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CHARACTERIZATION AND REGULATION OF THE OIL PALM *(Elaeis guineensis)* STEAROYL-ACP DESATURASE GENES

Keywords: *Elaeis guineensis,* stearoyl-ACP desaturase and gene regulation.

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Two different stenroyl-ACP desaturase genes are expressed in the mesocarp of oil palm (E. **guineensis) fruits.** *Their nucleotide sequences share 93% and 76% homologies within the coding and 3' untranslated regions, respectively. Southern blot analysis showed that there are at least two copies of stearoyl-ACP desaturase genes in the oil palm genome. Northern blot analysis using gene specific probes showed that the two genes from the mesocarp are differentially regulated. One gene is constitutively expressed with a high level of expression in various oil palm tissues. The othergene was found to be developmentally regulated with its expression pattern correlating with the pattern of oil synthesis in both the mesocarp and kernel tissues. Polyclonal antibodies were raised againts a peptide containing the N-terminal sequence of the mature oil palm stearoyl-ACP desaturase. The antibodies recognized the predicted size protein of 37kDa in total protein fractions of oil palm mesocarp after separation on denaturing polyacrylamide gels. Western blot analysis using the polyclonal antibodies showed the enzyme level is high in the mesocarp at late stage of ripening and remains high in ripe fruits. The leaf form of the enzyme appeared to be about 2kDa larger. High levels of enzyme and gene expression were detected in young mesocarp tissue consistent with the requirement for high levels of unsaturated fatty acids for membrane lipid biosynthesis.*

INTRODUCTION

In plants, stearoyl-ACP desaturase is an important fatty acid biosynthetic enzyme responsible for the production of oleic acid. It is a soluble enzyme in the plastid which introduces a cis double bond into saturated stearoyl-ACP (18:0-ACP) at the A9 position to produce monounsaturated oleoyl-ACP (18:1-ACP). Its catalytic activity requires O_2, NAD(P)H, NAD(P)H ferredoxin oxido-reductase and ferredoxin (Nagai and Bloch, 1968). This enzyme is found in all plant tissues, where it has a vital housekeeping role for producing unsaturated fatty acids required in membrane lipid biosynthesis. In oil accumulating tissues like anthers, seeds and mesocarp, it is also involved in the developmentally regulated process of storage lipid biosynthesis (Murphy and Piffanelli, 19981. Olive oil and palm oil, for example, contain high levels of oleic acid which has a great influence on the physical and nutritional properties of these vegetable oils. These properties are important in determining their various applications as food product. Oleic acid is also a valuable feedstock for the rapidly growing oleochemical industry which uses plant oils as renewable resources. Oleic acid and its chemical derivatives are required for the production of high value-added products such as cosmetics, pharmaceutics and polymers (Pryde and Rothrus, 1989).

cDNA clones encoding stearoyl-ACP desaturase have been isolated from various plant species such as castor (Shanklin and Somerville, 19911, rape (Slocombe et al., 1992) and recently, the oil palm (Shah and Rashid, 1996). The cDNAs encode precursor proteins containing N-terminal leader peptide required for targeting to the plastid. The sequence of the mature protein is highly conserved with greater than 80% identity between different plant species. The enzyme is a homodimer with a subunit mass of approximately 37kDA (Shanklin and Somerville, 1991; Thompson *et* al., 1991). A great advance in understanding the chemical mechanism of double bond insertion was achieved with the recent availability of the x-ray crystallographic structure of recombinant homodimeric castor stearoyl-ACP desaturase (Lindqvist et al., 1996). The three-dimensional structure serves as a model for analysing amino acid residues required in catalysis and electron transport for stearoyl-ACP desaturase and other closely related soluble plant desaturases. Furthermore, the overall conformation and, particularly, the orientation of the iron-binding residues of this desaturase, are remarkably similar to those of microbial monooxygenases and ribonucleotide reductases, implying that all of these proteins form a related group of diiron-oxo enzymes with similar reaction mechanisms (see Murphy and Piffanelli, 1998 for a recent review).

The current oil palm genetic engineering programme is aimed at increasing the level of oleic acid in palm oil at the expense of palmitic acid (16:0). One strategy of genetic manipulation is to antisense palmitoyl-ACP thioesterase and increase the expression of β-ketoacyl-ACP synthetase II in order to reduce palmitic production and channelling towards 18 carbon acyl chains (Cheah et *al.*, 1995). Manipulation of the stearoyl-ACP desaturase gene may also be required to cope with the possible accumulation of stearic acid so that more oleic acid can be produced. Furthermore, there is also an interest in producing high stearate palm oil which is achievable by introducing an antisense construct of the stearoyl-ACP desaturase gene as has been reported in rape (Knutson et al., 1992). An understanding of the structure and regulation of stearoyl-ACP desaturase gene in the oil palm would provide valuable background knowledge for any genetic manipulation effort. Such information is essential for altering the fatty acid composition of the storage oil with minimal perturbation to membrane lipids which can otherwise lead to detrimental agronomic effect on the transgenic plants produced (Thompson and Li, 1996).

The aim of this study is to identify different stearoyl-ACP desaturase genes from oil palm mesocarp and to study their regulation in different tissues with special emphasis on the oil-bearing mesocarp and kernel tissues. Investigations were performed at both transcriptional and post-transcriptional levels by studying gene expression patterns and monitoring the enzyme levels in these tissues.

MATERIALS AND METHODS

Plant Materials

Different tissues from *E. guineensis, tenera* (*dura* x *pisifera*) *variety were used.* Inflorescences were tagged at anthesis and the fruit bunches were harvested at various weeks after anthesis (WAA) for RNA and protein extractions from the mesocarp and kernel tissues. Leaves from 3-4 months polybag seedlings were obtained for RNA and protein preparations. Germinated seedlings after one week undergoing germination process were used for RNA extraction.

Sreening of cDNA Library for Oil Palm Stearoyl-ACP Desaturase Gene

Screening of a 15-week oil palm mesocarp lambda ZAP II cDNA library was carried out based on the methods described in Sambrook *et* al. (1989). Duplicate plaque lifts consisting of 200 000 recombinant clones were prepared for screening using a heterologous probe, pRCD1 (fall length stearoyl-ACP desaturase clone from castor). About 200 000 recombinant clones were plated for isolating the full length clone using partial oil palm stearoyl-ACP desaturase cDNA clone as probe.

cDNA inserts to be used as probes were purified and subsequently labelled *using* the Megaprime DNA labelling system from Amersham. Hybridization of the probes to membranes was carried out in hybridization buffer containing 5X SSPE (1X SSPE is 0.18M NaCl, 10mM NaH_2PO_4, pH 7.5, 1mM EDTA), 5X Denhardt's solution (1X Denhardt's solution is 0.02% each Ficoll 400, bovine serum albumin, and polyvinylpyrrolidone), 0.5% SDS, 100mg ml-*denatured salmon sperm DNA, and 1X 10^6 - 5X 10^6cpm ml-i probes. After the hybridization using heterologous probes, the membranes were washed twice in 2X SSPE, 0.1% SDS at 65°C for 15min. When oil palm probes were used, an additional wash in 0.1X SSPE, 0.1% SDS at 65°C for 20min was carried out. The membranes were subsequently exposed to x-ray films for 48hr at -80°C.

