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Full Length Research Paper

Detection of mesocarp oleoyl-thioesterase gene of the South American oil palm *Elaeis oleifera* by reverse transcriptase polymerase chain reaction

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The thioesterase enzyme functions in lipid synthesis by cleaving the acyl-ACP bond and liberating the fatty acid. Thioesterases have been isolated from a number of plant sources. The gene for this enzyme was detected in *Elaeis oleifera* by reverse transcriptase polymerase chain reaction (RT-PCR), cloned and sequenced and found to have considerable sequence similarity with other previously cloned thioesterases. Its highest homology is to the *Brassica napus* oleoyl-ACP thioesterase, 72% at the nucleotide level over the coding region examined, and 83% identity (90% positives) at the amino acid level.

Key words: *Elaeis oleifera*, mesocarp, oleoyl-ACP thioesterase, RT-PCR.

INTRODUCTION

During *de novo* fatty acid synthesis, the growing acyl chain is attached to acyl-carrier-protein (ACP) followed by sequential addition of (C2). The end products of this plastidal fatty acid synthesis, in plants, are palmitoyl-ACP (C16:0) and stearyol-ACP (C18:0) (Shanklin and Somerville, 1991), the stearyol-ACP being subsequently desaturated to oleoyl-ACP (C18:1). The fatty acid chain is then hydrolyzed and is either retained for plastid membrane synthesis or is transported to the cytoplasm and subsequently used for glycerolipid synthesis. The enzyme responsible for the hydrolysis and liberation of the acyl chain from acyl-ACP is the thioesterase. Investigations of thioesterase activity in plants have consistently revealed thioesterase enzyme activity specific for oleic acid, the enzyme being oleoyl-ACP thioesterase, which is believed to be widely distributed in the plant kingdom (Voelker, 1996). However, some plants (for example avocado, the oil and coconut palms) have oil storage tissues in which other classes of fatty acids, such as the medium chain fatty acids, (C8-C14), sometimes predominate. In these tissues, corresponding thioesterase activity, usually specific for the given substrate fatty acid chain length, have been found. Available data suggest therefore that thioesterase activity is responsible, at least in part, for the chain length distribution of the products of fatty acid synthesis in plants, and especially in the oils of seed storage tissues (Dehesh et al., 1996, Voelker et al., 1992). Evidence in support of this have been supplied by experiments in which plants that were transformed with medium chain thioesterases had their fatty acid profiles altered to reflect the activity of the newly incorporated thioesterase gene (Voelker et al., 1992). Thioesterases are therefore important chain length determining enzymes in oil seed crops. They have been isolated from a number of plant sources. In every plant species examined so far, oleoyl-thioesterase enzyme activity has been demonstrated.

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The genus *Elaeis* contains two very closely related palms of high economic value *Elaeis guineensis* and *Elaeis oleifera* (Hartely, 1995). Their oils are also very similar in fatty acid composition but the relative proportions of the major fatty acids are different. While palmitic acid, (C16:0), is the predominant fatty acid in *E. guineensis*, in *E. oleifera*, the predominant fatty acid is oleic acid, (C18:1). This difference in fatty acid proportions also affects their physical properties such that while the oil from *E. guineensis* has a fluid fraction of almost 50% at room temperature, that from *E. oleifera* has a fluid fraction of about 80%. This fluidity is in direct proportion to the content of oleic acid, which is unsaturated.

As part of studies of oil synthesis within the developing mesocarp in these two related species, it is important to find out how this enzyme functions in this tissue of these plants. As the thioesterase enzyme plays an important role in the fatty acid composition, this study was undertaken to isolate and characterize this enzyme in *E. oleifera* within the larger framework of isolating the gene(s) for subsequent use in fatty acid modification through genetic engineering.

MATRIALS AND METHODS

RNA extractions

The mesocarp tissue was harvested from the developing fruit into liquid nitrogen. At extraction, the frozen mesocarp was ground in liquid nitrogen. The extraction buffer contained 0.1 M Tris-HCl, pH 7.6, 0.1 M NaCl, 6% p-aminosalicylic acid, 1% SDS, 0.35% β-mercaptoethanol and 5% phenol. The buffer was added at the rate of 4 ml/g plant tissue, vortexed for thorough mixing and extracted with phenol/chloroform. The supernatant was precipitated twice by 3 M LiCl followed by ethanol precipitation. The pellet was redissolved in DEPC treated distilled water and again precipitated with 3 M LiCl followed by another round of ethanol precipitation. The precipitate was finally dissolved in DEPC treated distilled water and stored at –70^{o}C.

