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DEVELOPMENT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) MARKER FOR OLEIC ACID CONTENT IN OIL PALM (*Elaeis guineensis* Jacq.)

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ABSTRACT

Oil palm (*Elaeis guineensis* Jacq.) is an important economic crop species due to the versatile applications of its crude and kernel oils. The necessity to improve oil palm (*Elaeis guineensis* Jacq.), which is the world's most productive oil producing plant under cultivation currently for high yield and oil quality to meet the growing global demand especially in term of its healthy component, oleochemical industrial needs and biodiesel utilization has become a major area of focus for oil palm breeders. Marker assisted selection have played a crucial role in oil palm breeding programs and single nucleotide polymorphism (SNP) maker is one of those technologies that has recently attracted breeders. High content of oleic acid (18:1) has proven to be that needed component of oil palm and other vegetable oils that can greatly contribute to oil quality improvement. Stearoyl Acyl-carrier-protein Desaturase (SAD) is a key enzyme for oleic acid biosynthesis which plays an important role in determining the composition of unsaturated fatty acids in oil palm. In this study, we identified 9 SNP loci (4 in the exon and 5 in the intron) in SAD gene fragment and developed an allele-specific single nucleotide amplified polymorphism (SNAP) marker for oleic acid content prediction. . Four out of the nine SNAP marker developed and tested on 25 oil palm accessions were polymorphic and reasonably informative. The average expected and observed heterozygosities were 0.391 and 0.404, respectively. The mean polymorphism information content (PIC) was 0.312. Our results showed that these SNAP markers will be useful if validated in larger oil palm population in predicting oleic acid composition and genetic variation in oil palm breeding programs based on SAD gene fragment.

Keywords: Allele-specific; expected heterozygosities; observed heterozygosities; oil quality; oleic acid; polymorphism information content (PIC)

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the most productive oil producing plant under cultivation, with typical yields of 4.1 tons of oil per hectare per year with Indonesia and Malaysia been the leading palm oil producing countries in the world contributing 50% and 34% respectively to the global palm oil production (Oil World 2016). Palm oil is a rich nutritional source of vitamins, carotenoids, iron, antioxidant activity as well as its demand for biodiesel production and it's use in the oleochemical industry (Sundram *et al.*, 2003). Oil palm is a perennial and diploid monocotyledon plant with 16 chromosome pairs and belongs to the genus *Elaeis* which consist of species namely, African oil palm (*Elaeis guineensis* Jacq.) and American oil palm (*Elaeis oleifera*) (Maria *et al*., 1995). Because of high oil yield, African oil palm is highly favored for the commercial basis of cultivation (Edem *et al.*, 2002 and Munira*et et al.*, 2008). However, the American oil palm species is also of economic interest; and it is an important source of different traits such as slow growth of the stem, resistance to bud rot disease, higher composition of unsaturated fatty acid (oleic acid) that can be exploited in traditional/molecular breeding and genetic engineering (Singh *et al*., 2007).

Palm oil composition and quality is based on it fatty acid component, of which palmitic acid (45%) and oleic acid (40%) are the two-abundant saturated and unsaturated fatty acid respectively (Sambanthamurthi *et al.*, 2000). Vegetable oils with a high content of oleic acid (18:1) are of interest for both food and industrial purposes. Stearoyl-ACP desaturase (SAD) gene has an important role in the process of biosynthesis of oleic acid and other unsaturated fatty acids in palm oil (Parveez *et al*., 2014). Its convert the stearoyl-ACP to oleoyl-ACP in the biosynthesis of oleic acid in oil palm (Sambanthamurthi *et al.*, 1996).

