Insights from computational analysis of full-length β-ketoacyl-[ACP] synthase-II cDNA isolated from American and African oil palms

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Abstract

Background: Palm oil derived from fruits (mesocarp) of African oil palm (*Elaeis guineensis* Jacq. Tenera) and American oil palm (*E. oleifera*) is important for food industry. Due to high yield, *Elaeis guineensis* (Tenera) is cultivated on commercial scale, though its oil contains high (~54%) level of saturated fatty acids. The rate-limiting activity of beta-ketoacyl-[ACP] synthase-II (KAS-II) is considered mainly responsible for the high (44%) level of palmitic acid ($C_{16:0}$) in the oil obtained from *E. guineensis*. **Objective:** The objective of this study was to annotate KAS-II cDNA isolated from American and African oil palms. **Materials and Methods:** The full-length *E. oleifera* KAS-II (*Eo*KAS-II) cDNA clone was isolated using random method of gene isolation. Whereas, the *E. guineensis* KAS-II (*Eg*TKAS-II) cDNA was isolated using reverse transcriptase polymerase chain reaction (RT-PCR) technique; and missing ends were obtained by employing 5'and 3' RACE technique. **Results:** The results show that *Eo*KAS-II and *Eg*TKAS-II open reading frames (ORFs) are of 1689 and 1721 bp in length, respectively. Further analysis of the both *Eo*KAS-II and *Eg*TKAS-II predicted protein illustrates that they contains conserved domains for 'KAS-I and II', 'elongating' condensing enzymes, 'condensing enzymes super-family', and '3-oxoacyl-[ACP] synthase II'. The predicted protein sequences shows 95% similarity with each other. Consequently, the three active sites (Cys, His, and His) were identified in both proteins. However, difference in positions of two active Histidine (His) residues was noticed. **Conclusion:** These insights may serve as the foundation in understanding the variable activity of KAS-II in American and African oil palms; and cDNA clones could be useful in the genetic engineering of oil palms.

Key words: African oil palm, American oil palm, cooking oil, edible oil, *Elaeis oleifera*, *Elaeis guineensis*, fatty acids, oil palm, palm oil

INTRODUCTION

Oil palm is an important and a major source of edible oil. The oil palms belong to the family Arecaceae which contains 202 genera and about 2600 species distributed in tropical, subtropical, and warm temperate climates. Genus *Elaeis* contains only two oil palm species namely, *Elaeis guineensis* Jacq. and *E. oleifera*. The *E. guineensis* and *E. oleifera* are referred as African oil palm and American oil palm, respectively. The *E. guineensis* Jacq. contains three fruit forms (also called as verities) namely, 'Dura', 'Pisifera', and 'Tenera' that are distinguished based on the shell thickness of fruits.[1,2] *Elaeis guineensis* Jacq. 'Tenera' a hybrid of 'Dura' (♀) and 'Pisifera' (♂) is highly preferred for the commercial cultivation because of its high oil-yielding fruits.[3]

In tropical countries like Malaysia and Indonesia, oil palm is a major agricultural crop and both countries are major

producers and exporters of the palm oil. *Elaeis oleifera* is not extensively cultivated for the palm oil due to low oil-yielding fruits.[4,5] However, palm oil obtained from *E. oleifera* fruit mesocarp is far superior to palm oil obtained from commercially cultivated *E. guineensis* Jacq. 'Tenera' fruit mesocarp.[4]

The palm oil obtained from *E. guineensis* (Tenera) and *E. oleifera* contains about 44% and 25% palmitic acid ($C_{16:0}$), respectively.[6] The level of the $C_{16:0}$ in palm oil is dependent on the palmitoyl-acyl carrier protein [ACP] thioesterase (PATE) (EC: 3.1.2.14) and beta-ketoacyl-[ACP] synthase-II (KAS-II) (EC: 2.3.1.179) enzymes efficiency.[4,7] However, with advanced tools and techniques available for the plant genetic transformation, fatty acid biosynthesis pathway can be manipulated genetically to improve palm oil quality or to add value in it. The level of $C_{16:0}$ content in *E. guineensis* oil can be reduced by PATE gene silencing and over expression of the KAS-II gene in a mesocarp tissue-specific manner.[3] Researchers are working hard to transform oil palm for PATE gene silencing and successful oil palm transformation is reported elsewhere.[4,8,9]

In addition to PATE, KAS-II gene is an important gene target for the genetic modification of oil palm to minimize the $C_{16:0}$ content. The KAS-II enzyme catalyzes the elongation of $C_{16:0}$-ACP to $C_{18:0}$-ACP in plastidial fatty acid biosynthesis pathway. The biological activity of KAS-II is considered as rate limiting in commercially cultivated *E. guineensis* compared to its activity in *E. oleifera*.[3,4] In order to understand the gene expression patterns in *E. oleifera* fruit mesocarp tissue, expressed sequence tags (ESTs) generation project was initiated at Melaka Institute of Biotechnology, Melaka; and until now, 3,205 ESTs are generated from 17-week-old *E. oleifera* fruit-mesocarp tissue cDNA library (unpublished work). The *Eo*KAS-II is one of the isolated cDNA (EST) clones.

The computational annotation of *Eo*KAS-II and *Eg*TKAS-II and its comparative analysis could be useful in elucidating similarities and differences between *Eo*KAS-II and *Eg*TKAS-II. This could also give some insights in in-depth understanding of fatty acid biosynthesis pathway in both *Elaeis* species. The *Eo*KAS-II and *Eg*TKAS-II gene's cDNA nucleotide sequences, deduced proteins sequences as well as annotations are reported in this paper.

MATERIALS AND METHODS

Materials
Bacto-Agar, Bacto-Trypton, and Yeast extracts were purchased from DIFCO Laboratories (Etroit MI, USA). Chloroform, EDTA, ethanol, iso-amyl alcohol and sodium

chloride were from BDH Laboratory, England. The 100 bp DNA ladder, λ DNA *Hind* III DNA marker, the deoxy-nucleotide mix (dNTPs), other PCR components and Wizard® Plus SV Minipreps DNA purification system (kit) were purchased from Promega Corporation, USA. Restriction enzymes used were procured from New England BioLabs® Inc. The CloneMiner™ cDNA Library Construction Kit was procured from Invitrogen Corporation; and all other chemicals except those are mentioned above were procured from the Sigma-Aldrich Corporation (St. Louis, MO), USA.

