globally significant supplier of vegetable oil, renowned for its high productivity/ha/yr. Oil palm has diverse uses in either edible or non-edible sectors (Xia *et al*., 2019). Oil palm is a cross-pollinated, monoecious plant belonging to the Arecaceae family (Zaki *et al*., 2021). The *Elaeis* genus has two species: *Elaeis guineensis* from West Africa, which is high-yielding and has high oil content and *E. oleifera* from Central and South America, which has high levels of oleic acid and carries the dwarf and disease resistance characteristics (Goh *et al*., 2017). Since oil palm is perennial, breeding for improved oil palm takes time and requires significant resources (Seyum *et al*., 2021). Therefore, the development and use of molecular markers for supporting oil palm breeding programs is necessary. One of the future oil palm breeding program objectives is developing oil containing high unsaturated fatty acids (Martin *et al*., 2022).

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[1] Applied Botany Research Center,
National Research and Innovation Agency (BRIN),
Science and Technology Park of Dr. (H.C.) Ir. Soekarno,
Jl. Raya Jakarta-Bogor KM 46 Cibinong 16911,
West Java, Indonesia.

[2] Department of Agronomy and Horticulture,
Faculty of Agriculture, IPB University,
Jl. Meranti - Darmaga Campus,
Bogor 16680, West Java, Indonesia.

[3] National Standardization Agency of Indonesia,
J1, Kuningan Barat Raya, No.1A, Kuningan,
Mampang Prapatan, Jakarta Selatan,
DK1 Jakarta 12710, Indonesia

[4] Biotechnology Laboratory,
BRIN, Building 630, Puspiptek Serpong,
South Tangerang, Banten 15314, Indonesia.

* *Correspondence author e-mail: rism004@brin.go.id*

The palm oil from mesocarp comprises saturated fatty acids (FA) (palmitic acid [31.1%-45.1%], stearic acid [2.8%-5.1%] and myristic acid [0.5%-2.1%]) and unsaturated fatty acids (oleic acid [19.1%-43.1%], linoleic acid [8.7%-32.4%] and linolenic acid [0.4%-10.4%] (Suresh & Behera, 2020). The wild oil palm species can be the source of beneficial genes for improving the commercial oil palm cultivars through a breeding program (Martin *et al*., 2022), including improving its unsaturated oil content. Moreover, *E. oleifera* has a high percentage of unsaturated fatty acids, ranging from 72.0%-81.0%, with an oleic acid content of 56.0%-70.0% (Latiff, 2000; Lieb *et al*., 2017). Therefore, *E. oleifera* can be used as a donor to increase unsaturated fatty acid in *E. guineensis*.

The stearoyl-*ACP* desaturase (SAD) is one of the enzymes involved in the oleic acid biosynthetic pathway. The SAD enzyme catalyse the desaturation of stearoyl-ACP (18:0-ACP) by introducing the first double bond, resulting in the production of oleic acid (C18:1) (Parveez *et al*., 2015). Understanding fatty acid metabolisms can help marker-based breeding for improved fatty acid compositions (Zafar *et al*., 2020). Single nucleotide polymorphisms (SNPs) are the most prevalent genetic variations and sound indicators for assessing genetic diversity. The SNPs are also valuable for marker development, selecting desirable breeding materials and identifying genes linked to economically significant phenotypes (Yirgu *et al*., 2023). The presence of genetic polymorphisms may have an impact on the genetic composition and variability within populations (Ali *et al*., 2018). This study aims to identify the *SAD* nucleotide sequence variations to develop SNAP primers and test the informativeness of the SNAP markers among diverse oil palm accessions. The generated SNAP markers could aid the future breeding of oil palm for increasing the oleic acid content.

MATERIALS AND METHODS

Plant Materials

Five oil palm accessions comprise an American oil palm species (*E. oleifera*), and four accessions of African oil palm species *E. guineensis* (*Dura*, *Pisifera* and *Tenera* and a virescens variant) were used to isolate the partial *SAD* gene. The *Dura*, *Pisifera* and *Tenera* types of *E. guineensis* were differentiated based on their fruit shell thickness. In contrast, the *Virescens* variant was identified based on its green immature fruits that turned to orange when matured (*Figure 1*). Meanwhile, the normal *E. guineensis* immature fruits are red to purple in colour or nigrescens. Fifty samples of oil palm accessions belonging to the five groups were collected from the field and tissue culture collection materials at the Biotechnology Laboratory of the National Research and Innovation Agency (BRIN), Indonesia. The selected oil palm samples represent various oil palm genetic backgrounds.