The rising economic importance of oil palm has led to increased efforts through the use of biotechnology for the genetic improvement of this crop. The genetic enhancement of oil palm has been aimed at producing new cultivars that carry elite traits, such as an increased oil yield, enhanced carotenoid synthesis, increased vitamin E (Wahid *et al*., 2005) and oleic acid (Parveez *et al*., 2004) contents. Despite the progress made in cultivation,

additional gains in agricultural productivity are needed at an ever-faster pace due to competition from other vegetable oils and fats. Although traditional breeding continues to play an important role in yield enhancement, it is impeded by the long selection cycle of approximately 7 to 10 years and the requirement of enormous resources. Therefore, many oil palm breeders and industries are interested in a technology that can help them achieve high yield and quality of oil at an earlier growth stage.

Single nucleotide polymorphism (SNP) is a variation at a single nucleotide in DNA sequence among individuals of same species and is the most abundant class of polymorphisms in plant, animals and human genomes and plays a major role in the induction of phenotypic variations (Buckler *et* a*l.,* 2002 and Rafalski *et al*., 2002). Compared to SSR markers, SNP analysis can be done without requiring DNA separation by size and therefore, can be automated in high throughput assay formats. The diallelic nature of SNPs offers much lower error rate in allele calling and raises the level of consistency between laboratories. These advantages have resulted in SNPs increasingly becoming the markers of choice for accurate genotype identification and diversity analysis in perennial crops, as recently demonstrated in Theobroma cacao (Fang *al et*., 20014), Citrus maxima and strawberry (Wu *al et.,* 2014). The development of high-throughput sequencing technologies in recent years has greatly assisted association studies that utilize SNP markers (Meuwissen *et al.,* 2007).

Several studies on SNP marker for oil palm have been conducted including SNP marker application in genetic diversity (Ong *et al.,* 2015), transcriptomic analysis in oil palm (Tangphatsornruang *et al.,* 2013) and intra-gene SNP base on cDNA of interspecific pseudo-backcross of two oil palm species (Montoyo *et al.,* 2013). However, studies on SNP marker for oleic acid content and diversity of DNA sequence as they relate to SAD gene in oil palm species is yet to be conducted. In this study, we isolated Stearoyl-ACP desaturase gene fragments from 3 sources of oil palm including the African oil palm (*Elaeis guineensis* Jacq.), the American oil palm (*Elaeis oleifera*) and the Hybrid (*Elaeis oleifera*× *Elaeis guineensis* Jacq.) and identified SNPs residing in SAD gene fragments. In addition, we carried on genetic diversity analysis and developed an allele-specific single nucleotide amplified polymorphism (SNAP) marker based on the 9 identified SNPs to allow direct marker assisted selection (MAS) for oleic acid content prediction in oil palm at an early growth stage, with less resources and time.

MATERIALS AND METHODS

Place and Time of the Research: This research was conducted at the Genomic and Transcriptomics Section laboratory, Biotechnology Department, Plant Production and Biotechnology Division PT SMART Tbk, in Sentul City, Republic of Indonesia from August 2016 to February 2017.

Extraction of genomic DNA: Genomic DNA were extracted from leaves sample of three source of oil palm namely *E. Oleifera*, *E. Guineensis* and the *Hybrid* (*E. Oleifera* × *E. Guineensis*) obtained from the Sinarmas oil palm plantation/breeding program in Kalimatant and Riau and the Bogor botanical garden using the NuceloSpin Plant II Kits (Macherey-Nagel, Germany). The genomic DNA quality was observed on 1 % agarose in 1× TAE buffer using gel electrophoresis method while the quantity was measured on a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA). Electrophoresis was performed at 100 volts for 30 minutes and the result was then visualized using ultraviolet (UV) light transiluminator gel doc (Bio-Rad Lab Laboratories U.S.A).

Design of SAD specific primers: Specific primers were designed by accessing sequence data of SAD gene of *Elaeis guineensis* deposited in the database of National Center for Biotechnology Information (NCBI) with accession numbers XP_0109267-34.1 and XP_010929790.1. Exon areas were mainly used with the addition of small component of introns to design the specific SAD primers using Primer3plus software (http://.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi).