EgTKAS-II and EoKAS-II cDNA isolation
Elaeis oleifera, 17-week-old [weeks after anthesis (WAA)] fruit mesocarp tissue cDNA library constructed using the 'CloneMiner cDNA library construction kit' for ESTs generation (unpublished work) was used to isolate *Eo*KAS-II. This *Eo*KAS-II cDNA clone was isolated by random method of cDNA isolation.[10] Whereas, the reverse transcriptase polymerase chain reaction (RT-PCR) technique was used to isolate *Eg*TKAS-II cDNA from *E. guineensis*. The forward (5'-AGAATTGCCGGAGAGATCAAGTC-3') and reverse (5'-GATGAGTTATGACCACCAAACC-3') primers were designed based on the conserved regions of KAS-II gene from *Cuphea wrightii* (U67317) and *Perilla frutescene* (AF026149). The RNA samples extracted from 17-week-old (WAA) fruit mesocarp-tissues of *E. guineensis* were used. The PCR for synthesis and amplification of *Eg*TKAS-II cDNA was completed using: hot start at 94ºC for 5 min, followed by 35 cycles of 33 sec at 94ºC, 1 min at 49ºC, 1.30 min at 72ºC and a final extension at 72ºC for 5 minutes. The 5' RACE and 3'-RACE techniques were used to isolate the missing 5' and 3'end of the *Eg*TKAS-II gene cDNA, respectively. The PCR cloning vector, pGEM®-T Easy was used for the cloning of the PCR products.

Plasmid DNA purification and cDNA sequencing
Separately, the *Escherichia coli* strain DH5α cells harboring *Eo*KAS-II and *Eg*TKAS-II cDNA were cultivated in 10-ml LB medium in dark at 37ºC, 160 rpm for 16-18 h. The cultures of *E. coli* cells harboring plasmid DNA with *Eo*KAS-II and EgTKAS-II cDNA were supplemented with Kanamycin (50 μg/ml) and Ampicillin (50 μg/ml), respectively. The *E. coli* cells were harvested from broth and plasmid DNA was isolated by using a commercial kit, Wizard® *Plus* SV Minipreps DNA purification system.

For *Eo*KAS-II cDNA, sequencing reactions were carried out for both strands using M13 (Forward); 5'-GTAAAACGACGGCCAG-3' and M13 (Reverse); 5'-GGATAACAATTTCACACAGG-3' primers. Whereas, for *Eg*TKAS-II cDNA, sequencing reactions were carried

out for both strands using T7 and SP6 universal primers available in PCR cloning vector, pGEM®-T Easy.

Computational analysis of nucleotide and protein sequence

The sequences of cDNAs were edited manually to eliminate vector and or adaptor sequences from 5' and 3' ends. Blastn and Blastx programs were used to confirm the identity of the both isolated cDNA. The general features of the *Eo*KAS-II and *Eg*TKAS-II cDNA, conserved domains, and multiple sequence alignment of protein sequences was carried out as described by Bhore *et al.*[11] The active sites (residues) of *Eo*KAS-II and *Eg*TKAS-II proteins were identified based on active sites of *E. coli* KAS protein (1FJ8_A) described by Price *et al.*[12]

RESULTS

A full-length *Eo*KAS-II cDNA clone was isolated from 17-week-old fruit-mesocarp tissue cDNA library. Its nucleotide sequence, deduced protein sequence, and other features are shown in Figure 1. The nucleotide sequence, sequence isolated using 5' and 3' RACE technique as well as the deduced protein sequence and other features of *Eg*TKAS-II cDNA are depicted in Figure 2. The nucleotide sequence of *Eo*KAS-II and *Eg*TKAS-II gene cDNA has been submitted to GenBank/DDBJ/EMBL under accession numbers FJ940767 and AF220453, respectively.