DNA Extraction

DNA was isolated from oil palm leaves using a CTAB method of Doyle and Doyle (1990). Leaf samples (0.5-1.0 g) were homogenised in liquid nitrogen and mixed with 700 µL of CTAB extraction buffer. The mixture was vortexed and incubated at 65°C for 30 min. After cooling, 700 µL of chloroform-isoamyl alcohol (CIA, 24:1) was added to the

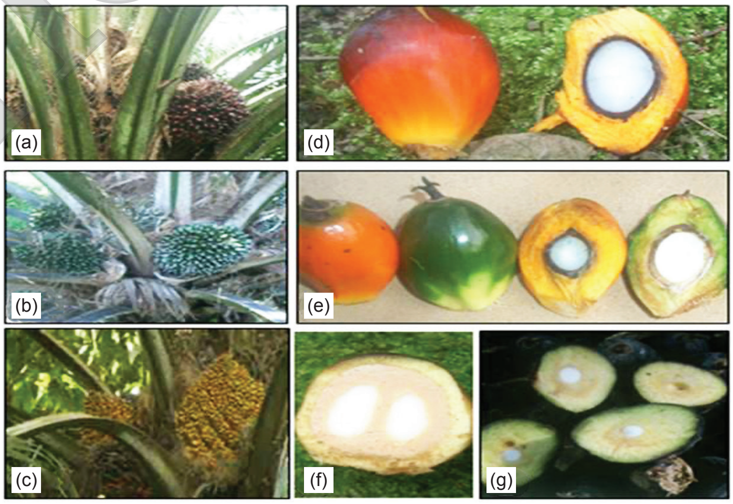


*Figure 1. The representative phenotypic characteristics of oil palm samples used for DNA isolation. (a) An African oil palm (*Elaeis guineensis*) with red to purple fruits (*nigrescens*), (b) a *virescens *variant of* E. guineensis *with green fruits (*virescens*), (c) an American oil palm (*E. oleifera*), Representatives of the (d)* Tenera *type fruits of* E. guineensis (nigrescens), *(e)* virescens *fruits of* E. guineensis (virescens), *(f)* Dura *type fruits of* E. guineensis *and (g)* Pisifera *type fruits of* E. guineensis.

mixtures and they were centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was transferred to a new tube. The CIA extraction procedure was repeated twice to obtain high-purity DNA. After absolute ethanol precipitation, the DNA pellet was washed with 70% ethanol and air dried. The dried DNA pellet was dissolved in 50 µL of TE buffer. The quality and quantity of isolated genomic DNA were checked using agarose gel electrophoresis and nanodrop.

Primer Design for *SAD*-Specific Amplicon

The *SAD* gene-specific primers were designed based on the *SAD* gene sequences available in the NCBI DNA Database, such as *SAD* gene (U68756) isolated from the *Tenera*; FJ940768 and EU057621 for the *E. oleifera* species. The primer design used Primer3 software (version 0.4.0) developed by Rozen and Skalestsky (2000). A Basic Local Alignment Search Tool (BLASTn) search was conducted as described by McGinnis and Madden (2004) to confirm that the complementary sequence of the primers were unique.

PCR Amplification of *SAD* Gene, Electrophoresis and DNA Sequencing

PCR reactions to amplify the *SAD* gene were conducted with a total volume of 20.0 µL, comprised 4.0 µL of 5x Phusion HF buffer, 0.4 µL of 10.0 µM dNTPs, 1.0 µL each of 10.0 µM forward and reverse primers, 0.2 µL of Phusion Hot Start II DNA polymerase, 100 ng of DNA template and the remaining volume filled with double distilled water (ddH$_2$O). The PCR reaction was done using the following steps: 98°C for 30 s for initial denaturation, followed by 30 cycles of 98°C for 5 s of template denaturation, 52°C for 30 s of primer annealing and 72°C for 15 s of primer extension. A final step of primer extension at 72°C for 5 min was added at the end of the cycles. The generated PCR amplicon were separated using 1% agarose gel electrophoresis performed in TAE buffer. The agarose gel's separated amplicon was visualised under UV light using a transilluminator and photographed. To estimate the amplicon size, 1 kb DNA ladders were included in the agarose gel electrophoresis as DNA size markers. The desired amplicon was cut from agarose gel, purified and sequenced using the ABI 3130 Genetic Analyser (Applied Biosystems). After trimming for the low-quality sequences, the identity of the determined amplicon fragment nucleotide sequences was subjected to the Basic Local Alignment Search Tool (BLAST) analysis against all nucleotide accessions in the NCBI GenBank DNA Database using the online BLAST 2.0.

SNP Identification, SNAP Primer Design and Analysis

The confirmed *SAD* gene sequences were aligned using CLUSTALW for SNP identification in the BioEdit software (Hall, 1999). The SNPs within the *SAD* gene coding regions and the amino acid changes were further analysed using Geneious software (Kearse *et al*., 2012). Subsequently, the identified SNP loci were used to design SNAP primers using the Web SNAPER program at https://pga.mgh.harvard.edu/cgi-bin/snap3/websnaper3.cgi (Drenkard *et al*., 2020). The designed SNAP primers for each SNP locus were used to PCR amplify the SNAP markers using five oil palm DNA templates and to validate the informativeness of the markers. PCR amplification of the SNAP markers was carried out using the Phusion Hot Start II DNA polymerase.

The PCR reaction was performed using the following steps: 98°C for 30 s for initial denaturation, followed by 30 cycles of 98°C for 5 s of template denaturation, 52°C for 30 s of primer annealing with annealing temperature ranging from 48°C-53.7°C (depending on the primers used), and 72°C for 15 s of primer extension. A final step of primer extension at 72°C for 5 min was added at the end of the cycles. The generated PCR amplicon was separated using 1% agarose gel electrophoresis in TAE buffer. The agarose gel's separated amplicon was observed under UV light using a transilluminator and photographed. To estimate the amplicon size, 100 bp DNA ladders were included in the agarose gel electrophoresis as size references.