RT-PCR

Prior to cDNA synthesis, 5 µg of the total RNA was treated with 1unit of Rnase free Dnase I (Gibco BRL), according to the supplier's instructions to eliminate possible genomic DNA contamination of the RNA.

The reverse primers, AAGCTTTTTTTTTTTA, AAGCTTTTTTTTTTTC and AAGCTTTTTTTTTTTG (supplied by Genehunter Corporation) were used to synthesize first strand cDNA from total RNA extracted from the developing mesocarp of the *E. oleifera* fruit.

Based on conserved regions from published sequences of the thioesterases from other plants, degenerate oligonucleotide primers were designed for the conserved amino acid sequence RYPAWSD as the forward primer, and TLDYRREC as the reverse primer. PCR products were amplified using these primers on *E. oleifera* cDNA. Amplification products were cloned in *E. coli* vector using a commercial Promega PCR products cloning kit and sequenced.

Northern analysis

20 µg of total RNA extracted from *E. guineensis* and *E. oleifera* mesocarp tissues at 5, 12, 15, 17 and 20 weeks after anthesis (WAA), 15 WAA kernel as well as leaf and root tissues of both species were electrophoresed in 1% agarose containing 2.2 M formaldehyde, 20 mM MOPS [N-(morpholino)propanesulphonic acid, 8 mM sodium acetate and 1 mM EDTA pH 8. The loading buffer contained the same constituents in addition to 50% formamide. After migration, the gel was blotted to nylon membrane (Amersham) and detected according to Sambrook et al. (1989). This filter was probed with α^{32}p dCTP labeled thioesterase fragment that was excised from the plasmid by restriction digest.

RESULTS AND DISCUSSIONS

Three conserved regions, at the amino acid level, exist in thioesterase proteins (Jones et al., 1995). We took advantage of this sequence conservation to amplify the region in between by PCR. For the initial cDNA synthesis, total RNA extracted from the developing mesocarp, 17 week after anthesis, served as template. Using the TLDYRREC as primer for initial cDNA synthesis followed by PCR amplification of target DNA with primers corresponding to YPTWGD and TLDYRREC, it was possible to amplify the 450 base pair fragment, the expected size from *E. oleifera*. The amplification product was cloned and sequenced to establish its identity. The nucleotide as well as the derived amino acid sequences is presented in Figure 1.

To determine the relatedness of the sequence to other known thioesterases, the sequence was compared with other thioesterases in the National Center for Biotechnology Information (ncbi.nlm.nih.gov) GenBank sequences using the 'BLAST' search programme. The results indicate that the amplified fragment from this species is indeed that of an oleoyl-ACP thioesterases with high homology to the *Brassica napus* oleoyl-ACP thioesterase gene (Figure 2). It has 72% homology at the nucleotide level, over the coding region examined, and 83% identity (90% positives) at the amino acid level.

Northern blot analysis of this transcrpt, shows, that the amplified *E. oleifera* mesocarp oleoyl-ACP thioesterase RNA is strongly expressed at 20 weeks after anthesis (WAA) in the *E. oleifera* fruit mesocarp (Figure 3). The transcript level detectable by northern hybridization was very low at 17 WAA, and could not be detected at any other time in the developing fruit.