Analysis of cDNAs general features [Table 1] revealed that guanine plus cytosine content (G + C %) in both *Eo*KAS-II and *Eg*TKAS-II gene cDNA was 49%. The alignment of the deduced proteins showing similarities and differences between *Eg*TKAS-II and *Eo*KAS-II is shown in Figure 3. Consecutively, the multiple sequence alignment was completed, and the three active sites were identified as shown in Figure 4a [for complete multiple sequence alignment, see Figure 5]. The phylogenetic tree was constructed to find out phylogenetic relationship among compared KAS-II protein sequences; and the radial phylogenetic tree is shown in Figure 4b.
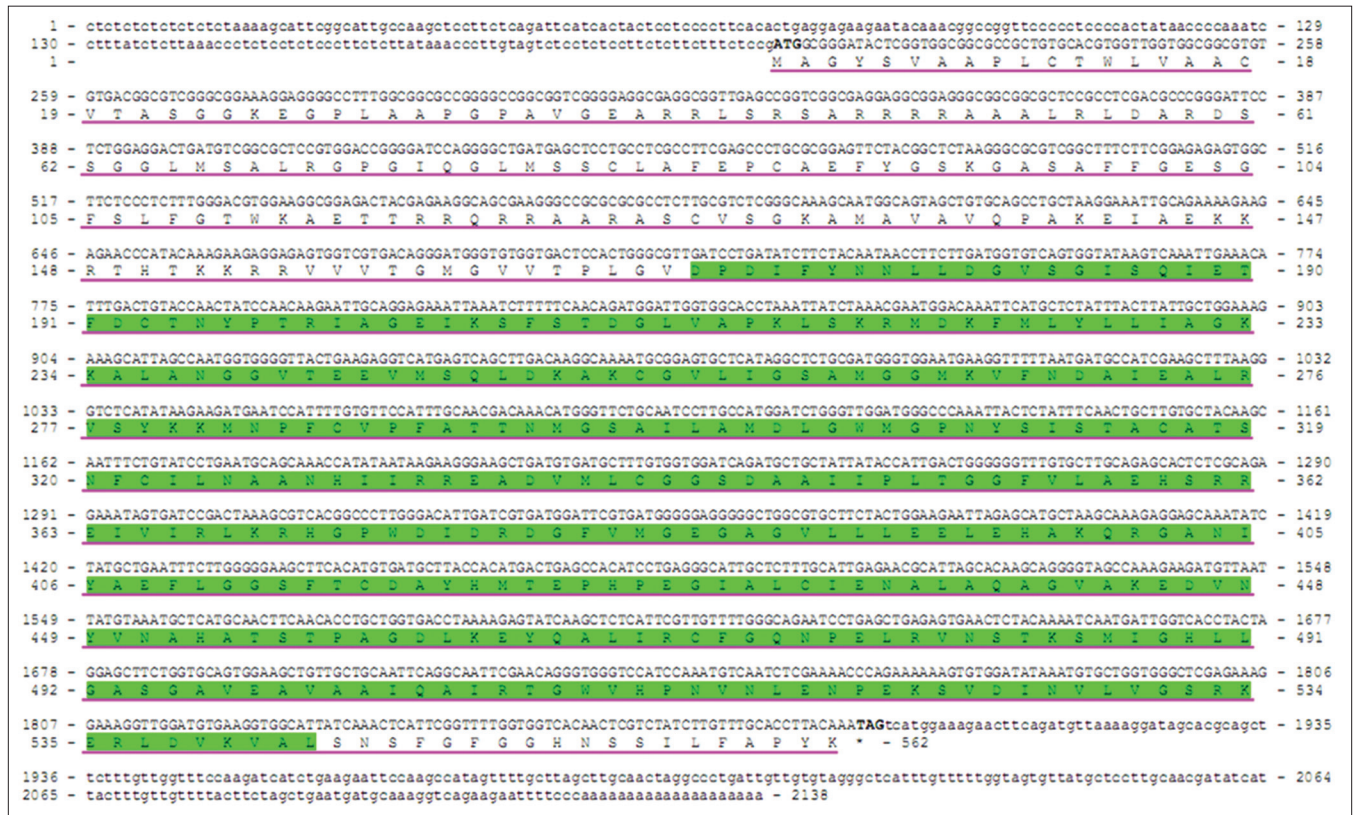

Figure 1: Nucleotide and deduced amino acid sequence of *E. oleifera* β-ketoacyl-[ACP] synthase II (*Eo*KAS-II) cDNA clone. An open reading frame and non-coding regions are shown in capital and small letters, respectively. The deduced amino acid sequence is given below the nucleotide sequence, which is numbered at the both ends of each sequence line. The open reading frame encodes for a protein of 562 amino acid residues. Amino acid residues are numbered beginning with the initial Methionine (m) till last Lysine (k) residue. Initiation and termination codons are shown in bold. The 'β-ketoacyl-[ACP] synthase (KAS) (type I and II)', 'Elongating' condensing enzymes and 'Condensing enzymes super-family conserved domain (multi-domain) residues (170 to 543) are highlighted in green color. Residues (1 to 562) underlined with pink color line are part of the '3-oxoacyl-[ACP] synthase II' domain (region name="PLN02787"). *represent the termination codon. This cDNA clone was isolated by random method of gene isolation from *E. oleifera* 17-week-old fruit-mesocarp tissue cDNA library
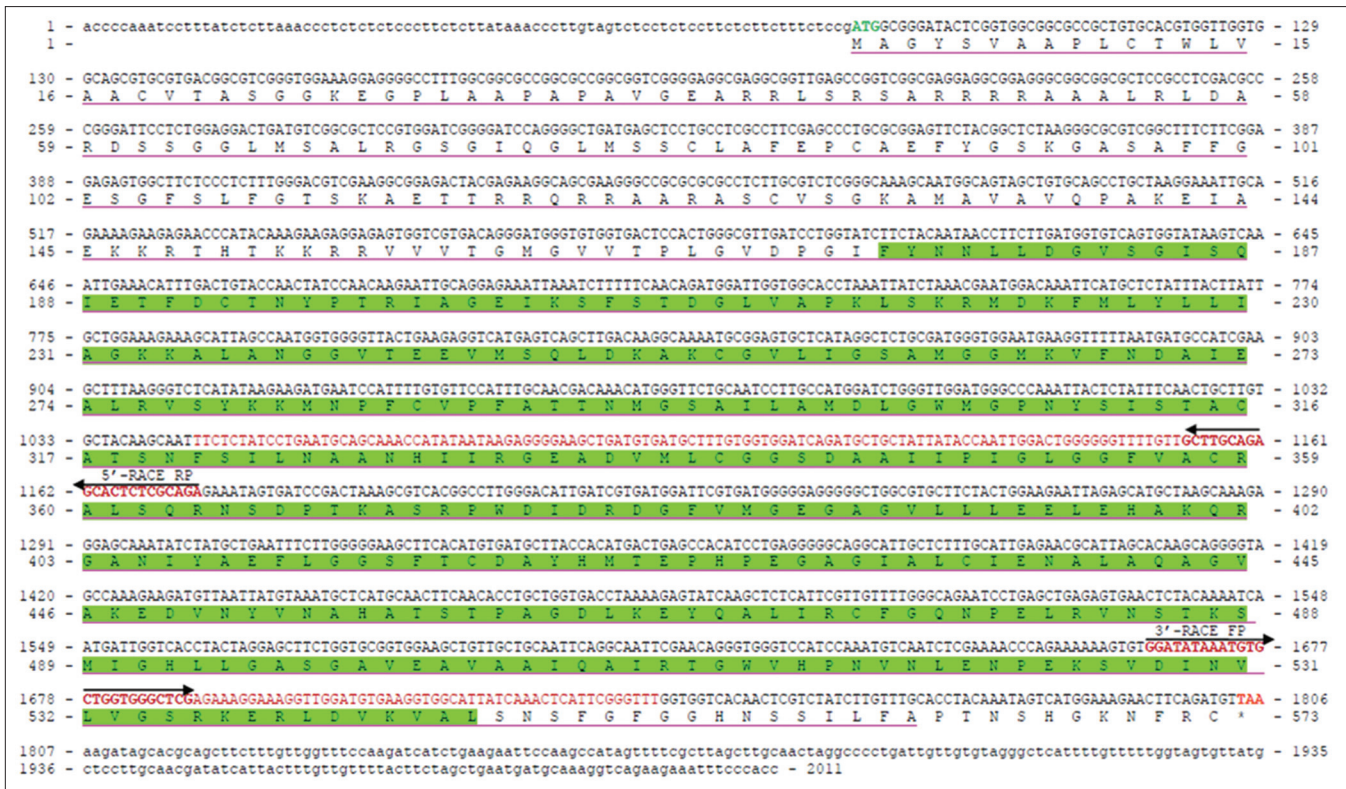
Figure 2: Nucleotide and deduced amino acid sequences of *E. guineensis* (Tenera) β-ketoacyl-[ACP] synthase II (*Eg*TKAS-II) cDNA clone. An open reading frame and noncoding regions are shown in capital and small letters, respectively. Nucleotides shown in bold brown color represent the location of the forward primer (3'-RACE FP) used in 3'-RACE and reverse primer (5'-RACE RP) used in 5'-RACE. Arrows indicate the 5'to 3' direction of the primer. Nucleotides shown in brown color represent the overlapping regions with cDNA fragments isolated using 5' and 3'-RACE. The deduced amino acid sequence is given below the nucleotide sequence, which is numbered at the both ends of each sequence line. The open reading frame encodes for a protein of 573 amino acid residues. Amino acid residues are numbered beginning with the initial Methionine (m) till last Cysteine (c) residue. Initiation and termination codons are shown in green and red color, respectively. The 'β-ketoacyl-[ACP] synthase (KAS) (type I and II)', 'Elongating' condensing enzymes and 'Condensing enzymes super-family conserved domain (multi-domain) residues (174 to 546) are highlighted in green color. Residues (1 to 563) underlined with pink color line are part of the '3-oxoacyl-[ACP] synthase II' domain (region name="PLN02787"). *represent the termination codon. This cDNA was isolated from *E. guineensis* 17-week-old fruit-mesocarp tissue by using RT-PCR, and 5' and 3' RACE techniques.