Sequence Analysis

Insert-carrying pBluescript SK-plasmids *were in vivo* excised from lambda ZAP II following the method described by the supplier (Stratagene). Three and two overlapping subclones were produced *in order to* get the complete nucleotide sequences of the classes 1 and 2 oil palm stearoyl-ACP desaturase gene, respectively. Phagemid DNA for sequencing was extracted using the Qiagen plasmid mini kit. DNA sequencing was carried out from both directions using the Automated Laser Fluorescent (A.L.F.) sequencer (Pharmacia). The DNASIS Sequence Analysis Software was used for sequence analysis and search for similarity between nucleotide and amino acid sequences.

Southern Blot Analysis

Genomic DNA was extracted from leaf tissue following the method of Dellporta *et* al. (1983). Digestion with restriction enzymes was carried out according to the manufacturer's instructions. Electrophoresis of digested samples was performed using 0.9% agarose gel in TAE buffer (40mM Tris-acetate pH 7.9, 1mM EDTA). Following electrophoresis, the gel was gently shaken in 0.25M HCl for 10min for depurination prior to transfer. The DNA was denatured during overnight transfer onto Hybond N^+ (Amersham) membrane under alkaline conditions using 0.4M NaOH as the transfer buffer. The membrane was rinsed in 2X SSPE before continuing with prehybridization.

Hybridization and preparation of labelled probes were carried out as described above. For medium stringency washes, the membrane was washed twice in 4X SSPE and 0.1X SDS at 50°C for 15min. The membrane was exposed to Kodak X-OMAT x-ray film for one week.

Northern Blot Analysis

Messenger RNA from various oil plam tissues were prepared as described in Siti Nor Akmar *et* al. (1994). Four microlitres of mRNA ($0.5\mu g\ \mu l^{-1}$) was denatured in 18µl of solution containing 78% (v/v) deionized formamide, 16% (v/v) deionized glyoxal, and 10mM NaH_2PO_4/

Na_2HPO_4 (pH 7.0) by heating for 15min at 55°C followed by immediate cooling. Denatured mRNA was separated on 1.2% agarose gel using 40mM Tris-acetate (pH 7.0) as electrophoresis buffer. Transfer to nylon membrane (Hybond-N Amersham) was carried out using a vacuum blotter (60cm H_2O, 4hr) in 20X SSC (1X SSC is 0.15M NaC1, 15mM trisodium citrate $2H_2O$, pH7.0). Hybridization and preparation of labelled probes were carried out as described above.

Western Blot Analysis

Protein was extracted by homogenizing about 10g of sliced oil palm tissues in 100ml of cold extraction buffer (40mM NaH_2PO_4, 60mM $NaHPO_4$, 0.1mM PMSF and 2mM dithiothreitol, pH '7.0). The supernatant was recovered by centrifugation at 1000xg. Polyvinylpyrrolidone was added until the solution became viscous and the mixture kept at 4°C overnight. The mixture was recentrifuged and the supernatant filtered through glass wool. The clear solution recovered was vacuum dried. Protein concentration was determined based on the method of Bradford (1976). Separation of total soluble protein extract on denaturing polyacrylamide gel was carried out as described by Laemmli (1972). Transfer to nitrocellulose Hybond-C membrane was performed using transfer buffer (25mM Tris pH 8.3, 192mM glycine, 20% (v/v) methanol 0.02% (w/v) SDS at 4°C for 3hr using a transblot apparatus (Biorad, USA). The membrane was blocked with 3% (w/v) dry milk powder in TBS (10mM Tris, 150mM NaC1, pH 7.4). Immunodetection of proteins was performed using 1:1000 dilution of the polyclonal antibodies raised in rabbit against multiantigenic peptide (MAP) synthesized using a 14-mer N-terminal sequence of the mature oil palm stearoyl-ACP desaturase. An anti-rabbit IgG conjugated alkaline phosphatase was used as secondary antibody. Colour development was performed using NBT/BCIP substrate (Western Blue; Promega, USA) according to the manufacturer's instructions.

MAP resin peptide synthesis (Applied Biosystem) columns were used for synthesizing the peptide antigen following the manufacturer's instructions. Two milligrams per millilitre purified MAP in 50% (v/v) Freund's complete adjuvant were used in the first rabbit injection. Subsequent boostings were performed using 2mg ml^{-1} MAP in 50% (v/v) Freund's incomplete adjuvant. Antiserum was collected two weeks after boosting.

RESULTS AND DISCUSSION

Sequence Analysis of cDNA Clones

A full length stearoyl-ACP desaturase clone from castor (Shanklin and Somerville, 1991) was used as a heterologous probe to screen 200 000 clones from a 15-week oil mesocarp cDNA library (Siti Nor Akmaret al., 1995). This resulted in the isolation of five partial genes whose nucleotide sequences had greater than 78% homology with the castor sequences. Based on their nucleotide sequences, the clones can be divided into two classes, three in the first and two in the second. The clones with the longest insert in classes 1 and 2 were designated pOPSN15 and pOPSN16, respectively. The overall sequence identity between pOPSN15 and pOPSN16 was 86% with 93% identity within the coding and 76% identity within the 3' untranslated regions. pOPSN15 which had a longer insert of 1350kb, was used as a probe in screening the same oil palm mesocarp cDNA library. Out of the 40 putative clones isolated, three clones, designated pOPSN18, 19 and 20 contained the longest inserts of approximately 1.7kb. The three clones were identical and belonged to class 1. They encoded a protein of 393 amino acids. Their 3' untranslated regions were also identical with the exception of pOPSN18 which contained 42 nucleotides less just before its poly $(A)^+$ tail. pOPSN19 had the longest 5' untranslated region. These class 1 clones were strongly homologous to the reported oil palm stearoyl-ACP desaturase sequence (Shah and Rashid, 1996) with 99% identity at both the nucleotide and amino acid levels. They are believed to be the same as the reported gene and sequencing errors have probably contributed to the slight differences observed.

When the nucleotide sequence of class 1, pOPSN19 was aligned with class 2, pOPSN16 and translated in the same frame, it was observed that the identity between the encoded

amino acids was about 95% (Figure 1). Several of the variant nucleotides in pOPSN16 occurred at the third position of the codons without any alteration to the encoded amino acids. The deduced amino acid sequence of pOPSN19 has two occurrences of the D/E EX_2H motif. Even though the sequence encoded by pOPSN16 lacks 168 N-terminal amino acids, it has five out of six of these conserved residues lacking only the conserved N-terminal E residue. Crystal structure of stearoyl-ACP desaturase showed that each enzyme subunit consists a diiron centre which forms the active site. The conserved E and H residues in the motif are involved in forming ligands with the diiron centre (Lindqvist et al., 1996).

Comparison of the deduced amino acid sequence of class 1 with the known sequences of higher plant acyl-ACP desaturase genes showed that it contains the complete sequence of the chloroplast transit and mature peptides (***Figure 2***). Like sequences of most other stearoyl-ACP desaturases, the oil palm sequence contains the MAST sequence surrounding the cleavage site of the transit peptide (Piffanelli, 1997). The proposed cleavage site is between the methionine and alanine residues, removing 31 N-terminal amino acids. The deduced amino acid sequence of the mature peptide was highly homologous to stearoyl-ACP desaturase from diverse plant species with 86%, 83% and 81% identities with castor (Shanklin and Somerville, 1991), rice (Akagi et al., 1995) and rape (Slocombe et al., 1992) sequences, respectively. If conservative substitutions are considered, the homology increases to 94%, 92% and 91%, respectively.