Fifty oil palm accessions were genotyped using the developed SNAP markers. The sample's genotypes were determined based on the presence or absence of the reference (Ref) or alternate (Alt) amplicons. The Ref-amplicon was PCR amplified for each SNAP locus using a pair of Ref forward and Reverse (Rev) primers. At the same time, the Alt-amplicon was PCR amplified using a pair of Alt forward and Rev primers. Finally, cluster analysis was done based on the score of the observed SNAP markers using DARWIN software (Perrier & Jacquemoud-Collet, 2006).

Three-Dimensional (3D) Protein Modeling

The SAD protein three-dimensional (3D) modeling was performed using the SWISS-MODEL, accessible at https://swissmodel.expasy.org (Waterhouse *et al*., 2018), based on the translated oil palm's partial SAD amino acid residues. The changes in amino acid residues because of the SNP loci within the coding region were included in the 3D target model.

RESULTS AND DISCUSSION

Oleic Acid Composition and *SAD* Gene Variation

The fatty acid composition of vegetable oil significantly impacts its nutritional and economic value (Zhu *et al*., 2019). Vegetable oils having the appropriate mix of fatty acids can offer health benefits and prevent cardiovascular disease (Liu *et al*., 2020; Lu *et al*., 2024). The oleic acid content has been associated with multiple genes *SAD*, *FabD*, *LACS6*, *BC*, *FabB* and *FabI* (Xu *et al*., 2025). The key gene related-to-oleic acid biosynthesis is the *SAD* gene (Ayed *et al*., 2018). The varying fatty acid composition in *E. guineensis* and *E. oleifera* could reflect the variety of gene sequences responsible for oleic acid production. The reported oleic acid content of palm oil derived from *E. oleifera* germplasm ranges from 56.5%-70.5%, significantly higher than *E. guineensis,* which is on average 39.0% (Latiff, 2000). Such information indicates that *E. oleifera* could be the donor to improve oleic acid content in *E. guineensis*. As such, the association between the *SAD* gene sequence variations between *E. oleifera* and *E. guineensis* is an interesting subject for investigation, and the nucleotide sequence variations can be used to develop the *SAD* gene-specific markers. Hence, the marker-assisted selection for high oleic acid content in oil palm breeding should be possible.

The total DNA from five oil palm samples was isolated and used as the template for PCR amplification of the *SAD* genes using a pair of the designed SAD1 and SAD2 primers (*Table 1*). The BLASTn results of the designed SAD1 and SAD2 primers show 99% sequence identity to the *SAD* reference sequences of *E. guineensis* (EGU68756) and *E. oleifera* (FJ940768).

The diagrammatic *Figure 2a* represents the *SAD* gene structures, the relative positions of the designed SAD-specific primers (SAD1 and SAD2 primers) and the expected sizes of the PCR amplicons. The amplicon size using the SAD1 primer pair is an 1,100 bp fragment comprising partial exon 2, intron 2 and partial exon 3 of the *SAD* gene. The amplicon size using the SAD2 primer pair is a 600 bp fragment, comprising partial exon 3 and 3'–untranslated region (UTR) (*Figure 2a*). The representative gel photograph of the SAD1 and SAD2 amplicons using the appropriate primers and the DNA template from four African oil palm species (*E. guineensis, Dura, Pisifera, Tenera* types and a *Virescens*) and an American oil palm (*E. oleifera*) is presented \in *Figure 2b.*

Partial *SAD* Amplicon Sequences

The assembled partial *SAD* gene from SAD1 and SAD2 amplicon sequences resulted in a combined fragment of 1,474-1,905 bp nucleotide sequences. The identity of the amplicon is confirmed by BLAST analysis against all available nucleotides in the NCBI GenBank DNA Database. The BLAST analysis results confirm that the amplicon sequences are partial sequences of the oil palm *SAD* gene with 99.6%-100.0% sequence identity and the date palm *SAD* gene with 93.6%-94.6%. These results confirm that the sequenced amplicon is a partial *SAD* gene from five oil palm samples.