Table 1: General features of American (Eo) and African (EgT) oil palms full-length ß-ketoacyl-[ACP] synthase II (KAS-II) gene's cDNA sequence

Features	*Eo*KAS-II cDNA	*Eg*TKAS-II cDNA
cDNA		
Size, bp	2138	2011
Molecular weight, Daltons	655722	623274
5'UTR, bp	204 (1.204)	84 (1.84)
Coding sequence length, bp (ORF)	1689 (205.1893)	1721 (85.1806)
3'UTR, bp	245 (1894.2138)	205 (1807.2011)
Stop codon	TAG (UAG)	TAA (UAA)
G+C content, %	49	49
Protein		
Length of peptide, AA	562	573
Molecular weight, Daltons	59878.13	60451.51
Isoelectric point (pI) (Theoretical)	8.95	8.99

ORF: Open reading frames , AA: number of amino acids, cDNA: Complementary deoxyribonucleic acid

DISCUSSION

Oil palm is an important oil producing crop and nutritional quality of its oil can be improved by using tools available for plant genetic engineering. However, in order to make a strategy for genetic engineering of socio-economically important crop, oil palm; understanding of its key genes involved in fatty acid biosynthesis pathway is necessary. Therefore, several key genes that encodes for enzymes involved in fatty acid biosynthesis pathway were isolated in our laboratory. In this study, we determined the full-length cDNA sequence of *Eo*KAS-II and *Eg*TKAS-II which are known for catalyzing the elongation of $C_{16:0}$ fatty acid-[ACP] to $C_{18:0}$ fatty acid-[ACP] in plastids.[13]

We performed sequencing for both positive and negative strands of both *Eo*KAS-II and *Eg*TKAS-II cDNA clones.

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EoKAS-II   MAGYSVAAPLCTWLVAACVTASGGKEGPLAAPGPAVGEARRLSRSARRRRAAALRLDARD 60
EgTKAS-II  MAGYSVAAPLCTWLVAACVTASGGKEGPLAAPAPAVGEARRLSRSARRRRAAALRLDARD 60
           **************************** .***************************

EoKAS-II   SSGGLMSALRGPGIQGLMSSCLAFEPCAEFYGSKGASAFFGESGFSLFGTWKAETTRRQR 120
EgTKAS-II  SSGGLMSALRGSGIQGLMSSCLAFEPCAEFYGSKGASAFFGESGFSLFGTSKAETTRRQR 120
           *********** .********************************** *********

EoKAS-II   RAARASCVSGKAMAVAVQPAKEIAEKKRTHTKKRRVVVTGMGVVTPLGVDPDIFYNNLLD 180
EgTKAS-II  RAARASCVSGKAMAVAVQPAKEIAEKKRTHTKKRRVVVTGMGVVTPLGVDPGIFYNNLLD 180
           ************************************************** .********

EoKAS-II   GVSGISQIETFDCTNYPTRIAGEIKSFSTDGLVAPKLSKRMDKFMLYLLIAGKKALANGG 240
EgTKAS-II  GVSGISQIETFDCTNYPTRIAGEIKSFSTDGLVAPKLSKRMDKFMLYLLIAGKKALANGG 240
           ************************************************************

EoKAS-II   VTEEVMSQLDKAKCGVLIGSAMGGMKVFNDAIEALRVSYKKMNPFCVPFATTNMGSAILA 300
EgTKAS-II  VTEEVMSQLDKAKCGVLIGSAMGGMKVFNDAIEALRVSYKKMNPFCVPFATTNMGSAILA 300
           ************************************************************

EoKAS-II   MDLGWMGPNYSISTACATSNFCILNAANHIIRREADVMLCGGSDAAIIPL-TGGFVLAEH 359
EgTKAS-II  MDLGWMGPNYSISTACATSNFSILNAANHIIRGEADVMLCGGSDAAIIPIGLGGFVACRA 360
           ********************* .**********   ****************:   ****  ..