More recently, a few genes corresponding to variant soluble plant acyl-ACP desaturases, such as the Δ4-palmitoyl (16:0)-ACP desaturase of coriander (*Coriandrum sativum*) seeds (Cahoon ***et al.,*** 1992) and Δ9-16:0-ACP desaturase of milkweed (*Asclepias syriaca*) seed (Cahoon et al., 1997) have been isolated from lipid accumulating tissues. These enzymes act on different chain length saturated acyl-ACP substrates and some insert the double bond at different position from stearoyl-ACP desaturase. Interestingly, their amino acid sequences have the same conserved motif and share substantial overall sequence homology of 60%-70% with A9 stearoyl-ACP desaturases from various plants. Similarly the oil palm sequence was found to share considerable homology with about 66% identities with both the Δ4-16:0-ACP desaturase from coriander and Δ9-16:0-ACP desaturase from milkweed (***Figure 2***). All of these soluble plant desaturases are believed to be evolutionarily related to stearoyl-ACP desaturases. However, the soluble desaturases form a separate group from the putative membrance-bound plant stearoyl-CoA desaturase recently identified in rose (Rosa *hybrida*) by Mizutani et al. (1995) which has very little sequence similarity as shown in the cluster alignment (***Figure 3***). The two oil palm sequences are closely related and they cluster together with the strongly homologous stearoyl-ACP desaturase sequences from castor and the other monocot species, rice.

Gene Copy Number Determination

The entire insert containing class 1 gene (pOPSN19) was used as a probe in Southern analysis to enable detection of closely related genes with substantial homology in the coding region. The hybridization signal was abolished with high stringency washing and, therefore, medium stringency washing was performed after hybridization. Two bands were observed in lanes 1, 2 and 3 where the oil palm genomic DNA was digested with *Eco*RI, ***Hind*** III and ***Xba*** I, respectively ***(Figure 4).*** One of the *Eco*RI and the ***Xba*** I bands was slightly more intense than the second. This more intense band could possibly correspond to the class 1 gene and the less intense one to the class 2 gene. As a whole, this result suggests that there are at least two copies of the stearoyl-ACP desaturase gene in the oil palm genome.

Gene Expression Patterns

Gene-specific probes of approximately 300bp were designed based on the 3' untranslated regions of classes 1 and 2 genes, excluding about 70 of the 3' terminal nucleotides near the polyadenylation site. These were used to probe Northern blots containing mRNA from six different stages of mesocarp (8-20 WAA), three different stages of kernel (10-14 WAA) development and from vegetative tissues using high

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   1 CACGAGAGACAAAGGAGGGGCCTTTCTCTCTTTCTCCTTCTTTCACTCCCTTCCTCCCTC  60

  61 TCTCTCTCTCTCCTAAGGGAGAAAAAAGGAAGGAAGAAAGGGAGGCGAAAAGAAGAAAGG 120

                M  A  S  M  V  A  F  R  P  E  A  F  L  C  F  S  P  P  K
 121 GCAATGGCGTCGATGGTGGCCTTCCGACCGGAGGCGTTCTTATGCTTCTCTCCTCCCAAG 180

                T  T  R  S  T  R  S  P  R  I  S  M  A  S  T  V  G  P  S  T
 181 ACCACGAGGAGTACCAGATCTCCCAGGATTTCCATGGCCTCCACCGTTGGCCCCTCCACC 240

                K  V  E  I  P  K  K  P  F  M  P  P  R  E  V  H  V  Q  V  T
 241 AAGGTTGAGATTCCAAAAAAGCCCTTCATGCCTCCGCGTGAGGTACATGTTCAGGTCACA 300

                H  S  M  P  P  Q  K  I  E  I  F  K  S  L  E  D  W  A  E  N
 301 CATTCAATGCCACCTCAGAAGATTGAGATTTTTAAGTCATTAGAGGACTGGGCAGAAAAT 360

                N  I  L  V  H  L  K  P  V  E  K  C  W  Q  P  Q  D  F  L  P
 361 AATATCTTGGTGCATTTGAAGCCTGTTGAGAAGTGTTGGCAGCCCCAGGATTTTCTGCCT 420

                D  P  S  S  E  G  F  H  E  E  V  K  E  L  R  E  R  S  K  E
 421 GATCCATCATCAGAAGGATTTCATGAAGAGGTTAAGGAACTGAGAGAACGCTCTAAGGAG 480

                I  P  D  D  Y  Y  V  C  L  V  G  D  M  I  T  E*E  A  L  P
 481 ATCCCTGATGATTATTATGTTTGCTTGGTTGGAGATATGATCACTGAAGAAGCTCTTCCT 540

                T  Y  Q  T  M  L  N  T  L  D  G  V  R  D  E  T  G  A  S  L
 541 ACATATCAAACAATGCTCAACACCCTTGATGGAGTTCGAGATGAGACAGGGGCAAGCCTT 600