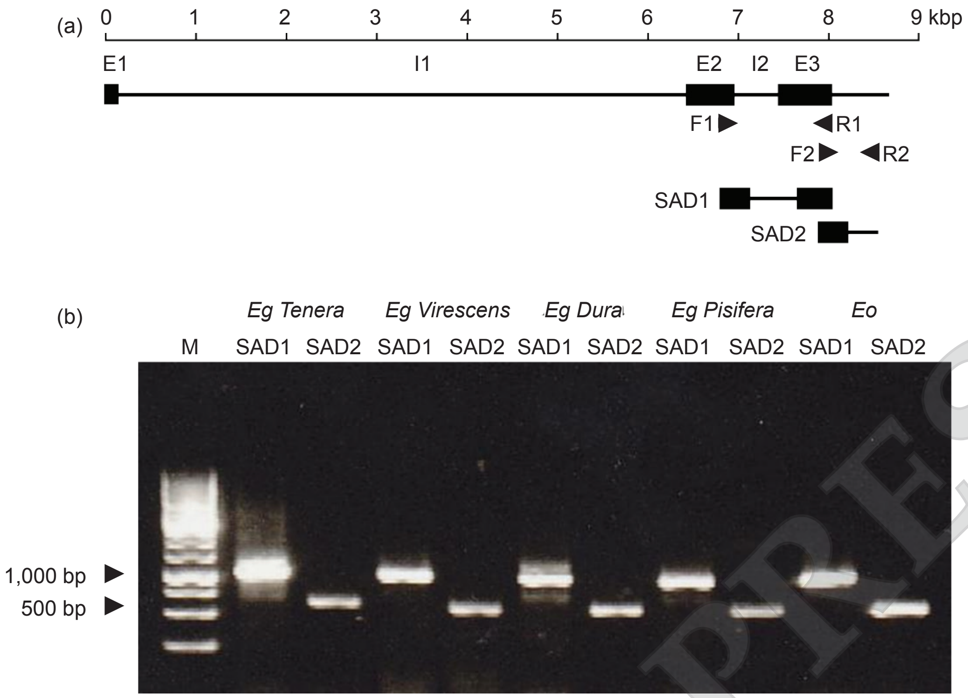
The position of the partially determined *SAD* gene sequences against the reference *SAD* gene (KM979557) is presented in *Figure 3a*. Meanwhile, representative sequences of the determined partial *SAD* gene from the Pisifera type of *E. guineensis* sample number P.46 are presented in *Figure 3*. The P.46 and the other four oil palm samples' partial *SAD* gene comprising partial exon 2 (148 bp) at nucleotide positions 6,835-6,982, intron 2 (508 bp), at 6,983-7,490, a full exon 3 (561 bp), at 7,491-8,051 and 3' UTR (varied in length), at position >8,051 of the *SAD* gene reference (KM979557). All the partial *SAD* gene sequences determined in this study have been submitted to the NCBI DNA Database under accession no. KR84119, KR84120, KR84121, KR84122 and KR184123.

SNP Loci Identification and Design SNAP Primer

Multiple sequence alignment (MSA) among the oil palm partial *SAD* sequences of five studied samples and four oil palm *SAD* sequences available in the NCBI GenBank DNA Database reveals the existence of 15 SNPs. There is no SNP in the partial 148 bp exon 2 of the *SAD* gene, but there are eight SNP loci in the 561 bp Exon 3 (*Figure 3*). Intron 2 (508 bp) of the studied partial *SAD* gene contains five SNPs, while the 3' UTR contains two (*Figure 4*).

TABLE 1. SEQUENCES OF STEAROYL ACP DESATURASE (SAD) GENE-SPECIFIC PRIMERS TO AMPLIFY PARTIAL SAD GENE FROM FIVE OIL PALM SAMPLES

Primer names	Forward primer sequences (5' – 3')	Reverse primer sequences (5' – 3')	Amplicon sizes (BP)
SAD1	ATGCTCAACACCCTTGATGG	GGCATAGTCCTTGGCTGTGT	1,100
SAD2	CCTGCCCATCTGATGTATGA	CGAGCAAGAACTAGGTTCCA	600



Note: Eg – *Elaeis guineensis*; Eo – *Elaeis oleifera*; *Tenera*; *Dura* and *Pisifera* are different types of *E. guineensis* oil palm – differentiated based on their shell thickness. Virescens is an *E. guineensis* variant having green immature fruits.

Figure 2. Diagrammatic representation of the stearoyl-ACP-desaturase (SAD) *gene structure and the generated partial SAD amplicons (SAD1 and SAD2). (a)* SAD *gene structure with three exons and two introns. The positions of SAD1 and SAD2 primer pairs and the expected amplicons are noted. (b) The gel photograph shows SAD1 and SAD2 amplicons from five oil palm samples.*