EoKAS-II   SRREIVIRLKRHGPWDIDRDGFVMGEGAGVLLLEELEHAKQRGANIYAEFLGGSFTCDAY 419
EgTKAS-II  LSQRNSDPTKASRPWDIDRDGFVMGEGAGVLLLEELEHAKQRGANIYAEFLGGSFTCDAY 420
             :.        *   ********************************************

EoKAS-II   HMTEPHPEG--IALCIENALAQAGVAKEDVNYVNAHATSTPAGDLKEYQALIRCFGQNPE 477
EgTKAS-II  HMTEPHPEGAGIALCIENALAQAGVAKEDVNYVNAHATSTPAGDLKEYQALIRCFGQNPE 480
           *********   *************************************************

EoKAS-II   LRVNSTKSMIGHLLGASGAVEAVAAIQAIRTGWVHPNVNLENPEKSVDINVLVGSRKERL 537
EgTKAS-II  LRVNSTKSMIGHLLGASGAVEAVAAIQAIRTGWVHPNVNLENPEKSVDINVLVGSRKERL 540
           ************************************************************

EoKAS-II   DVKVALSNSFGFGGHNSSILFAPYK-------- 562
EgTKAS-II  DVKVALSNSFGFGGHNSSILFAPTNSHGKNFRC 573
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Figure 3: Comparison between *Eo*KAS-II and *Eg*TKAS-II amino acid sequence depicts the similarity and difference. Amino acid sequences are numbered at the end of each sequence row. (*), (:) and (.) indicate identical residues, residues with conserved strong groups and residues with conserved weak groups, respectively. *Eg*TKAS-II and *Eo*KAS-II represents the *Elaeis guineensis* Jacq. (Tenera) and *Elaeis oleifera* β-ketoacyl-[ACP] synthase II amino acid sequence, respectively

The complete *Eo*KAS-II and *Eg*TKAS-II cDNA sequence was 2138 and 2011 bp in length, respectively [Figures 1 and 2]. The *Eo*KAS-II and *Eg*TKAS-II cDNA's open reading frame (ORF) encodes for 562 and 573 amino acids, respectively. Both, *Eo*KAS-II and *Eg*TKAS-II ORF contains conserved domains for 'KAS-I and II', which is responsible for the elongation steps in fatty acid biosynthesis process.[14] The ORFs of *Eo*KAS-II and *Eg*TKAS-II gene also contain 'Condensing enzymes super-family'[15] and '3-oxoacyl-[ACP] synthase II'[16] conserved domains.

From protein BLAST hits analysis, we observed that *Eo*KAS-II and *Eg*TKAS-II proteins are 95% identical

with each other [Figure 3]. However, *Brassica napus* KAS-II (AF244520) protein was the closest to *Eo*KAS-II and *Eg*TKAS-II proteins. It was reflected in multiple sequence alignment of the proteins [see Figure 5] as well as in the phylogenetic tree of the KAS-II proteins [Figure 4b]. The KAS sequence from *Chlamydomonas reinhardtii* (DS496157)[17] and *E. coli* (1FJ8_A)[12] showed maximum phylogenetic distance with the *Eo*KAS-II and *Eg*TKAS-II proteins [Figure 4b]. The phylogenetic distance of *Eo*KAS-II and *Eg*TKAS-II with their counterparts from *C. reinhardtii* and *E. coli* is in line with the evolution of living organisms. However, three active sites (Cys-His-His)[12,16] were fully conserved in *Eo*KAS-II (Cys316, His453, and His489),
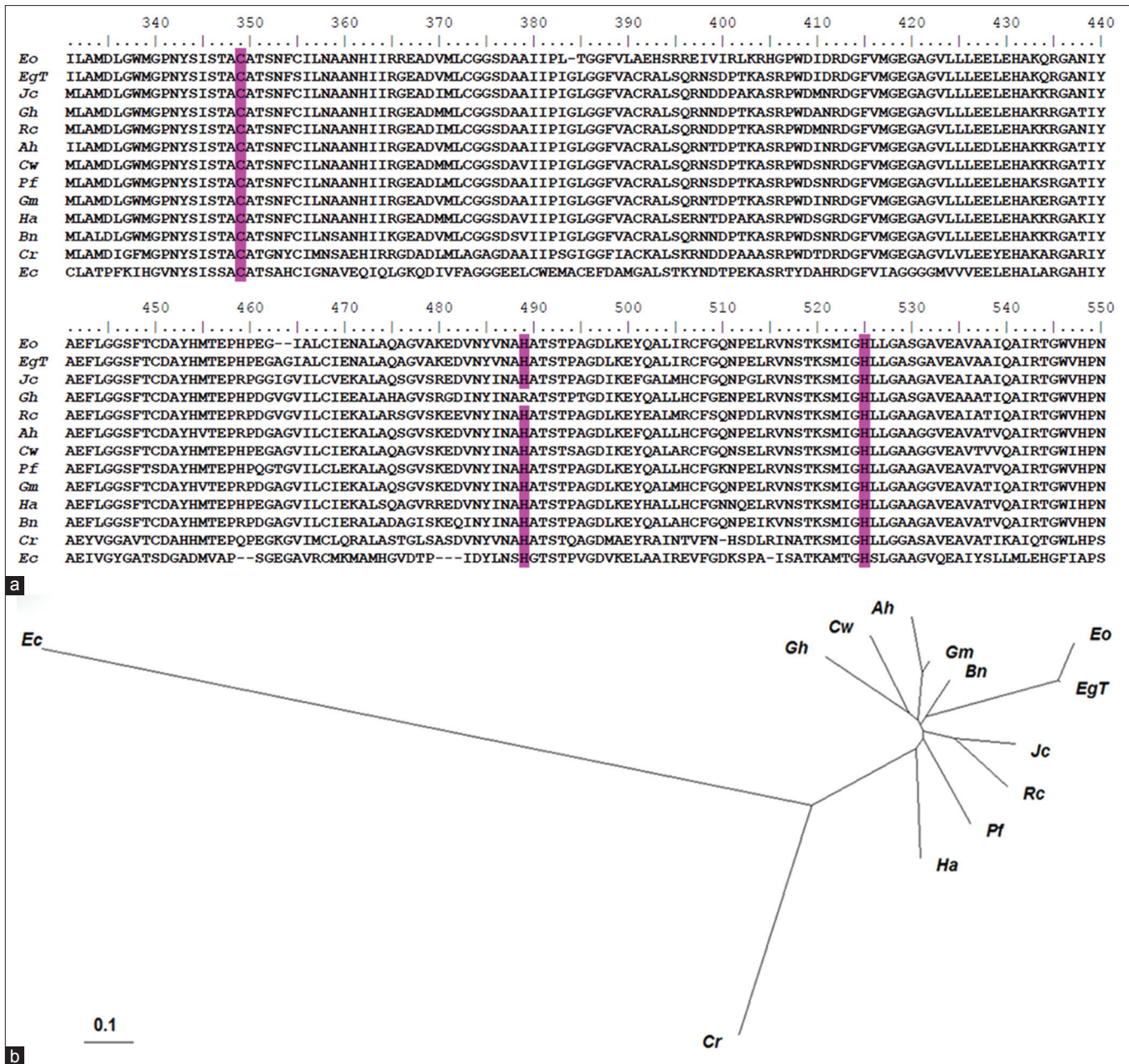
Figure 4: (a) Multiple sequence alignment of *Eo*KAS-II and *Eg*TKAS-II amino acid sequences with their selected counterparts from other organism. Protein sequences were aligned using the CLUSTALW alignment algorithm. The three active sites (residues) of *Eo*KAS-II and *Eg*TKAS-II proteins corresponding to *E. coli* KAS, Cys163, His298, and His333 are highlighted in pink color; the numbers at top indicate the residue ruler for whole alignment. GenBank accession numbers are as follows: *Eo*, FJ940767; *Eg*T, AF220453; *Jc*, DQ987700; *Gh*, HM236494; *Rc*, EQ973802; *Ah*, EU823327; *Cw*, U67317; *Pf*, AF026149; *Gm*, AY907523; *Ha*, DQ835562; *Bn*, AF244520; *Cr*, DS496157; *Ec*, 1FJ8_A. Abbreviations: *Eo*, *Elaeis oleifera*; *Eg*T, *Elaeis guineensis* (Tenera); *Jc*, *Jatropha curcas*; *Gh*, *Gossypium hirsutum*; *Rc*, *Ricinus communis*; *Ah*, *Arachis hypogaea*; *Cw*, *Cuphea wrightii*; *Pf*, *Perilla frutescens*; *Gm*, *Glycine max*; *Ha*, *Helianthus annuus*; *Bn*, *Brassica napus*; *Cr*, *Chlamydomonas reinhardtii*; *Ec*, *Escherichia coli*. This alignment is produced by CLUSTAL W multiple sequence alignment program. (b) Radial phylogenetic tree of amino acid sequence relationship among the proteins aligned in (a). The phylogenetic distance scale at bottom is proportional to the divergence between the sequences.