                T  S  W  A  V  W  T  R  A  W  T  A  E  E*N  R  H*G  D  L
 601 ACTTCTTGGGCAGTCTGGACGAGGGCTTGGACTGCTGAAGAGAACAGACATGGGGACCTT 660
                                  ||||||||||| |||||||||||||| ||||||
                                  TGGACTGCTGAGGAGAACAGACATGGAGACCTT
                L  N  K  Y  L  Y  L  S  G  R  V  D  M  K  Q  I  E  K  T  I
 661 CTCAACAAGTATCTATACCTTTCTGGTAGAGTGGACATGAAGCAAATTGAGAAAACAATC 720
                ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
                CTCAACAAGTATCTATACCTTTCTGGTAGAGTGGACATGAGGCAAATTGAGAAAACAATC
                Q  Y  L  I  G  S  G  M  D  P  M  T  E  N  S  P  Y  L  G  F
 721 CAATATCTGATAGGTTCTGGAATGGATCCTATGACAGAGAACAGCCCCTACCTTGGTTTT 780
                |||||||||||||||| |||||||||||| |||||||||||||| ||||||||||||
                CAATATCTGATAGGTTCCGGAATGGATCCTAGGACAGAGAACAGCCCATACCTTGGTTTT
                I  Y  T  S  F  Q  E*R  A  T  F  I  S  H  G  N  T  A  R  H
 781 ATATACACCTCATTTCAGGAGAGGGCGACTTTCATTTCCCATGGGAATACTGCCAGGCAT 840
                |||||||||| ||||| ||| ||||||| ||||||||||||||||||||||||||||
                ATATACACCTCGTTTCAAGAGCGGGCGACCTTCATTTCCCATGGGAATACTGCCAGGCAT
                A  K  E  H  G  D  V  K  L  A  Q  I  C  G  T  I  A  S  D  E*
 841 GCCAAGGAACATGGGGACGTGAAGTTGGCTCAGATATGTGGTACAATTGCCTCAGATGAG 900
                |||||||||||||||| | |||||||||||||||||||| ||||||||||||||||
                GCCAAGGAACATGGGGACCTAAAGTTGGCTCAGATATGTGGTATAATTGCCTCAGATGAG
                K  R  H*E  T  A  Y  T  K  I  V  E  K  L  F  E  I  D  P  D
 901 AAACGCCATGAGACAGCTTATACTAAGATCGTGGAGAAACTGTTTGAGATCGACCCGGAT 960
                |||||||||||||||| ||||| ||||| |||||||||||||||||||| ||||| |||
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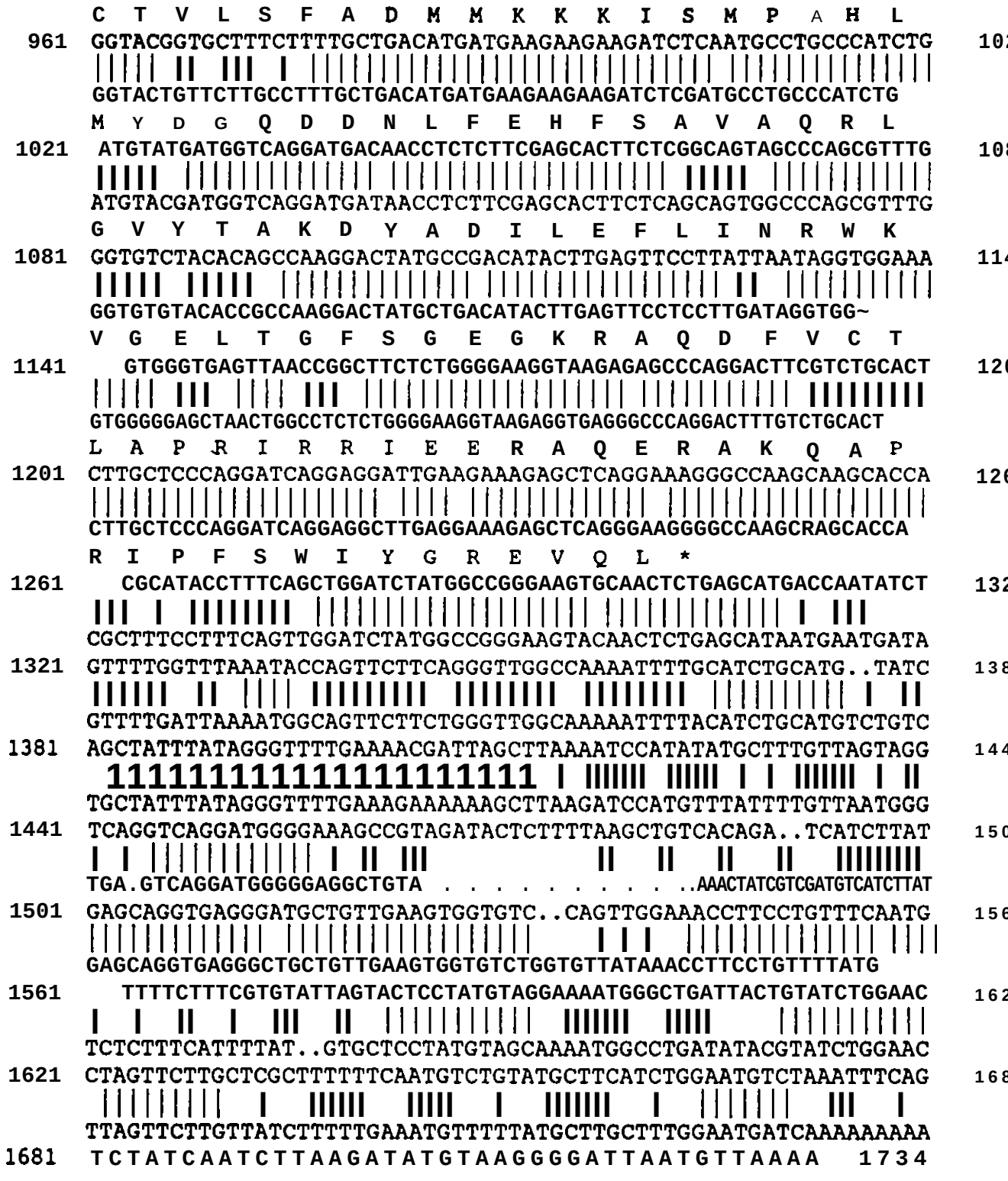
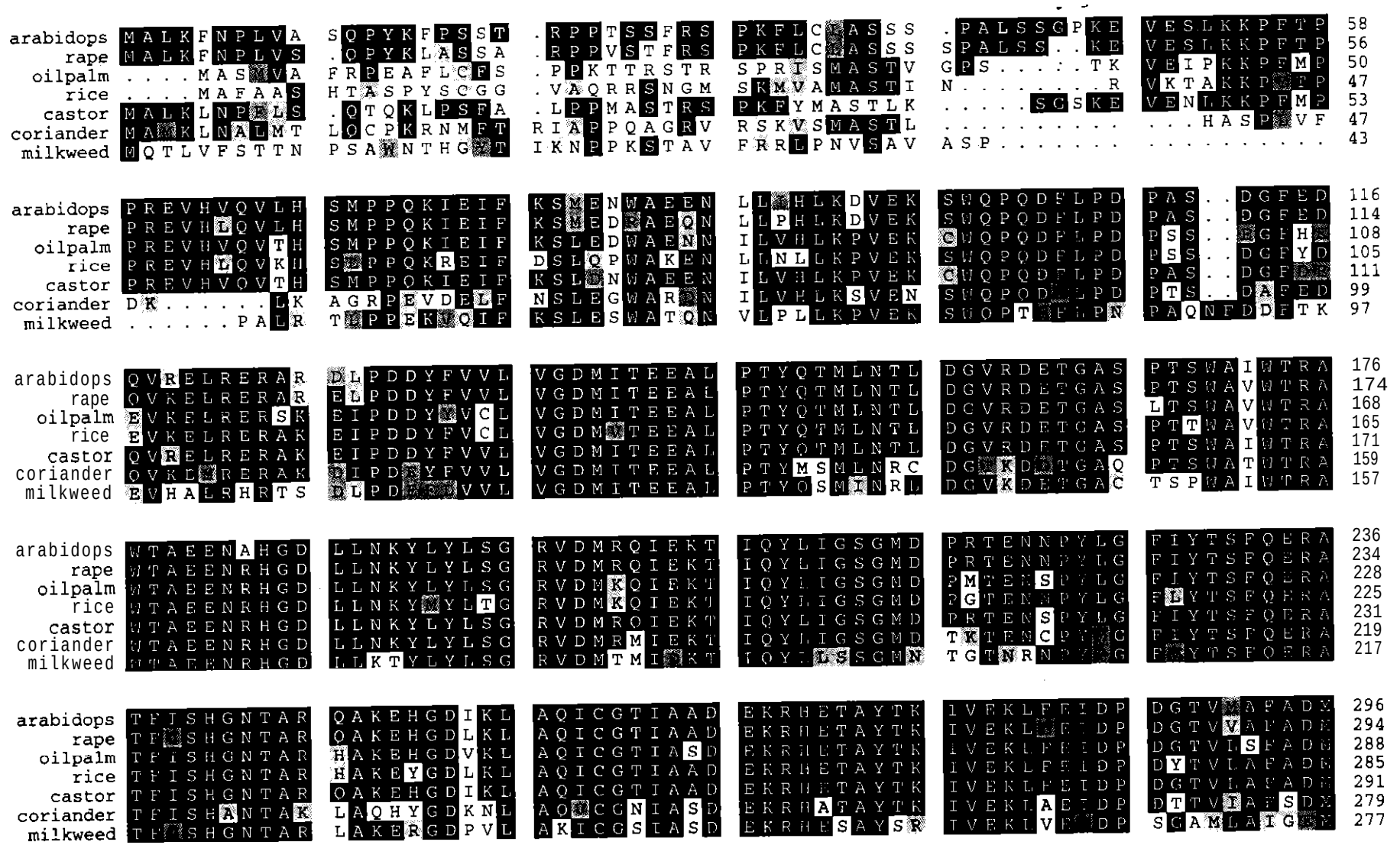