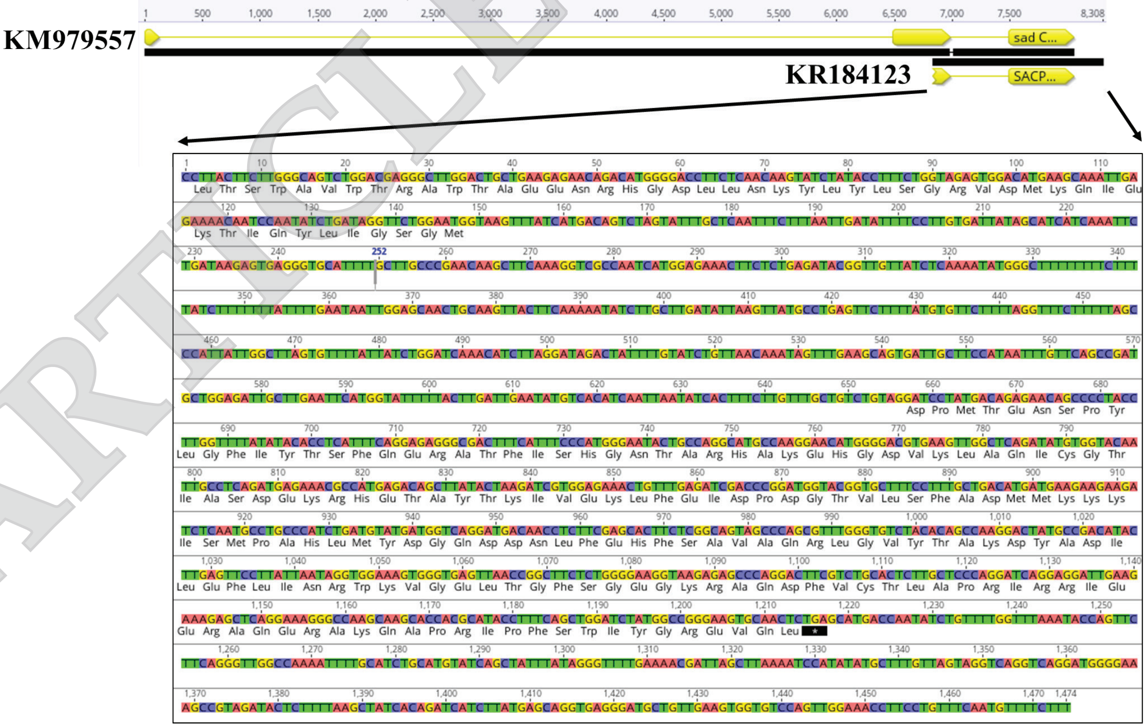


Figure 3. Assembled partial stearoyl-ACP desaturase (SAD) *gene nucleotide sequences from* Pisifera *type of* Elaeis guineensis *sample No. P.46. (a) The position of the determined sequences against the reference of the SAD gene (KM979557). (b) Nucleotide sequences and the translated amino acid residues of the partial SAD gene. The partial* E. guineensis *sample P.46 partial SAD gene sequences have been submitted to the NCBI DNA Database under accession No. KR184123.*

Out of eight SNPs in exon 3 of the *SAD* gene, seven SNP loci are non-synonymous and one locus is synonymous. The nucleotide substitution in the non-synonymous SNPs results in amino acid residue changes, while no amino acid residue changes in the synonymous SNPs. Moreover, four out of seven non-synonymous SNP loci in exon 3 contain *E. oleifera*-specific alleles (*Figure 3*). One out of five SNP loci in the intron 2 of the oil palm's partial *SAD* gene contains an *E. oleifera* specific allele, and so do the two SNP loci in the 3' UTR.

The identified SNP loci in the partial *SAD* gene of oil palm were used to design the SNAP primers. However, only 12 SNAP primer sets were successfully designed out of the 15 SNPs (*Table 2*). The designed SNAP primers were subsequently used to generate SNAP markers by PCR. Out of 12 SNAP primer sets, 11 generate informative SNAP markers and one failed (*i.e.*, SNAP7937). Representative samples of the generated SNAP marker profiles are presented in *Figure 5*. The genotyping using 11 SNAP marker loci indicated that some of the evaluated samples are homozygous for the Reference allele (Ref/Ref homozygous), some are homozygous for the Alternate allele (Alt/Alt homozygous) and the others are heterozygous for both alleles (Ref/Alt heterozygous, *Figure 5*). The identified *E. oleifera*'s specific SNPs may be used to develop *E. oleifera*'s specific SNAP markers, and they can be evaluated for their association with oleic acid content in the *E. oleifera* fruits and their hybrids. Evaluation of such associations is needed in the future.