*Eg*TKAS-II (Cys316, His456, and His492) and their counterpart proteins from other species, except *Gossypium hirsutum* KAS-II (HM236494) protein in which one active residue (His) was missing [Figure 4a].

Both, *Eg*TKAS-II and *Eo*KAS-II gene cDNA clones were isolated from 17-week-old fruit-mesocarp tissue;

and this indicated that *Eg*TKAS-II and *Eo*KAS-II genes were expressed in developing fruit mesocarp tissue of both *Elaeis* species. However, it is also expressed in leaf, roots (unpublished data) and flower tissues, and embryogenic callus.[18] It also suggests that *Eg*TKAS-II and *Eo*KAS-II genes are not tissue-specific in their expression.
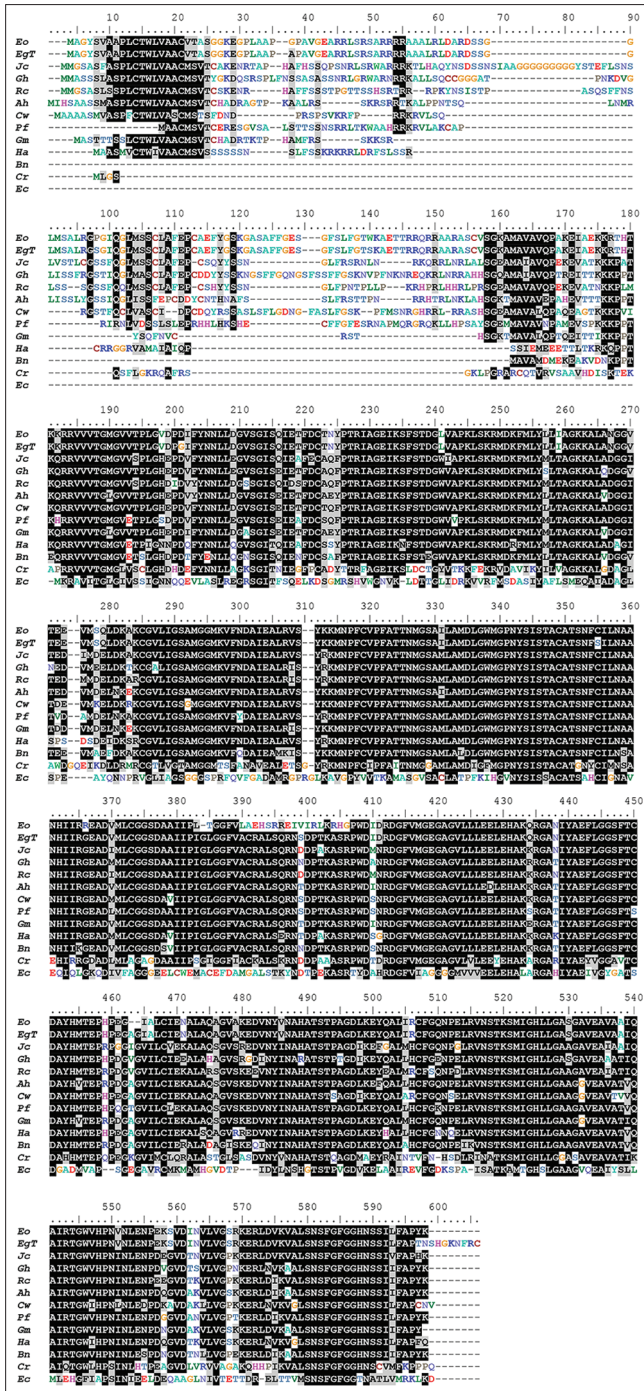
Figure 5: Multiple sequence alignment of *EoKAS*-II and *EgTKAS*-II proteins with their selected counterparts from other organism. Protein sequences were aligned using the CLUSTALW alignment algorithm. Identical and conserved residues are shaded black and grey, respectively; the numbers at top indicate the residue ruler for whole alignment. GenBank accession numbers are as follows: Eo, FJ940767; EgT, AF220453; Jc, DQ987700; Gh, HM236494; Rc, EQ973802; Ah, EU823327; Cw, U67317; Pf, AF026149; Gm, AY907523; Ha, DQ835562; Bn, AF244520; Cr, DS496157; Ec, 1FJ8_A. Abbreviations: Eo, *Elaeis oleifera*; EgT, *Elaeis guineensis* (Tenera); Jc, *Jatropha curcas*; Gh, *Gossypium hirsutum*; Rc, *Ricinus communis*; Ah, *Arachis hypogaea*; Cw, *Cuphea wrightii*; Pf, *Perilla frutescens*; Gm, *Glycine max*; Ha, *Helianthus annuus*; Bn, *Brassica napus*; Cr, *Chlamydomonas reinhardtii*; Ec, *Escherichia coli*. This alignment is produced by CLUSTAL W multiple sequence alignment program