Figure 1. Nucleotide and deduced amino acid sequences of the full length clone, pOPSN19 (class 1) and the nucleotide sequence of the partial gene, pOPSN16 (class 2). Identical nucleotides between pOPSN19 and pOPSN16 are shown with matching lines. Dots have been introduced to optimize alignment. Proposed cleavage site for plastid transit peptide is indicated by a downward arrow. The conserved E and H residues in the higher plant acyl-ACP motif are indicated by asterisks.


arabidops
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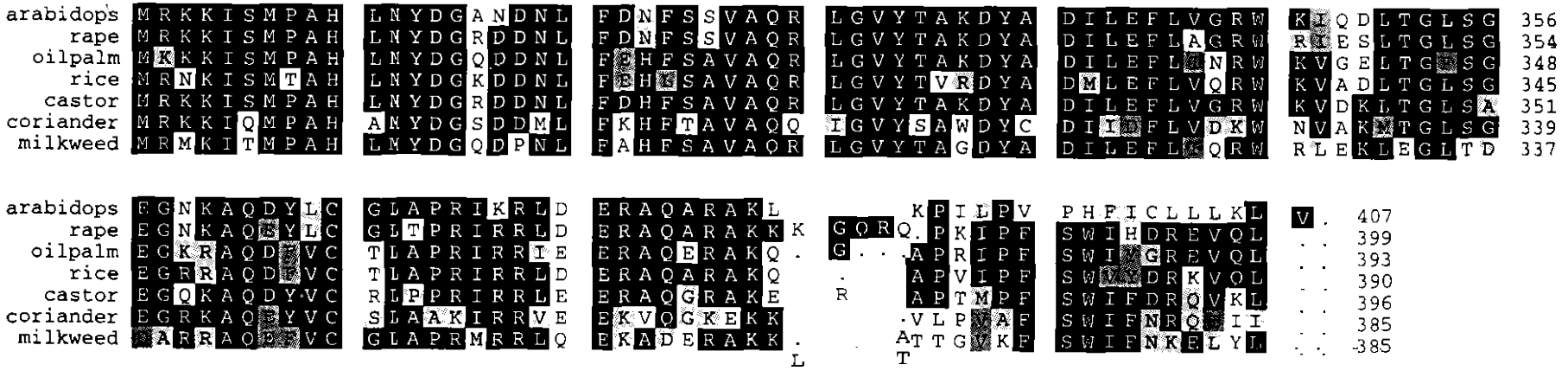

Figure 2. Multiple alignment of the deduced amino acid sequences of higher plant acyl-ACP desaturase genes. The sequences are Δ9-stearoyl (18:0)-ACP desaturases from A. thaliana, *rape* (B. napus), *oil palm* (E. guineensis), *rice* (Oryza sativa), *castor* (Ricinus communis), *Δ4-palmitoyl (16:0)-ACP desaturase from coriander* (Coriandrum sativum) *and Δ9-16:0-ACP desaturase from milkweed* (Asclepias syriaca). *Dark shaded areas represent identical amino acids and light shaded areas represent similar amino acids. Dots have been introduced to optimize alignment.*