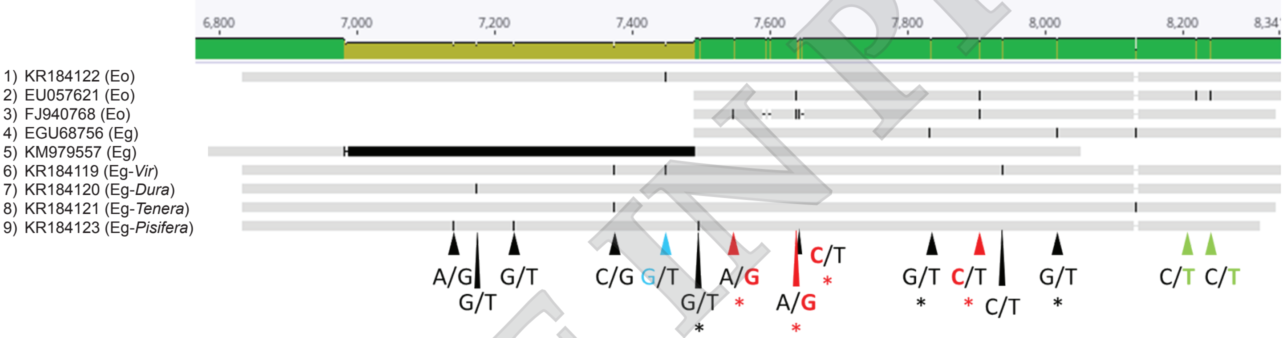


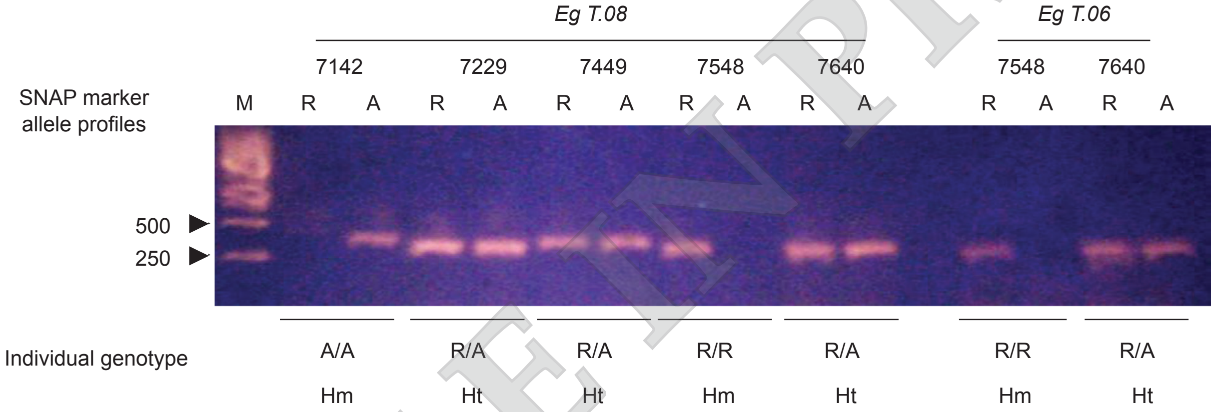
Figure 4. Identification of the SNP loci in the partial SAD *gene. Five SNPs are identified in the intron-2, 8 in the exon-3 and 2 in the 3'-untranslated regions (3' UTR). The SNPs in the exon-3 consist of 6 non-synonymous (*) and 2 synonymous and 2 SNPs in 3' UTR. The alleles in blue, red and green are the* Elaeis oleifera*-specific alleles in intron 2, exon 3 and 3' UTR.*

TABLE 2. IDENTIFIED SNPs, ALTERNATE ALLELES, DESIGNED SNAP PRIMER SETS AND EXPECTED AMPLICON SIZES

SNP location	SNP loci	Alternate alleles	Forward primer (5' – 3')	Common reverse primer (5' – 3')	Amplicon sizes
Intron-2	7142	Ref (A)	GGAGAAACTTCTCTGAGATGCA	GATCCTACAGACAGCAAACAAGA	374
		Alt (G)	GGAGAAACTTCTCTGAGATCCG		374
	7229	Ref (G)	AAAAGAACTCAGGCATAACTTAATATC	TACCTTTCTGGTAGAGTGGACATGAAG	354
		Alt (T)	AAAAGAACTCAGGCATAACTTAATTTA		354
	7375	Ref (G)	CAAATTATGGAAGCAATCGC	TCAAATTCTGATAAGAGTGAGGGT	340
		Alt (C)	GAACAAATTATGGAAGCAATGAG		340
	7449	Ref (T)	CATGGTATTTTTACTTGATTGAACAT	TTGTCATCCTGACCATCATACA	374
		Alt (G)	CATGGTATTTTTACTTGATTGAACAG		374
Exon-3	7498	Ref (G)	ATAGGTTCTGGAATGGATCCGAG	TGGGCTACTGCCGAGAAGTG	344
		Alt (T)	TGATAGGTTCTGGAATGGATCCTCT		344
	7548	Ref (A)	TATGTGGTACAATTGCCGCA	GAGCAAGAGTGCAGACGAAG	330
		Alt (G)	TATGTGGTACAATTGCCCCG		330
	7640	Ref (A)	TGGGAAATGAAAGTCGTCCT	TTATTATGTTTGCTTGGTTGGAG	330
		Alt (G)	TGGGAAATGAAAGTCGCACC		330

TABLE 2. IDENTIFIED SNPs, ALTERNATE ALLELES, DESIGNED SNAP PRIMER SETS AND EXPECTED AMPLICON SIZES (continued)

SNP location	SNP loci	Alternate alleles	Forward primer (5′ – 3′)	Common reverse primer (5′ – 3′)	Amplicon sizes
Exon-3	7833	Ref (T)	GCATAGTCCTTGGCTGGGTA	GGACAGAGAACAGCCCCTAC	354
		Alt (G)	GCATAGTCCTTGGCTGAGTC		354
	7905	Ref (T)	GTAAGAGAGCCCAGGACGTT	GCTCATAAGATGATCTGTGATAGCT	366
		Alt (C)	GGTAAGAGAGCCCAGGAGTTC		366
	7937	Ref (C)	AGTGGGTGAGTTAACCGGAC	GCTCATAAAATGATCTGTGATAGCT	361
		Alt (T)	AGTGGGTGAGTTAACCGCCT		361
	8017	Ref (T)	CAAGCACCACGCATACATTT	TTTTCCTACATAGGAGTACTAATACACG	341
		Alt (G)	CAAGCACCACGCATACCATG		341
3′ UTR	8240	Ref (C)	CATAAAATGATCTGTGATAGCTTAGAAG	AGGTAAGAGAGCCCAGGACTT	328
		Alt (T)	ATAAAATGATCTGTGATAGCTTACAAA		328



Note: R – reference, A – alternate alleles, Hm – homozygous, Ht – heterozygous individuals and Eg – *elaeis guineensis*.

Figure 5. Gel photographs of the generated SNAP marker allele profiles of various SNAP marker loci from representative Elaeis guineensis *(Eg) sample No. T.06 and T.08. Lanes 1-2 are SNAP marker profiles of homozygous A/A individual genotypes. Lanes 7-8 and 11-12 are homozygous R/R individual genotypes, while the rest are profiles of heterozygous R/A individual genotypes.*

Oil Palm Genetic Diversity Based on the SNAP Markers

Genotyping of the 50 genetically diverse oil palm species was done by PCR using 11 SNAP primer sets. The possible oil palm genotypes based on the SNAP marker profiles are presented in *Figure 5*. The genotype frequencies of the evaluated oil palm samples are presented in *Table 3*.