Palm oil derived from fruit mesocarp tissue of *E. oleifera* contains about 68.6% oleic acid ($C_{18:1}$) and 25% $C_{16:0}$. However, $C_{18:1}$ and $C_{16:0}$ content in the palm oil obtained from fruit mesocarp of commercially cultivated *E. guineensis* Jacq. Tenera is 32.5% and 44%, respectively.[6] The total content of saturated fatty-acids in palm oil from commercially cultivated *E. guineensis* is 52%; and it is significantly high in comparison to other vegetable oils and fats [Figure 6].[8] The PATE enzyme is known to have $C_{16:0}$-ACP substrate specificity;[19-21] and its high efficacy is responsible in part for high (44%) content of $C_{16:0}$ in palm oil derived from *E. guineensis*. Hence, the nutritional quality of the palm oil obtained from commercially cultivated *E. guineensis* can be improved by minimizing the $C_{16:0}$ content. This can be done by silencing the expression of PATE gene and overexpression of the *EgTKAS*-II gene. Therefore, the isolated *EgTKAS*-II cDNA clone will be useful in genetic transformation of oil palms in general and commercially cultivated *E. guineensis* in particular. The *EoKAS*-II cDNA clone could be useful in genetic transformation experiments as a reference so that its efficiency can be compared with efficiency of *EgTKAS*-II.

As a part of oil palm molecular biology project, previously, we have isolated mesocarp tissue-specific[7] and kernel tissue-specific[22] cDNA clones in order to study the genes expressed during oil palm fruit development and to have tissue-specific gene promoters for oil palm genetic transformation. For the PATE gene silencing, various novel expression cassettes were constructed[3] and genetic transformation work was initiated in our laboratory.[8,9] However, *EgTKAS*-II gene expression cassette for it's over expression and PATE gene-silencing
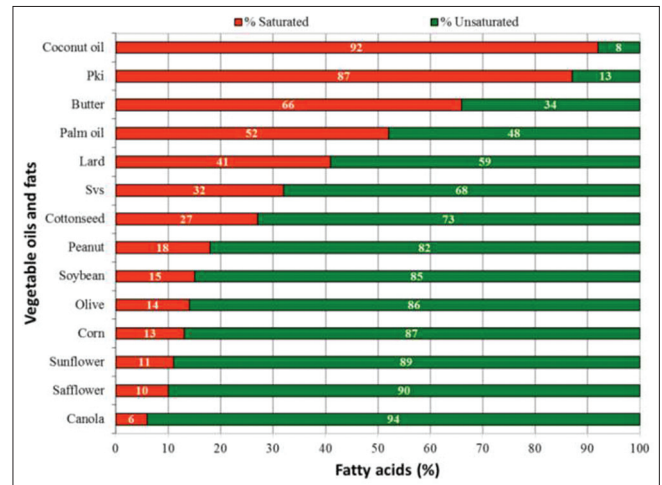

Figure 6: Bar diagram showing saturated and unsaturated fatty acids (%) in edible vegetable oils and fats. Palm oil represented is from commercially cultivated oil palm, *Elaeis guineensis* Jacq. Tenera; Svs, solid vegetable shortening; Pki, Palm Kernel oil.[8]

expression cassettes can be incorporated in a single vector to utilize them in genetic transformation of commercially cultivated *E. guineensis*. It may help to save the time in producing genetically engineered oil palm (*E. guineensis*) which can produce more stearic acid ($C_{18:0}$) and or $C_{18:1}$ at the expense of $C_{16:0}$.

The use of *Eg*TKAS-II and *Eo*KAS-II gene promoters or oil palm fruit mesocarp tissue-specific gene promoters for their overexpression could be an advantage. Due to commercial importance of oil palm, Malaysian Palm Oil Board has completed oil palms (*E. oleifera* and *E. guineensis* Jacq. including the Pisifera and Dura) genome sequencing; however, the data is not available in the public domain.[23] The *Agrobacterium tumefaciens*-mediated[24] or biolistic mediated[25] mode of genetic transformation for oil palm can be used once the expression cassettes are ready for the over expression of KAS-II. However, the alternative of chloroplast transformation can also be considered for the genetic transformation of oil palm.[26,27]

CONCLUSIONS

In conclusion, we determined the full-length cDNA sequence of the KAS-II gene from American and African oil palms. We showed three active sites (catalytic triad) and the conserved domains in both *Eo*KAS-II and *Eg*TKAS-II deduced protein. These two cDNA clones are important for the genetic engineering of fatty acid biosynthesis pathway in oil palm to improve nutritional quality of the palm oil. However, the biochemical characterization of the *Eo*KAS-II and *Eg*TKAS-II proteins is not done yet and need further study in this line to understand the difference in the efficacy of this enzyme in two *Elaeis* species. Furthermore, an investigation of the structural differences between *Eo*KAS-II and *Eg*TKAS-II protein is warranted.