Evaluation of effectiveness of SAD specific primers: To test the specificity of the SAD primers, 2 genotypes of *E. Oleifera*, 2 genotypes of *E. Guineensis* and 2 genotypes of *Hybrid* of oil palm were amplified using PCR method. PCR amplification was carried out using KAPA HiFi Hot Start Enzyme Kit (KAPA BIOSYSTEMS, USA). A total PCR mix was 50µL prepared according to manufacturer's instruction which included 10µL of KAPA HiFi 5X Buffer, 1.5µL of KAPA dNTP, 15µL of forward and15µL of reverse primers (SADE 27, SADE 29 and SADE 39), 1µL of KAPA Hot Start DNA polymerase, 1.0µL DNA template, and 5.5µL of dH_2O. The PCR amplification was carried out as follows, initial denaturation at 95^0C for 5 min, denaturation at 98^0C for 20 s, annealing at 66-68^0C for 15 s, extension 72^0C for 15s with final extension at 72^0C for 1min and run for 32 cycles.

Qualitative evaluation of PCR product: Amplified product was qualitatively analyzed using agarose gel electrophoresis and gel doc apparatus. 0.8grams of agarose (1%) was added to 80 ml of 1x

TAE buffer, then homogenized using a microwave. 2µL PCR DNA sample and 2µL loading dye was mixed and then loaded into wells of the agarose gel. Electrophoresis was performed at a voltage of 100 volts for 30 minutes and the result was then visualized using ultraviolet (UV) light transiluminator gel doc (Bio-Rad Lab Laboratories U.S.A). The size of the amplified product was estimated using 1kb DNA ladder (Bio-Rad Lab Laboratories USA).

Purification of PCR product and sequencing: PCR products (single band) were purified using QIAquick PCR Purification Kit (QIAGEN Group). Purified samples were sent for sequencing to Malaysia (1st Base Malaysia). Sequencing data obtained for targeted gene (SAD) and were used for alignment analysis using Geneious software.

Prediction of amino acids and SNP discovery: The sequence of nucleotide obtained from sequencing result, was analyzed to enables us predict the amino acid of said sequence. To identify SNPs in the SAD gene fragment among 6 different genotypes of oil palm, the sequences were aligned using Geneious software, version 10.0.3 (Biomatters Ltd USA).

Allele specific SNP primer design and SNAP-PCR: To obtain primers specific to the identified SNPs, the segments of the SAD sequence containning the SNP sites were entered the Web-available SNAPER program (http://ausubellab.mgh.harvard.edu/). Nine primers (18 primer pairs) corresponding to the SNPs were tested using standard PCR amplification. PCR amplification was carried out using KAPA HiFi Hot Start Enzyme Kit (KAPA BIOSYSTEMS, USA). The total PCR mix composition was 50µL prepared according to manufacturer's instruction which included 10µL of KAPA HiFi 5X Buffer, 1.5µL of KAPA dNTP, 15µL of forward and 15µL of reverse primers (SADE 27, SADE29 and SADE 39), 1µL of KAPA HotStart DNA polymerase, 2.0 µL DNA template, and 5.5µL of dH_2O. The PCR amplification was carried out as follows, initial denaturation at 95^0C for 5 minutes, denaturation at 98 ^{0}C for 20 s, annealing at 66^0C -68^0C for 15 s, extension 72ºC for 15 s with final extension at 72^0C for 1min and run for 30 cycles Amplified products were separated on a 1% agarose gel to estimate each allele in the SNP site as presence or absence of a band. Result obtained from the SNAP-PCR was processed through binary data with Darwin software while genetic diversity analysis was done using Cercus software.