Genotyping *E. oleifera* using two SNAP primer sets (SNAP7375 and 7498) generates no amplicon. Therefore, those two SNAP primer sets cannot be used to genotype *E. oleifera*. PCR using four SNAP primer sets (SNAP 7833, 7548, 7640 and 8017) and the *E. oleifera* DNA template generated amplicon only from the Ref primer pair and did not generate amplicon from the Alt primer pair. Therefore, the genotype of *E. oleifera* for those four loci is Ref/Ref homozygous (*Figure 5*). On the other hand, PCR using the SNAP7142 primers and the *E. oleifera* DNA template generates an amplicon only for the Alt primer pair but not for the Ref primer combination. Therefore, the genotype of *E. oleifera* for the SNAP7142 primers is an Alt/Alt homozygous. In contrast, PCR using the other four primer sets (SNAP7229, 7449, 7905 and 8240) generates amplicons for both the Alt and Rev primer pairs, indicating *E. oleifera* genotypes are Ref/Alt heterozygous that for those loci (*Table 3*). Meanwhile, for the O×G hybrid, the genotypes of the samples are either Ref/Ref homozygous (5 loci) or Ref/Alt heterozygous (6 loci). The genotype frequencies of *E. guineensis*, *Dura*, *Pisifera* and *Tenera* types, and the virescens variant are also presented in *Table 3*.

TABLE 3. GENOTYPING OF OIL PALM SAMPLES USING 11 SNAP MARKER LOCI AND GENOTYPE FREQUENCIES BASED ON THE MARKERS

Oil palm samples (sample numbers)	Average genotype frequency (Loci no.: 11)			
	Unidentified genotype	Ref/Ref homozygous	Alt/Alt homozygous	Ref/Alt heterozygous
E. guineensis (G):				
Dura type (5)	3	3	1	4
Pisifera type (10)	2	3	1	5
Tenera type (29)	2	3	1	5
Virescens variant (4)	2	3	1	5
E. oleifera (O, 1)	2	4	1	4
O x G hybrid (1)	0	5	0	6

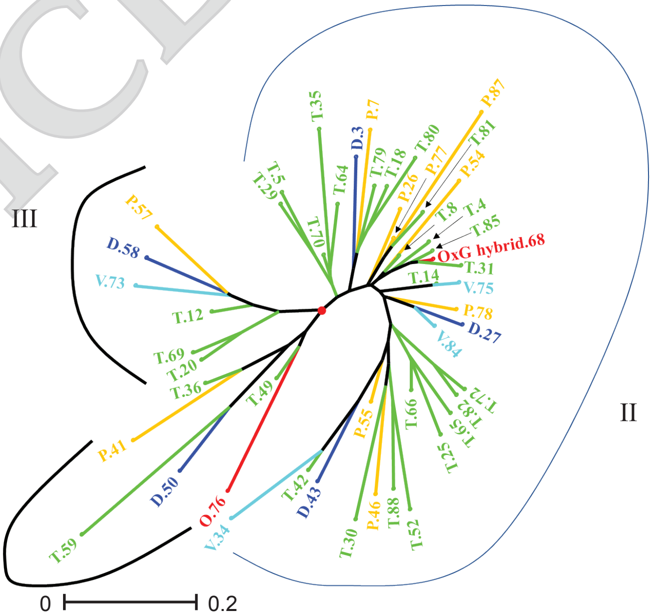
Note: Ref - reference allele, Alt - alternate allele.

Subsequently, the genotyping results were used to construct a phylogenetic tree of 50 oil palm samples using 11 SNAP marker loci, and the results are presented in *Figure 6*. Based on the phylogenetic analysis results using 11 loci genetic data, 50 oil palm samples are grouped into Groups I, II and III. The Group I member includes *E. oleifera* O.76, *E. guineensis* D.50, P.41, T.36, T.49 and T.59. The Group II members include one O×G hybrid, 3 *Dura*, 8 *Pisifera*, 23 *Tenera* types and 3 virescens of *E. guineensis*, while Group III members are *E. guineensis* D.58, P.57, T.12, T.20, T.69 and V.73.

The SAD Protein 3-D Structure and SNPs

The SNP loci identified in the exons of the *SAD* gene's nucleotide sequences are either synonymous or non-synonymous. Four SNPs at 7548, 7640, 7643 and 7905 were detected in the coding region of the *SAD* gene related to the *E. oleifera* species (*Figure 6*), at 7548 loci arginine (R) change to glycine (G), at 7640 serine (S) change to glycine (G), at 7643 aspartic acid (D) change to arginine (R) and at loci 7905 phenylalanine (F) change to leucine (L), leading to distinct amino acid variations compared to *E. guineensis.* The non-synonymous SNP changes the amino acid impacts the protein structure of the *SAD* gene. The SNPs responsible for variations in amino acid sequences influence protein structure changes, which impact specific characters (Gulzar *et al.*, 2023).