ACKNOWLEDGMENTS

REFERENCES

1. Abdullah N, Rafii Yusop M, Ithnin M, Saleh G, Latif MA. Genetic variability of oil palm parental genotypes and performance of its' progenies as revealed by molecular markers and quantitative traits. C R Biol 2011;334:290-9.
2. Cristancho RJ, Hanafi MM, Omar SR, Rafii MY. Variations in oil palm (*Elaeis guineensis* Jacq.) progeny response to high aluminium concentrations in solution culture. Plant Biol (Stuttg) 2011;13:333-42.
3. Bhore SJ, Shah FH. Construction of efficient and effective transformation vectors for palmitoyl-acyl carrier protein thioesterase gene silencing in oil palm. Bioinformation 2011;6:212-20.
4. Parveez GK, Masri MM, Zainal A, Majid NA, Yunus AM, Fadilah HH, *et al*. Transgenic oil palm: Production and projection. Biochem Soc Trans 2000;28:969-72.
5. Parveez GK, Bahariah B. Biolistic-mediated production of transgenic oil palm. Methods Mol Biol 2012;847:163-75.
6. Rajanaidu N, Jalani BS, Kushairi A. Crop improvement for the 21st century. New Delhi: Research Signpost; 1997. p. 11.
7. Shah FH, Cha TS. A mesocarp-and species-specific cDNA clone from oil palm encodes for sesquiterpene synthase. Plant Sci 2000;154:153-60.
8. Bhore S, Shah F. Genetic Engineering of Oil Palm: Novel transformation vectors for PATE gene silencing and genetic transformation of oil palm. Germany: LAP LAMBERT Academic Publishing Gmb H and Co. KG; 2012.
9. Bhore SJ, Shah FH. Genetic transformation of the American oil palm (*Elaeis oleifera*) immature zygotic embryos with antisense palmitoyl-acyl carrier protein thioesterase (PATE) gene. World Appl Sci J 2012;16:362-9.
10. Lange BM, Wildung MR, Stauber EJ, Sanchez C, Pouchnik D, Croteau R. Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. Proc Natl Acad Sci U S A 2000;97:2934-9.
11. Bhore SJ, Kassim A, Loh CY, Shah FH. Analysis of beta-carotene hydroxylase gene cDNA isolated from the American oil-palm (*Elaeis oleifera*) mesocarp tissue cDNA library. Bioinformation 2010;5:104-12.
12. Price AC, Choi KH, Heath RJ, Li Z, White SW, Rock CO. Inhibition of beta-ketoacyl-acyl carrier protein synthases by thiolactomycin and cerulenin. Structure and mechanism. J Biol Chem 2001;276:6551-9.
13. Hakozaki H, Park JI, Endo M, Takada Y, Kazama T, Takeda Y, *et al*. Expression and developmental function of the 3-ketoacyl-ACP synthase2 gene in *Arabidopsis thaliana*. Genes Genet Syst 2008;83:143-52.
14. Rock CO, Jackowski S. Forty years of bacterial fatty acid synthesis. Biochem Biophys Res Commun 2002;292:1155-66.
15. Staunton J, Weissman KJ. Polyketide biosynthesis: A millennium review. Nat Prod Rep 2001;18:380-416.
16. von Wettstein-Knowles P, Olsen JG, McGuire KA, Henriksen A. Fatty acid synthesis. Role of active site histidines and lysine in Cys-His-His-type beta-ketoacyl-acyl carrier protein synthases. FEBS J 2006;273:695-710.
17. Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, *et al*. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 2007;318:245-50.
18. Low ET, Alias H, Boon SH, Shariff EM, Tan CY, Ooi LC, *et al*. Oil palm (*Elaeis guineensis* Jacq.) tissue culture ESTs: Identifying genes associated with callogenesis and embryogenesis. BMC Plant Biol 2008;8:62.
19. Ohlrogge JB, Jaworski JG. Regulation of fatty acid synthesis. Annu Rev Plant Physiol Plant Mol Biol 1997;48:109-36.
20. Pirtle RM, Yoder DW, Huynh TT, Nampaisansuk M, Pirtle IL, Chapman KD. Characterization of a palmitoyl-acyl carrier protein thioesterase (FatB1) in cotton. Plant Cell Physiol 1999;40:155-63.
21. Othman A, Lazarus C, Fraser T, Stobart K. Cloning of a palmitoyl-acyl carrier protein thioesterase from oil palm. Biochem Soc Trans 2000;28:619-22.
22. Cha TS, Habib Shah F. Kernel-specific cDNA clones encoding three different isoforms of seed storage protein glutelin from oil palm *Elaeis guineensis*. Plant Sci 2001;160:913-23.
23. Bhore SJ. Biomalaysia 2009: News and Views from Biotech Industry. Global J Biotech Biochem 2010;5:206-10.

24. Izawati AM, Parveez GK, Masani MY. Transformation of oil palm using *Agrobacterium tumefaciens*. Methods Mol Biol 2012;847:177-88.
25. Kadir Ahmad Parveez G. Biolistic mediated production of transgenic oil palm. Methods Mol Biol 2008;477:301-20.
26. Uthaipaisanwong P, Chanprasert J, Shearman JR, Sangsrakru D, Yoocha T, Jomchai N, *et al*. Characterization of the chloroplast genome sequence of oil palm (*Elaeis guineensis* Jacq.). Gene 2012;500:172-80.
27. Wani SH, Haider N, Kumar H, Singh NB. Plant plastid engineering. Curr Genomics 2010;11:500-12.

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