African Journal of Biotechnology Vol. 3 (3), pp. 199-201, March 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Short communication

Isolation of a kernel oleoyl-ACP thioesterase gene from the oil palm *Elaeis guineensis* Jacq.

Omorefe Asemota*, Cha Tye San, Farida H. Shah

Department of Genetics, Faculty of Life Sciences, Universiti Kebangsaan Malaysia, 43600 Bangi, Malaysia.

Accepted 22 December 2003

Thioesterases play a central role in determining chain lengths of fatty acids in oil storage tissues and have been isolated from a number of plant sources. While in some species enzymes that are specialized for the predominant fatty acids in the tissues examined have been found, in others, enzymes that are active over a broad range were observed. We have isolated a cDNA clone from the developing kernel of the oil palm *Elaeis guineensis* which encodes a thioesterase enzyme. Its highest homology was to the *Brassica napus* oleoyl-ACP thioesterase with which it had 72% homology at the nucleotide level, over the coding region examined, and 83% identity (90% positives) at the amino acid level.

Key words: *Elaeis guineensis*, kernel, oleoyl-ACP thioesterase, cDNA.

INTRODUCTION

The oil palm, *Elaeis guineensis* Jacques, is the highest producing oil seed crop, on a per hectare basis, in the world (Rajanaidu *et al*, 1997) and it has the distinction of producing two different but economically important oils in its fruit; palm oil from its mesocarp and palm kernel oil from its kernel. The mesocarp oil is rich in palmitic acid (C16.0), which constitutes about 50% of the oil but it also contains oleic acid (C18.1), which is about 33% in the mature fruit. In the kernel, the oil is mainly constituted by lauric acid (C12.0), about 50%. While the mesocarp largely contains long chain fatty acids, the kernel contains mainly medium chain fatty acids. A physical barrier, the shell separates these two tissues in the fruit (Hartely, 1988).

The growing acyl chain is linked, during fatty acid synthesis, by a thioester bond, to an acyl carrier protein (ACP), from which the thioesterase enzyme, at the end of synthesis finally releases it. This hydrolytic enzyme has been purified from a number of species including the oil palm (Sambanthamurthi and Oo, 1990). The corresponding cDNA has also been obtained for some species such as *Brassica napus* (Loader et al., 1993). The analysis of these clones has shown that there are two types of thioesterases that function in plants, the type A and type B fatty acids thioesterases (Jones et al., 1995). Both enzymes are believed to be widespread in plants having arisen, perhaps, from an ancient gene duplication (Voelker, 1996). Fat A, oleoyl-ACP thioesterase, is expressed in tissues which store oleic acid (C18.1) and is believed to be responsible for the predominant C16.0-C18.1 fatty acid content of plant membranes. Fat B on the other hand has been found in tissues that store saturated and short length fatty acids C8.0-C14.0. While thioesterases with activities that are specific for particular fatty acid chain lengths have been isolated (Dehesh et al., 1996), others with broader specificities have also been found (Voelker et al., 1997).

In the oil palm, *E. guineensis*, the fatty acid composition of the kernel at maturity is about 47% C12.0, 17% C14.0, 9% C16.0 and 16% C18.1 with a few other fatty acids in minor proportions. As thioesterases are considered to be responsible, at least for the most part, for fatty acid chain length determination in oil storage tissues, it is of importance to find out how this enzyme functions in this tissue. The isolation and characterization of the thioesterase gene from the kernel of the oil palm was thus the object of the study reported here.