RESULTS AND DISCUSSION

Specific Primers designed and amplification of stearoyl-ACP desaturase (SAD) gene fragment: Based on sequence data of stearoyl-ACP desaturase (SAD) for oil palm obtained from NCBI, there are 8 areas of exon and this gene is located in chromosome 7, 8, and 9 in oil palm. The size of SAD in chromosome 7 is 8686 bp, chromosome 8 account for 1906 bp while chromosome 9 account for 5481. This study focuses on SAD in chromosome 7 and 9. Three primer pairs specific for stearoyl-ACP desaturase gene including SADE 27, SADE29 and SADE 39 were designed to amplify mostly the exon region and taking into consideration the intron region. Detail description and specification of the designed primer see table 1. All three primers pairs were successful in amplifying the 6 genomic DNA isolated from three sources of oil palm (*E. Oleifera*, *E. Guineensis* (Deli dura), the *Hybrid* (*E. Oleifera* × *E. Guineensis*).

Table-1: Three specific primer pairs used in the amplification of SAD fragment

Primer ID	Forward sequence	Reverse sequence	Tm	Product size
SADE27	ATC ATA TTA GGG TTG AGA TTC CAA AA	ATG ATT GGC GAC CTT TGA AGC	61.^{0}C	720bp
SADE29	TCC TAG TTT TAG AGA GCC AAA ATG	GTT TAA CAC TCC TCT AAC CCC TCT	60.^{0}C	680bp
SADE39	TGC ATG GCC TCC AAA TGT	TAC CAT GAC AAA CAA CTC GAA GC	64.^{0}C	640bp

SAD primers were designed to obtain fragments of SAD genes from isolated genomic DNA of oil palm. This SAD gene is key gene in the pathway of the formation of unsaturated fatty acids in oil palm especially it initiates the formation of oleic acid that is the major component of unsaturated fatty acid. Oil palm fatty acid biosynthesis is controlled by genes encoding key enzymes that play an important role in the formation of fatty acids in all plants. Fatty acid biosynthesis generally result in the production of saturated fatty acid of which palmitic acid and stearic acid are the major products (Nishida 2004) and unsaturated fatty acid which mainly has oleic acid, linoleic acid and linolenic acid as its major products (Browse *et al*., 1993). Oleic acid formation process is associated with SAD gene which encode enzymatic activity that convert stearic acid to unsaturated fatty acid (oleic acid). Therefore, obtaining fragment of this SAD gene in oil palm for molecular purposes such as developing marker required the specificity of the primer (s). In consistent with a model proposed for SNP discovery by Jamali *et al., (*2005), these primers could also be used to amplify plant family with high similarity of SAD gene order and gene sequences such as coconut (*Cocos nucifera*).

SNP identification and amino acids prediction analysis: Base on the alignment result of nucleo-

tide sequence of SAD gene fragment obtained from sequencing, there were four SNP sites identified in the exon region and at least five sites were identified in the intron region. These SNP identifications showed that there are more SNP loci in the intron region than that of the exon region of SAD gene of oil palm. The objective to identify a sequence change can then become the basis for a marker that is specific for that allele and such markers will often be based on a SNP (Jamali *et al.,* 2005). Judith *et al.*, (2007) reported higher number of SNP in the noncoding (intron) region in Sunflower plant than in the coding region. Selecting amplicons in the non-coding regions, such as introns or 3' untranslated regions (UTRs), usually increases the frequency of polymorphisms found by up to three- fold (Zhu *et al.,* 2003).

Intron has been proven to increase the efficiency of transcription of many genes in different organisms. For example, an early study in rats showed that transgene transcription without introns showed 10-100 times lower than those which contain introns. Similarly, the efficiency of the gene encoding transcription in Drosophila, where alcohol dehydrogenase decreased after the removal of introns. One way that introns may affect the transcription of plant and other organisms is to act as the recipient of transcriptional regulatory elements (Mckenzie *et al.,* 1996). SNP may result in changes in amino acids sequence in the exon of a gene meaning it is non-synonymous. SNP can also be silent that means it is present in the coding region but does not cause a change in the amino acid sequence (synonymous). For the SNPs identified in exon region of the SAD gene fragment in this study, there were three non-synonymous SNP loci (SADSNP1, SADSNP7 and SADSNP9) and one synonymous locus (SADSNP8). In the SADSNP1 locus, a single-base mutation in *E.oleifera* change a glutamine codon (CAG) to arginine codon (CGG). At SADSNP7 locus, a mutation in also *E. oleifera* changes an aspartic codon (CGA) to an asparagine codon (CAA). In the hybrid (O × G), SADSNP9 locus leads to a change of serine codon (AGC) to a cysteine codon (TGC) due to a mutation in the nucleotide sequence. At SADSNP8 locus (synonymous SNP), a change in codon CGC to CAC does not cause an alteration in the amino acid sequence for the hybrid (O×G) therefore no functional change in the SAD gene protein between the three sources of oil palm that were aligned.