Variations in the genetic code can lead to diverse amino acid sequences that influence protein structure and gene expression. These changes can occur at the level of base pairs, a single piece of



Note: D - *Dura*, P - *Pisifera*, T - *Tenera* types and V - a virescens mutant of *E. guineensis*. O - *E. oleifera* and O×G hybrid - A hybrid between *E. oleifera* × *E. guineensis*.

Figure 5. Phylogenetic analysis of 50 oil palm accessions constructed using genotype data from 11 SNAP marker loci. The SNAP marker loci were developed based on the SNPs in the SAD *gene of* Elaeis guineensis, E. oleifera *and their hybrid.*

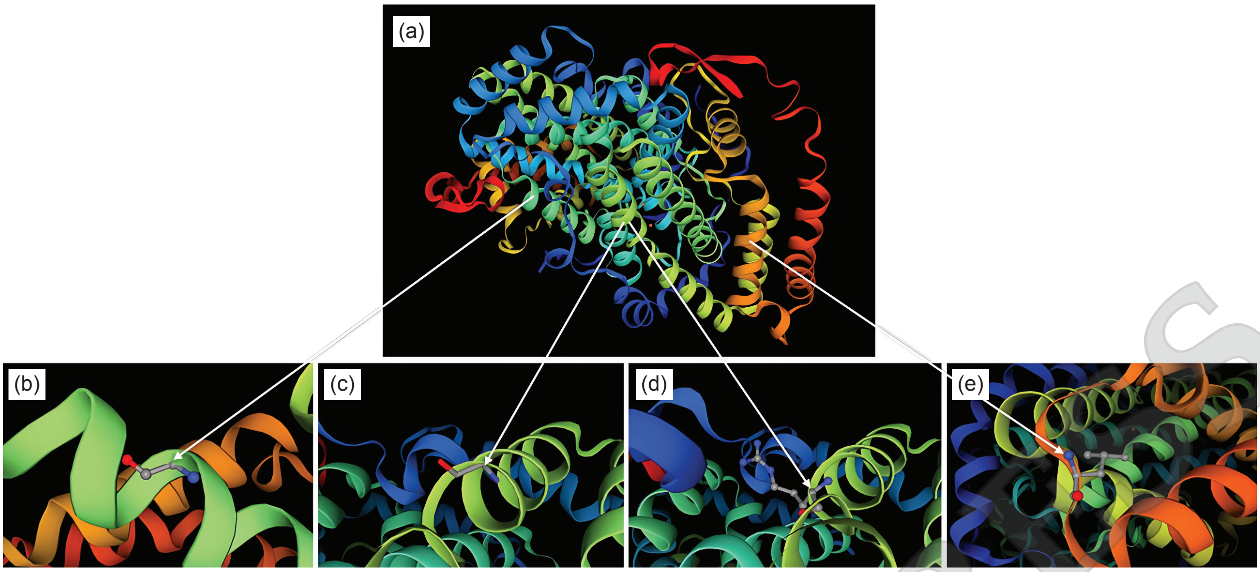


Figure 6. (a) Three-dimensional protein structure homology models of the oil palm SAD *gene, using the amino acid of the* Elaeis oleifera *FJ940678 accession with the template delta9 stearoyl-acp desaturase of the castor seed with 83.73% sequence identity. The position of non-synonymous amino acids at 4 loci, 7548, 7640, 7643 and 7905, change protein structure and may influence gene function. (b) at 7548 arginine (R) change to glycine (G), (c) at 7640 serine (S) change to glycine (G), (d) at 7643 aspartic acid (D) change to arginine (R) and (e) at loci 7905 phenylalanine (F) change to leucine (L). The models were generated and visualized by the SWISS-MODEL Molecular Graphics System. The models were generated and visualized by the SWISS-MODEL Molecular Graphics System.*

DNA, or even a cell. The variety of mutations is influenced by different rates of DNA repair and the presence of transposable elements. Mutation rates vary significantly depending on factors such as the type of mutation, location within the genome, gene function, epigenomic settings, external influences, race and species (Quiroz *et al*., 2023).

SNP markers are critical in the selection process to enhance fatty acid content (Bueno *et al*., 2018). It assists with quality control analysis for closely related species and germplasm management programs (Gouda *et al*., 2021). SNP sequence target is more competitive than existing genotyping techniques because of its high efficiency and low cost, and it shows promising application possibilities in genetic research and plant breeding (Zhang *et al*., 2020). These studies provide a breeding option that allows for targeted breeding at specific loci of the gene sequence to develop oil palms with high oleic acid, and they also serve as tools for early detection of high-oleic acid genotypes. It can be utilised to further study the potential role of the identified *SAD* SNPs in enhancing oleic acid content in oil palm to alter the fatty acid composition.

CONCLUSION

This study identified a total of 15 SNPs in the *SAD* gene, 5 in intron, 8 in exon and 2 loci in 3′ UTR region. This information can be utilised in future studies, especially on the potential role of identified *SAD* SNPs in enhancing oleic acid content in oil palm. The study emphasises the regulatory function of the combined SNPs in the coding region of the exon with the non-coding region, the intron, and 3′-UTR for oleic acid biosynthesis. Future studies could also use these markers for an expanded breeding population aimed at changing oleic acid content, and advancing comprehensive knowledge of the genetic control of oleic acid biosynthesis.

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