*Corresponding author. Present address: Nigerian Institute for Oil Palm Research, P.M.B. 1030, Benin City, Nigeria. E-mail: efe_asemota@hotmail.com.

```
1   T GGG TTC GCC ACG ACC CCT ACC ATG AGG AAA CTT CGC CTC ATA TGG    46
1      G   F   A   T   T   P   T   M   R   K   L   R   L   I   W    15

47  GTG ACT TCT CGC ATG CAC ATT GAA ATA TAT AAG TAT CCT GCT TGG GGT   94
16   V   T   S   R   M   H   I   E   I   Y   K   Y   P   A   W   G   31

95  GAT GTT GTT GAG ATT GAA ACA TGG TGC CAA GGG GAA GGA AGA ATA GGC  142
32   D   V   V   E   I   E   T   W   C   Q   G   E   G   R   I   G   47

143 ACC AGG CGT GAT TGG ATT ATC AAG GAT TTG GCT ACT GGT GAA GTT ATT  190
48   T   R   R   D   W   I   I   K   D   L   A   T   G   E   V   I   63

191 GGT AGA GCC ACC AGC AAG TGG GTA ATG ATG AAC CAA GAT ACT AGG AAA  238
64   G   R   A   T   S   K   W   V   M   M   N   Q   D   T   R   K   79

239 CTT CAG CGA GTA AGT GAT GAA GTG AGG GAA GAA TAT CTT CTC TTC TGG  286
80   L   Q   R   V   S   D   E   V   R   E   E   Y   L   V   F   C   95

287 CCG AGA ACT CCT AGA TTA GCA TTT CCA GAG GAG GAT AAT CGC AGC GTG  334
96   P   R   T   P   R   L   A   F   P   E   E   D   N   G   S   V  111

335 AAG AAA ATT CCT AAA CTT GAA GAG CCT GCA GAT TAT TCA CGA TCA GAA  382
112  K   K   I   P   K   L   E   E   P   A   D   Y   S   R   S   E  127

383 CTT GTT CCC AGG AGA GCC GAT TTG GAC ATG AAC CAA CAT GTA AAC AAT  430
128  L   V   P   R   R   A   D   L   D   M   N   Q   H   V   N   N  143

431 GTA ACT TAT ATC GGA TGG GTC CTT GAA AGC ATG CCT CAA GAA ATT ATC  478
144  V   T   Y   I   G   W   V   L   E   S   M   P   Q   E   I   I  159

479 GAT ACC CAT GAA CTC CAG ACA ATC ACC CTG GAT TAC AGG AGA GAA TGC  526
160  D   T   H   E   L   Q   T   I   T   L   D   Y   R   R   E   C  175

527 CAG CAT AAT GAC ATG GTT GAT TCT CTT ACT AGT CTG GAA TTG GCT GAT  574
176  Q   M   N   D   M   V   D   S   L   T   S   L   E   L   A   D  191

575 GAT TAT AGC ACT AAT GGG TCT GCT ATT GGA AAG CAA CAC AAG AAA GAG  622
193  D   Y   S   T   N   G   S   A   I   G   K   Q   H   K   K   E  207

623 CAC CCA TCG CTT TTT GCA TTT CTT GAG ATT GTC CAG CAC TGC ACT TGA  670
208  H   P   S   L   F   A   F   L   E   I   V   Q   H   W   T   *  223

671 AAT AAA TCG AGG TCG CAC TGA GTG GAG GAA GCT AGT TCG ATG AGG TTT  718
719 TGT GTT GGA AGC TAC TTT GTT CTC CCT CTT TTG TCT CTG CTC TTT AGA  766
767 ATG CAT GTA TTA TGC TGT CAT TTC GCT GTT GTT TTC TTC CAT TTG CCA  814
815 ATT ATG TCA TGT TAC TTG TAA GCG CTC AGA TTT TGC AGC AAT TTG CAT  862
863 GAT TGA TTG GAT GGT GAG CTA TTT TTA TGC AAG AGA TAT ATA GAC AAC  910
911 TTT                                                              913
```

Figure 1. Nucleotide sequence (GenBank accession number AF110462) and amino acid translation of pKTT1 (*Tenera* kernel oleoyl-ACP thioesterase) clone.

MATERIALS AND METHODS

Plant Material

The oil palm fruits used for the RNA extractions were of the tenera fruit form and were obtained from a commercial plantation.

cDNA library construction

An oil palm, *E. guineensis* kernel cDNA library was constructed from mRNA extracted from 15 weeks after anthesis (WAA) *Tenera* kernel using the λ ExCell vector system (Pharmacia).

RT-PCR amplifications

Three conserved regions, at the amino acid level, are known to exist in thioesterase proteins (Jones et al., 1995). We decided to take advantage of this sequence conservation to amplify the intervening regions by PCR. This was aimed at obtaining homologous probes for the thioesterase gene in the oil palm.

Using the primer for the TLDYRREC amino acid sequence 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 expected 450 base pair fragment (Jones et al., 1995) for thioesterase genes from *Tenera*. The amplification product was cloned and sequenced to establish its identity. The presence of all three conserved regions of thioesterase genes confirmed its identity as a thioesterase fragment.

Kernel library screening

The *Tenera* mesocarp oleoyl-ACP fragment recovered from RT-PCR, was excised by restriction enzyme digestion after cloning, and was used to screen an *E. guineensis* 15 WAA kernel library.