When SNP are present in the coding sequences, they may or may not determine the mutant phenotype, but will show 100% association with the trait and will therefore, be very useful, both for MAS and for gene isolation (Nasu *et al.,* 2002). When found in the proximity of coding sequences, although the association of SNPs with traits will be less than 100%, the association with the economic traits will still allow their use in MAS, and in positional cloning (Nasu *et al.,* 2002). We therefore can say that in plants, most SNPs will not be genetic determinants and therefore asso-ciations among SNPs and the traits of economic value will be of major interest to the plant breeders and oil palm breeders are of no exception. In a study conducted by Hu *et al.,* (2006) in canola to map the *fad2* and *fad3* gene which are related oleic acid, single nucleotide mutations were identified by sequencing the genomic clones of these genes and subsequently SNP markers were developed. Allele-specific PCR assays were developed to enable direct selection of desirable *fad2* and *fad3* alleles in marker assisted trait introgression and breeding.

SNPs that occur in regions upstream of the protein-encoding gene regions might influence the binding of promoters or repressors, resulting in differential regulation of transcription (Carlson *et al*., 2005). Polymorphisms at intron/exon boundaries may affect exonic or intronic splicing enhancer or silencer positions, or especially conserved GT donor or AC acceptor positions, modifying the resulting polypeptide (Fairbrother *et al*., 2002, Maniatis and Tasic *et al*., 2002). There is even demonstrated potential for phenotypic effects from non-coding or synonymous SNPs through alteration of RNA secondary structure (Shen *et al*., 1999). Similarly, untranslated distal 3′ differences may have additional effects, including interruption of poly-adenylation, which would alter the effecttiveness of the template.

SNAP marker development based on SAD gene for Oleic acid prediction: The identified SNP between *E. guineensis*, *E. oleifera* and their hybrid (O×G) was used to develop an allele specific PCR-based SNP marker which will facilitate marker assisted selection (MAS) in oil palm breeding program. Nine primers (18 primer pairs) for 9 SNP loci identified were developed but only 4 of said primers (8 primer pairs) were polymorphic (data not included). The SNP-specific primers were designed so that the 3'-terminal nucleotides of a primer should be complementary to one allele of a SNP, and such that the primer should contain an artificial mismatch within 4 bp of the site of the SNP (Drenkard *et al.,* 2000).

Twenty-five accession of oil palm consisting of *E. guineensis*, *E. oleifera* and the *hybrid* (O×G) were evaluated with the 9 SNAP markers. Out of the 9 SNAP markers, 4 (SADSNP4, SADSNP5, SADSNP6, and SADSNP7) were polymorphic

producing products for either reference allele or alternative allele the 25 accession while 5 (SADSNP1, SADSNP2, SADSNP3, SADSNP8 and SADSNP9) were monomorphic producing products for both reference allele and alternative allele in all the 25 accession. Figure 1 show representtation amplification patterns of the SNAP informative/polymorphic marker for SAD/oleic acid in 6 genotypes of oil palm using SADSNP4 primer.

Mutation at 1600 (SNP4) locus in the SAD sequence generated SNAP markers that distinguish the hybrid (O×G) from both *E. guineensis* and *E. oleifera.* This difference in sequence could be attributed to results of crossing and backcrossing in the oil palm breeding program which has suggested that the hybrid (O×G) fatty acid composition especially the oleic acid content is relatively higher than *E. guineensis* but lower than *E. oleifera* (Singh *et al*., 2009). This further supported the assertion of Dahot et al., (2012) that mutations are the tools used to study the nature and function of the genes, which are the building blocks and basis of plant growth and development as it relates to yield and quality.