This is the first time that the presence of oleoyl-ACP thioesterase is demonstrated in this species by isolation and cloning. This finding is consistent with the fact that in every plant species so far examined, oleoyl-thioesterase enzyme activity has been found. This gene for this enzyme in *E. oleifera* was found to have considerable sequence similarity with other previously cloned thioesterases from other species. The fact that the fruit fully ripens at about 28 WAA whereas this particular transcript is expressed only strongly at 20 WAA suggests

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TAT CCA ACT TGG GGT GAT GTT GTT GAG ATT GAA ACA TGG TGC CAA GGG    48
 Y   P   T   W   G   D   V   V   E   I   E   T   W   C   G   G    16
GAA GGA AGA ATA GGC ACC AGG CGT GAT TGG ATT ATC AAG GAT TTG GCT    96
 E   G   R   I   G   T   R   R   D   W   I   I   K   D   L   A    32
ACT GGT GAA GTT ATT GGT AGA GCC ACC AGC AAG TGG GTA ATG ATG AAC   144
 T   G   E   V   I   G   R   A   T   S   K   W   V   M   M   N    48
CAA GAT ACT AGG AAA CTT CAA CGA GTA AGT GAT GAA GTG AGG GAA GAA   192
 Q   D   T   R   K   L   Q   R   V   S   D   E   V   R   E   E    64
TAT CTT GTC TTC TGC CCG AGA ACT CCT AGA TTA GCA TTT CCA GAG GAG   240
 Y   L   V   F   C   P   R   T   P   R   L   A   F   P   E   E    80
GAT AAT GGC AGC GTG AAG AAA ATT CCT AAA CTT GAA GAG CCT GCA GAT   288
 D   N   G   S   V   K   K   I   P   K   L   E   E   P   A   D    96
TAT TCA CGA TCA GAA CTT GTT CCC AGG AGA GCT GAT TTG GAC ATG AAC   336
 Y   S   R   S   E   L   V   P   R   R   A   D   L   D   M   N   112
CAA CGT GTA AAC AAT GTA ACT TAT ATC GGA TGG GTC CTT GAA AGC ATG   384
 Q   R   V   N   N   V   T   Y   I   G   W   V   L   E   S   M   128
CCT CAA GAA ATT ATC GAT ACC CAT GAA CTC CAG ACA ATC ACT CTG GAT   432
 P   Q   E   I   I   D   T   H   E   L   Q   T   I   T   L   D   144
TAT AGA AGA GAA TGC                                              447
 Y   R   R   E   C                                              149
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Figure 1. Nucleotide and deduced amino acid sequences of *E. oleifera* mesocarp oleoyl-ACP thioesterase RT-PCR product.

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B. napus      YPAWSDVVEIETWCQSEGRIGTRRDWILRDSATNEVIGRATSKWVMMNQDTRRLQRVTDE   210
E. oleifera   **T*G**********G**********IK*L**G****************K****S**   180

B. napus      VRDEYLVFCPREPRLAFPEENNSSLKKIPKLEDPAQYSMLELKPRRADLDMNQHVNNVTY   270
E. oleifera   **E********T********D*G*V*******E**D**RS**V*****************   360

B. napus      IGWVLESIPQEIIDTHELQVITLDYRREC                                  299
E. oleifera   *******M*********P*T*********                                  447
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Figure 2. Alignment of derived polypeptide sequence of *E. oleifera* oleoyl-ACP thioesterase with the oleoyl-ACP thioesterase of *B. napus* (conserved regions are underlined).

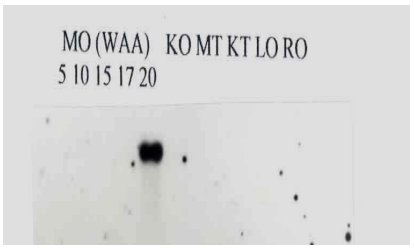

Figure 3. Northern blot analysis of the expression of *E. oleifera* mesocarp oloeyl-ACP thioesterase. RNA was isolated from *oleifera* mesocarp (MO) at 5, 10, 15, 17 and 20 weeks after anthesis (WAA), *oleifera* kernel (KO), *Tenera* mesocarp (MT), *Tenera* kernel (KT), *oleifera* leaves (LO) and *oleifera* roots (RO).

however, that there might be other thioesterases that are active in this species at other stages of maturity in the fruit. There could also be expression control at the level of translation to make high levels of the corresponding protein available even at relatively lower transcript levels. We intend to check further by southern blot hybridization how this gene is organized, if it is also a small family of genes in this species, as suggested for *Arabidopsis thaliana* (Dormann et al., 1995). Experiments are also in progress to isolate full-length clones of this gene in these species and to find out some regulatory mechanisms that control the expression of the gene in these crops.

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