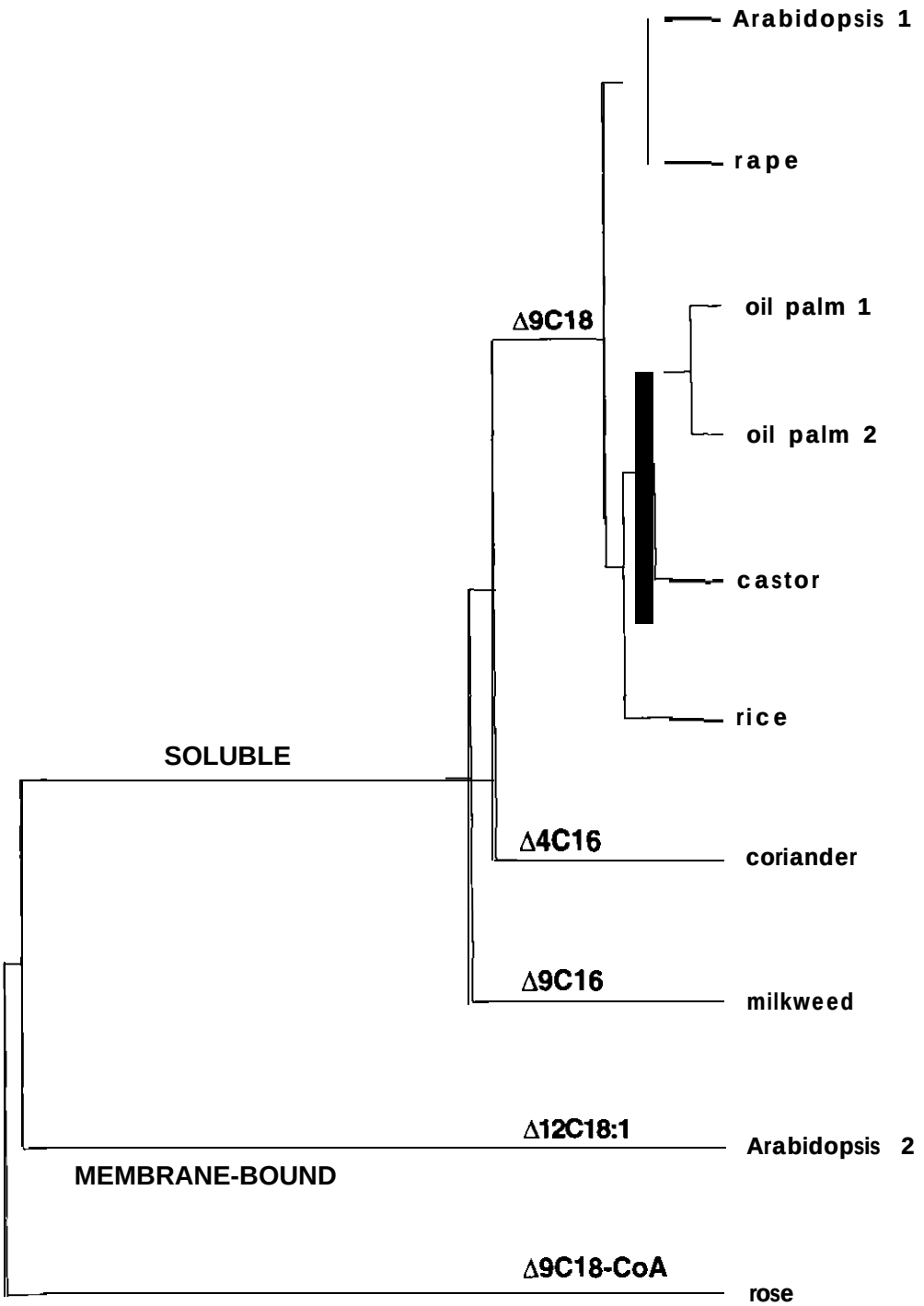

Figure 3. Cluster alignment Of the mature proteins Of higher plant acyl-ACP desaturases and membrane bound desaturases. The **sequences are** *soluble Δ9-stearoyl (18:0)-ACP desaturase* **from A. thaliana,** *rape, oil palm (class 1), oil palm (class 2), rice, castor, Δ4-palmitoyl (16:0)-ACP desaturase from coriander and Δ9-16:0ACP desaturase* ***from*** *milkweed. The membrane-bound desaturases are putative plant stearoyl-CoA desaturase from* **rose (Rosa hybrida) and** *Δ12-oleoyl (18:1) desaturase* ***from*** **A. thaliana.**

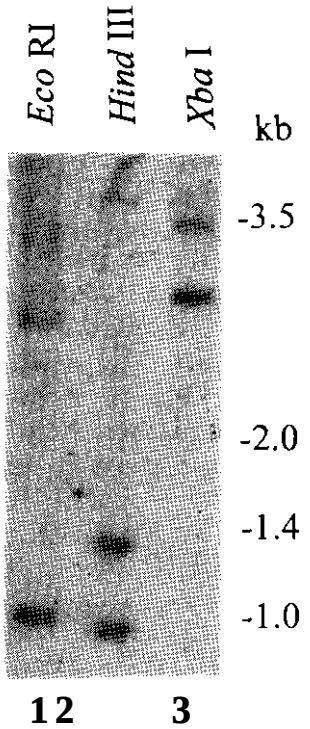

Figure 4. Southern blot analysis of stearoyl-ACP desaturase gene in the oil palm. Lanes 1, 2 and 3 represent 10µg E. guineensis *genomic DNA digested with* Eco*RI*, **Hind** *III and* Xba *I, respectively after separation on 0.9% agarose gel, and probing with* ^{32}*P-labelled entire insert of pOPSN19.*

stringency conditions. It was shown that the two probes, each hybridized specifically to transcripts of about 1.7kb. The expression pattern of class 1 was shown to be different from class 2 (Figure 5). Class 2 is highly expressed and at approximately the same levels in the *E.* guineensis leaves, kernel at 12 and 14 WAA and mesocarp at the different stages of development with the exception of 17-week mesocarp where the expression was approximately three-fold higher. Interestingly, negligible expression levels were detected in lo-week kernel and germinated seedlings.

On the other hand, the expression of class 1 was found to be much lower in general and appeared to be developmentally regulated. The highest level of expression in the mesocarp tissue was around 15 WAA while the expression level in ripe fruits was significantly reduced. A high level of expression was also detected in young mesocarp tissue at eight WAA. The gene was expressed at very low levels in kernel tissues between 10 WAA and 14 WAA with the maximum at 12 WAA. The genes were also weakly expressed in leaves but the expression was not detected in germinated seedlings.

Both the class 1 and class 2 gene-specific probes hybridized only very weakly with mRNA from 15-week mesocarp of *E. oleifera.* However, the 3' untranslated regions of *E. oleifera* stearoyl-ACP desaturase genes may be different from those of *E. guineensis,* and it may not be accurate to correlate the poor hybridization signals observed using both probes with low expression levels.

The different patterns of expression of the two *E.* guineensis genes indicates that the encoded proteins play different roles in the oil palm. Class 2 which is constitutively expressed, most likely encodes an enzyme with housekeeping functions, possibly a stearoyl-ACP desaturase involved in producing unsaturated fatty acid for membrane lipid biosynthesis. It seems likely that class 1 encodes a different isoform of stearoyl-ACP desaturase found in lipid rich tissues which contain high levels of oleic acid.

In developing oil palm fruits, accumulation of oil in the kernel occurs earlier at between 11-14 WAA than in the mesocarp which starts at about 15 WAA and stops when the fruits ripen at 20 WAA (Oo *et* al., 1986; Hartley, 1988). The mesocarp oil contains 39% and kernel oil contains only 15% oleic acid (deMan and deMan, 1994). As the expression of class 1 correlates closely with the pattern of oil synthesis in the mesocarp and kernel, it would seem likely that these genes encode a desaturase directly involved in producing oleic acid for incorporation into triacylglycerol. The high level of expression in young mesocarp tissue is believed not to be related to oil synthesis but rather to production of oleic acid for membrane and cellular lipids for these actively dividing young tissues.

These results suggest the possibility that the oil palm has two differentially regulated stearoyl-ACP desaturase genes. Occurrence of differentially regulated genes for proteins involved in fatty acid synthesis has been reported for genes encording acyl carrier protein in *Arabidopsis thaliana* (Baerson and Lamppa, 1993) and *Brassica napus* (deSilva *et al.*, 1992)