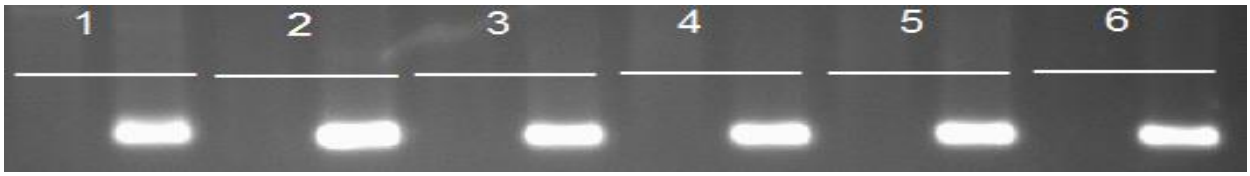

Figure-1: Amplification patterns of allele-specific SADSNP5 informative marker for SAD in 6 oil palm genotypes. For each genotype, the left lane represents the reference allele (C) and the right lane for the alternative allele (A). 1&2 *E. guineensis* genotype 1, 2; 3&4 *hybrid* genotype 1, 2 and 5&6 *E. oleifera* genotype 1, 2.

At locus 3553 (SNP7), all the accession produced only bands for the alternative allele (A) while at locus 1883 (SNP6), *E. guineensis* and hybrid (O×G) produced bands for both allele while the *E. oleifera* produced band for the reference allele only. These results may account for the taxonomy classification for the two species and distinct difference in oleic acid composition.

SAD SNP and genetic diversity analysis in 25 oil palm accession: Allele specific SNAP marker is a co-dominant marker which indicates that it can be used to differentiate homozygous from heterozygous genotypes. We used present or absent banding profile and then translated said banding profile into nucleotide base (A, T, G, and C) scoring profile. Based on the 9-allele specific SNAP marker tested on 25 oil palm accession, 4 loci (SADSNP4, SADSNP5, SADSNP6 and SADSNP7) revealed polymorphism.

The summary of genetic diversity statistics is presented in Table 3. The average observed heterozygosity and expected heterozygosity values were 0.391 and 0.404, respectively. The average polymorphic information content (PIC) was 0.312 ranging from 0.232 (SADSNP4) to 0.373 (SADSNP6). Although there is no current information on genetic diversity information as it relates to SAD gene in oil palm, our PIC result is comparable to the PIC value (0.315) reported in oil palm by Ong *et al.*, (2015). Hayden *et al.,* (2010) have classified PIC value into three classes: slightly informative (PIC < 0.25), reasonably informative (0.5 > PIC > 0.25), and highly informative (PIC > 0.5). Based on this classification, these SNPs were classified as reasonably informative which also suggest their potential use for linkage disequilibrium and association mapping studies in addition to their use as molecular markers.

Table-2: Heterozygosity and polymorphic summary based on 4 informative SNP markers.

Locus	HObs	HExp	PIC	HW
SADSNP4	0.413	0.310	0.232	***
SADSNP5	0.417	0.325	0.301	**
SADSNP6	0.330	0.536	0.373	**
SADSNP7	0.406	0.448	0.342	***

HObs=Observed heterozygosity; HExp=Expected heterozygosity; PIC = Polymorphic information content; HW= Hardy-Weinberg equilibrium; *** PIC < 0.001; **PIC < 0.01; PIC < 0.05

Furthermore, the expected heterozygosity recorded in this study is greater than several reports on SNP marker for other plants including tongkatali (*Eurycoma longifolia*, 0.216; Osman et al., 2003), castor bean (*Ricinus communis*, 0.220; Foster *et al*., 2010) and maize (*Zea mays*, 0.319; Hamblin *et al*., 2007). We therefore suggest that these results would indicate the suitability of the SNP markers developed for analyzing the diversity of oil palm populations as they related to important agronomic traits.

Dendrogram revealed the *hybrid (O×G)* and *E. oleifera* in one cluster *while E. guineensis* in another cluster. The UPGMA dendrogram showed *hybrid8* nearer *E. oleifera1* (Figure 3). Accession belonging to *E. guineensis* were classified into cluster I, whereas cluster II was made up of accession of *hybrid (O×G)* and *E. oleifera*. Cluster II was further divided into two sub-clusters (A and B). Sub-cluster IIA consist predominately the hybrid with the exception *hybrid*8 while sub-cluster IIB contain *E. oleifera*2 & *E. oleifera*3 on one side and *E. oleifera*1 & *hybrid*8 on the other side. The dendrogram shows the closeness of the *E. oleifera* and *hybrid (O×G)* especially *hybrid8* which may support past researches that have indicated that the *hybrid* has an intermediate composition of unsaturated fatty acid with oleic acid been the highest (Ong et al., 1981) and signified considerable genetic similarity of SAD enzymatic in both *E. oleifera* and *hybrid (O×G).*

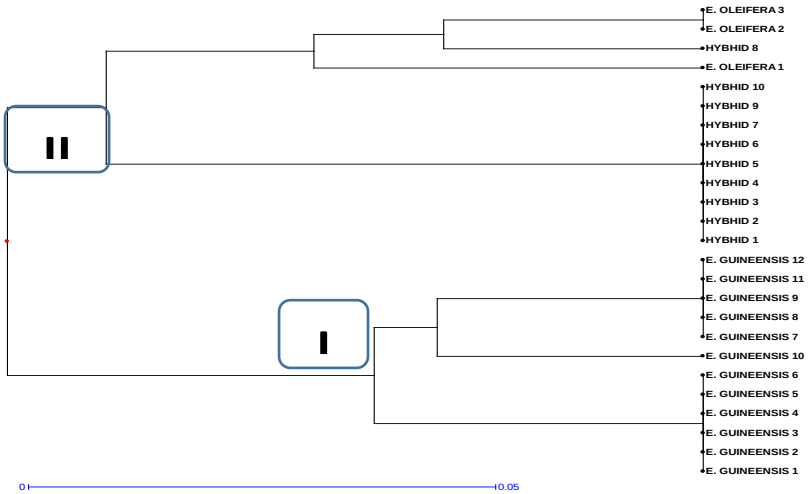

Figure 2. Dendrogram of genetic diversity of 25 oil palm accession tested by 9 SNAP makers

Hardon *et al.*, (1969) and Ong *et al.*, (19 81) reported that most of the fatty acid proportions and unsaturated fats in *Elaeis* interspecific *hybrid* are intermediate between the parents' proportion and as such the potential of the hybrid to accumulate more unsaturated fatty acid is achievable through molecular genetics and breeding programs. This dendrogram analysis further support these finding as it relates to genetic diversity of SAD in the oil palm. It also useful to plant breeders who are always interested in genetic diversity among their existing germplasm with the desired objectives, like high grain yield, early maturity with increased grain filling period, resistance to biotic (diseases) and abiotic stresses (drought tolerance and high temperatures) through conventional breeding (hybridization), mutagenesis, tissue culture and molecular techniques (Sial *et. al.*, 2006).

CONCLUSION

This research succeeded in isolating DNA fragment of Stearoyl-ACP desaturase (SAD), identified 9 SNP loci in three oil palm sources and predicted the alteration of amino acid t due to the identified SNP loci. Based on genetic diversity analysis we obtained observed heterozygosity and expected heterozygosity values that are in line with previous research on oil palm. We also developed 9 SNAP markers for oleic acid prediction. However, only 4 of the 9 markers revealed polymerphism henceforth informative. Due to less population of genotypes (25 accessions) used in this research, these makers need to be validated and tested on large oil palm population before their application in oil palm breeding programs.

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