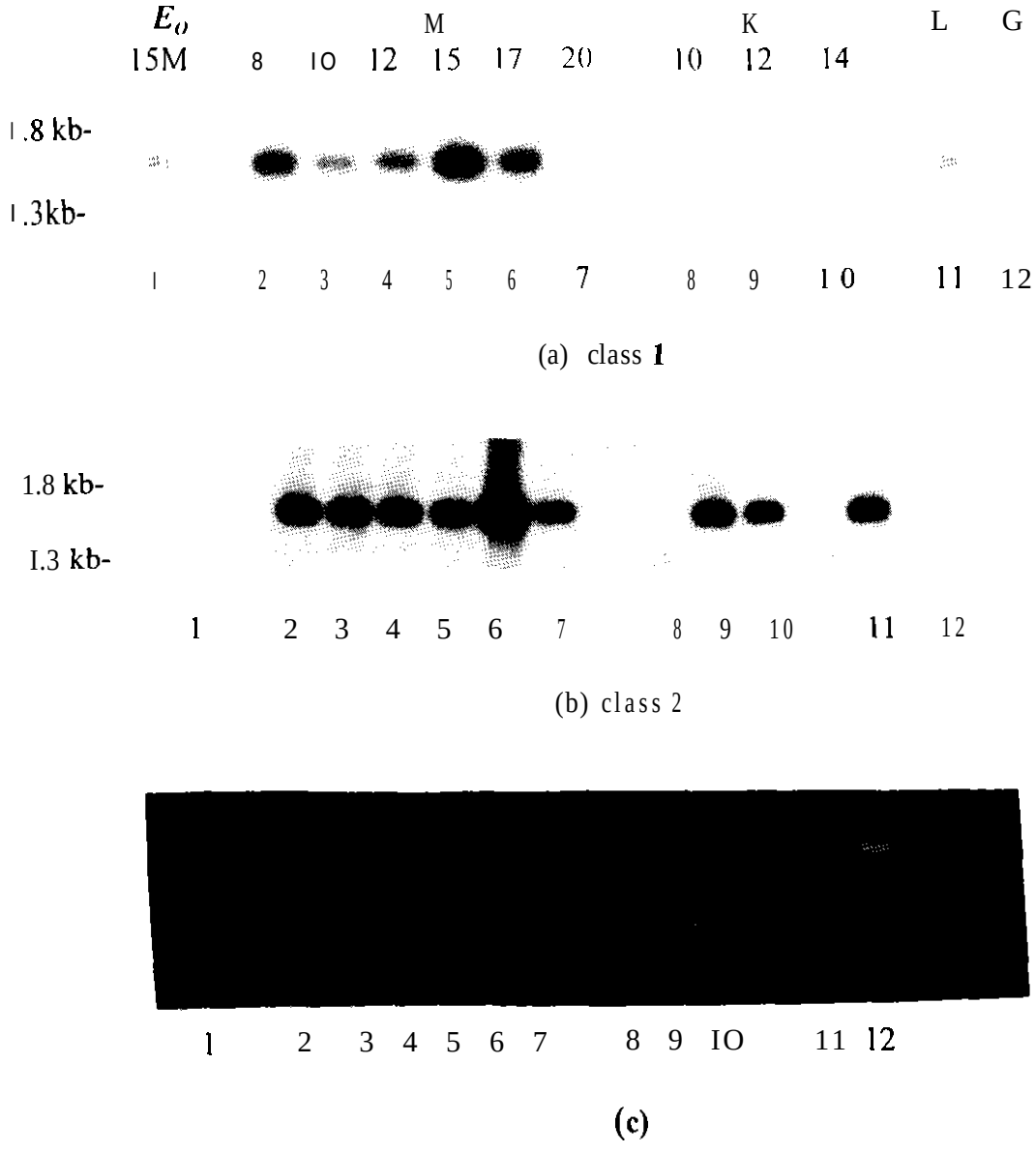

Figure 5. Northern blot analysis of oil palm stearoyl-ACP desaturase genes. Northern blot containing 2µg poly (A)+RNA from various oil palm tissues was probed with ^{32}P-labelled gene specific probes based on 3' untranslated regions of (a) pOPSN19 and (b) pOPSN16. Lane 1 represents 15-week E. oleifera mesocarp. All other samples were from E. guineensis tissues. Lanes (2-7) represent 8, 10, 12, 15, 17 and 20-week mesocarp, respectively; lanes (8-10) represent 10. 12 and 14-week kernel, respectively; lanes 11 and 12 represent leaves and germinated seedlings. Ethidium bromide stained gel (c) showing approximately equal loading of poly (A)+RNA.

where the promoter of the first gene produced ubiquitous expression while the second produced 100-fold higher expression in seeds compared to leaves in transgenic tobacco plants. The results of the transgenic studies were consistent with the pattern of ACP transcipts in the *Arabidopsis thaliana* and *Brassica napus* plants. Differential regulation of stearoyl-ACP desaturase genes has been implicated in *Brassica napus* where the promoter of a stearoyl-ACP desature gene produced about 2-3 fold higher expression in seeds compared to in leaves of transgenic tobacco (Slocombe *et al.*, 1994). Previous studies using Northern analysis had shown the existence of seed-specific gene whose expression at maximal level was

100-fold higher in seeds than leaves (Slocombe *et al.*, 1992). However, more recent studies have shown that the *Brassica napus* stearoyl-ACP desaturase genes involved in housekeeping functions are often upregulated to supply the additional stage-specific and tissue-specific requirements for unsaturated fatty acids (Piffanelli et *al.*, 1997). This study showed that the situation may be different in the oil palm where one of the desaturase genes (class 1) may be 'induced to cater to the high demand for stearoyl-ACP desaturase activities in oil accumulating tissues at specific stages of development, The gene with the housekeeping role (class 2) may still be upregulated to some extent as shown by the three-fold higher expression in oil palm mesocarp at 17 WAA.

Post-transcriptional Regulation

It was not possible to use the polyclonal antibodies previously raised against rape stearoyl-ACP desaturase (Piffanelli, 1997) because no cross reaction was detected with the oil palm mesocarp extract. Based on the full length oil palm stearoyl-ACP desaturase amino acid sequence, a 14-residue peptide near the N-terminal of the mature protein TVGPSTKVEIPKKP which contains three charged amino acids (see *Figure 1*) was selected for synthesizing a multiantigenic peptide (MAP) for raising antibodies. MAP consists of four or eight copies of the same peptide attached to a branchinglysine core matrix. The 14mer peptide was synthesized directly onto the branching lysine arms to form an immunogenic macromolecular structure. The MAP approach proved capable of producing high titre anti-peptide antibodies that recognized the native protein (Tam, 1992). Figure 6 shows that the polyclonal antibodies produced were highly specific for a 37kDa protein from the oil palm mesocarp extract. This was the expected size for stearoyl-ACP desaturase consistent with that observed from other plant species (Shanklin and Somerville, 1991; Thompson et al., 1991). There was no cross reaction detected with the rape embryo protein extract. This was not surprising because the homology between the peptide used for raising the antibodies and rape sequence was very weak (Figure 2).

The polyclonal antibodies were used to detect levels of stearoyl-ACP desaturase in different oil palm tissues (Figure 7). In the mesocarp, the highest level observed was in the young tissue of eight weeks, similar to the Northern blot data (Figure 5). Levels in 12- and 15-week tissues were very low but it increased again at 17 weeks and remained high in the ripe 20-week mesocarp. The enzyme was not detectable in the kernel tissue while in the leaves, it appeared with a slightly higher apparent molecular weight of about 39kDa. In the photosynthetic tissues of *Arabidopsis thaliana* and *Brassica napus*, two distinct isoforms of stearoyl-ACP desaturase were detected; a 38kDa isoform, the same size as that found in seeds and an additional and large 40 kDa isoform (Piffanelli, 19971. *There* is no evidence as yet for a gene encoding stearoyl-ACP desaturase with specific expression in leaves which could lead to a distinct isoform from that in the seed. The studies in Brassica *napus* suggest that the isoform in leaves with slower mobility on denaturing protein gels, could be the result of post-translational modification of the enzyme found in both leaves and seeds. Since the oil palm leaf desaturase was also apparently 2kDa larger than the mesocarp and kernel isoforms, a similar post-translational modification may also be occurring here.

The antibodies may recognize the encoded products of both oil palm classes 1 and 2 genes or just the former depending on their homologies in the region used for antibody production, Comparison of the N-terminal amino acid sequences encoded by the three different stearoyl-ACP desaturase genes isolated from *Thunbergia alata* (Cahoon *et* al., 1994) seed showed quite a high level of variability, but for two different *Brassica napus* genes (Piffanelli, 1997), the sequences in this region are fully identical. If the N-terminal sequence is also conserved in the encoded products of both classes 1 and 2 oil palm genes, we may expect quite high basal levels of enzyme in different tissues due to high and ubiquitous expression of the class 2 gene. There is a possibility that the class 2 gene may not be efficiently translated and thus, the high abundance of the class 2 transcripts in different tissues was not reflected in the levels of the gene product.

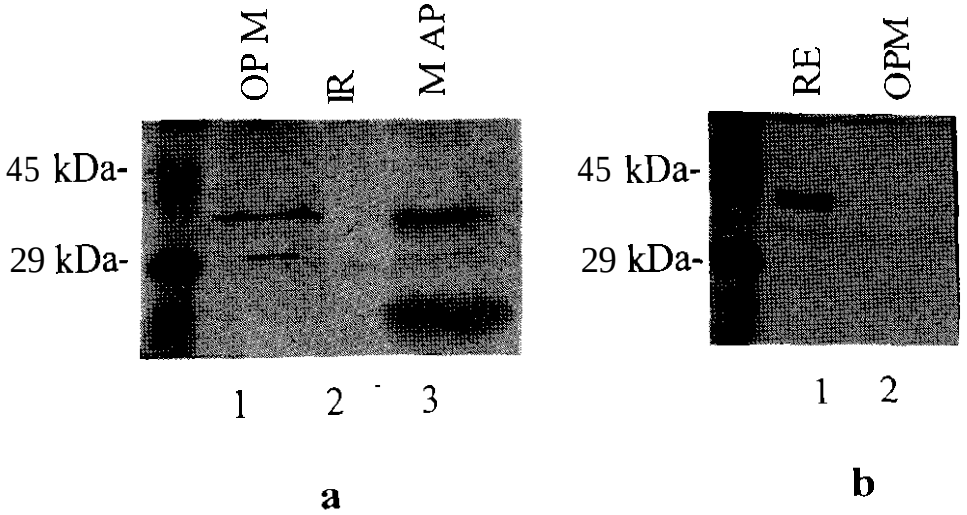

Figure 6. Analysis of polyclonal antibodies raised against synthetic multiantigenic peptide from the oil palm stearoyl-ACP desaturase N-terminal sequence. The serum obtained after the third boost was diluted 1:1000 and used for protein detection on a Western blot (a). Lanes I-3 respectively represent 20μg total protein from oil palm mesocarp, rape embryo and of the multiantigenic peptide separated using 12% denaturing polyacrylamide gel. For comparison, protein on a Western blot (b) were detected using polyclonal antibodies raised against B. napus *(rape) stearoyl-ACP desaturase. Lanes 1 and 2 represent 7μg total protein from rape embryo and oil palm mesocarp, respectively.*

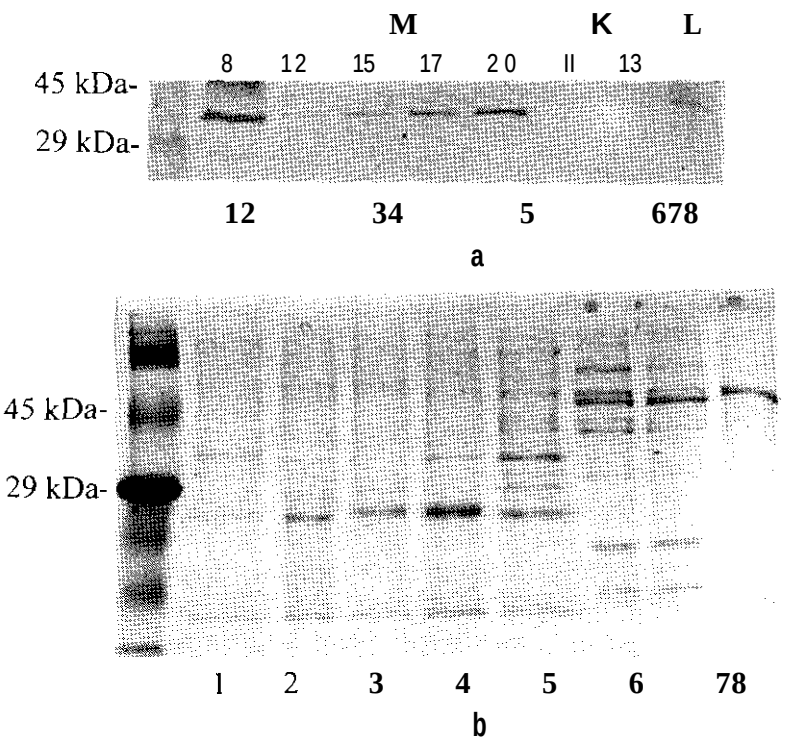

Figure 7. Western blot (a) was analysed using polyclonal antibodies specific for oil palm stearoyl-ACP desaturase. Ten micrograms total protein from oil palm mesocarp and kernel at different stages of development and from leaves were separated on 12% denaturing polyacrylamide gel and detected using 1:1000 dilution of the serum. Lanes (1-5) represent 8, 12, 15, 17 and 20-week mesocarp, respectively; lanes (6 and 7) represent 11 and 13-week kernel and lane 8 represents leave. Duplicate Coomassie Blue stained gel (b) showing equal loading of protein.

In young rapidly growing mesocarp tissues, there is a high requirement for unsaturated fatty acids for membrane and cellular lipids. High levels of stearoyl-ACP desaturase observed in young mesocarp tissue are related to production of oleic acid which serves as the substrate for futher desaturation to polyunsaturated. linoleic (18:2) and linolenic (18:3) acids required for membrane lipid biosynthesis. Gene expression always precedes enzyme production. In the oil palm mesocarp, the enzyme levels start increasing at 17 weeks following gene expression which has maximal expression at 15 weeks for class 1 and 17 weeks for class 2. Active oil accumulation in E. *guineensis* mesocarp starts around 15 WAA and the fruits ripen at about 20 WAA. The major components of the final oil are palmitic and oleic acids. Studies by Aziz et al. (1986) showed that the oil composition at 19 WAA includes about 42.5% palmitate and 36.5% oleate and has not yet reached the final composition of about 44% and 39%, respectively. Thus, the enzymes for producing these fatty acids are still required even at very late stages of ripening. Therefore, the observation that the enzyme level has not significantly dropped in ripe fruits is not totally unexpected and the level may only eventually drop in over ripe fruit when there are no more changes in the fatty acid composition and oil production has stopped.

CONCLUSION

Two differentially regulated stearoyl-ACP genes were identified in the oil palm. Constitutive expression of one of the genes (class 2) suggests a possible housekeeping role in membrane lipid biosynthesis. The other gene (class 1) which is induced in lipid-rich mesocarp and kernel tissues in phase with oil synthesis, is believed to have a direct involvement in storage oil synthesis. This study also showed that regulation at transcriptional level is important in controlling the levels of stearoyl-ACP desaturase in oil accumulating tissues. Based on these findings, it may be possible to manipulate the level of stearoyl-ACP desature in the mesocarp to produce oil with the desired composition by introducing sense or antisense constructs of the class 1 gene without interfering with membrane lipid biosynthesis. The promoter of this gene would be useful in regulating expression of heterologous genes required for oil modification. Furthermore, the polyclonal antibodies specific for oil palm stearoyl-ACP desaturase produced during this study would be a valuable tool to monitor successful production of translational product encoded by the introduced oil palm stearoyl-ACP desaturase gene, and also to check the effects produced on endogenous stearoyl-ACP desaturase in transgenic oil palm.

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