RESULTS AND DISCUSSION

Altogether, about 140000 plaques were screened from the kernel cDNA library and one clone was isolated after two rounds of plaque purification (Sambrook et al., 1989). This plaque was *in vivo* excised according to Pharmacia protocols and then sequenced. The resulting clone lacking the 5' end of the transcript contained an insert of 915 base pairs. The nucleotide sequence together with the amino acid translation is presented in Figure 1. The

```
Tenera:   1  GFATTPTMRKLRLIWVTSRMHIEIYKYPAWGDVVEIETWCQGEGRIGTRRDWIIKDLATG  180
             GFATT TMRKL  LIWVT+RMHIEIYKYPAW  DVVEIETWCQ  EGRIGTRRDWI++D  AT
B.napus:125  GFATTLTMRKLHLIWVTARMHIEIYKYPAWSDVVEIETWCQSEGRIGTRRDWILRDSATN  184

Tenera:181EVIGRATSKWVMMNQDTRKLQRVSDEVREEYLVFCPRTPRLAFPEEDNGSVKKIPKLEEP 360
          EVIGRATSKWVMMNQDTR+LQRV+DEVR+EYLVFCPR  PRLAFPEE   N  S+ KKIPKLE+ P 360
B.napus:181EVIGRATSKWVMMNQDTRRLQRVTDEVRDEYLVFCPREPRLAFPEENNSSLKKIPKLEDP 360

Tenera:361ADYSRSELVPRRADLDMNQHVNNVTYIGWVLESMPQEIIDTHELQTITLDYRRECQHNDM 540
          A   YS    EL    PRRADLDMNQHVNNVTYIGWVLES+PQEIIDTHELQ   ITLDYRRECQ    + D+
B.napus:245AQYSMLELKPRRADLDMNQHVNNVTYIGWVLESI PQEIIDTHELQVITLDYRRECQQD DI   304

Tenera:  361  VDSLTSLELADD - -- - -YSTNGSAI  600
              VDSLT+  E+   DD            TNGSAI
B. napus:  361  VDSLTTSEI PDDPISKFTGTNGSAM  329
```

Figure 2. Alignment of derived amino acid sequences of *Tenera* kernel oleoyl-ACP thioesterase with the *B. napus* oleoyl-ACP thioesterase.

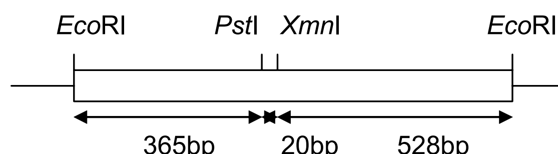

Figure 3. Restriction map of clone pKTT1.

sequence has been deposited at the gene bank and has accession number AF110462. Sequence analysis shows that it is a thioesterase cDNA clone. A gene bank BLAST search established the gene to be an oleoyl-ACP thioesterase with highest homology to the *Brassica napus* oleoyl-ACP thioesterase (E.C. 3.12.14). The amino acid sequence alignment with the *B. napus* homologue is presented in Figure 2. Restriction enzyme analysis of this clone revealed no internal *Eco*RI*, Hind*III or *Bam*HI sites (Dormann *et al*, 1995), but it has an internal *Pst*I site at position 365 as well as an *Xmn*I site at position 385 (Figure 3).

Although the predominant fatty acid in *E. guineensis* kernel is lauric acid (about 50%), this tissue does contain about 17% oleic acid. The isolation of an oleoyl-ACP thioesterase cDNA clone from a kernel cDNA library, therefore reflects this fatty acid composition.

ACKNOWLEDGEMENTS

We wish to thank the Universiti Kebangsaan Malaysia for the postdoctoral research position at UKM granted to O. A., and the Director, Nigerian Institute for Oil Palm Research, for granting him leave to carry out this project. This project was funded by a Malaysian government IRPA grant no 010-02-010.

REFERENCES

Dehesh K, Edwards P, Hayes T, Cranmer AM, Fillatti J (1996). Two novel thioesterases are key determinants of the bimodal distribution of acyl chain length of *Cuphea palustris* seed oil. Plant Physiol. 110: 203-210.

Dormann P, Voelker TA, Ohlrogge JB (1995). Cloning and expression in *Escherichia coli* of a novel thioesterase from *Arabidopsis thaliana* specific for long chain acyl-acyl carrier proteins. Arch. Biochem. Biophys. 316: 612-618.

Hartely CWS (1988). The oil palm. Longman publishers, London (UK).

Jones A, Davies HM, Voelker TA (1995). Palmitoyl acyl carrier protein (ACP) thioesterase and the evolutionary origin of plant Acyl-ACP thioesterases. Plant Cell 7: 359-371.

Loader NN, Woolner EM, Helleyer A, Slabas AR, Safford R (1993). Isolation and characterization of two *Brassica napus* embryo acyl-ACP thioesterase cDNA clones. Plant Mol. Biol. 23: 769-778.

Rajanaidu N, Jalani BS, Kushairi A (1997). Genetic improvement of oil palm. Crop improvement for the 21st century. Signpost Publishers, New Delhi, (India).

Sambanthamurthi R, Oo K (1990). Thioesterase activity in the oil palm (*Elaeis guineensis*) mesocarp. In seed storage compounds, PJ Quinn, JL Harwood (Eds), Portland Press, London (UK), pp. 166-168.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: a laboratory manual. 2nd edition, Cold Spring Habour, N.Y. Press (USA).

Voelker TA (1996). Plant Acyl-ACP thioesterases: chain length determining enzymes in plant fatty acid biosynthesis. Genetic Eng. 18: 111-133.

Voelker TA, Jones A, Cranmer AM, Davies HM, Knutzon DS (1997). Broard range and binary range acyl-acyl carrier protein thioesterase suggest an alternative mechanism for medium chain production in seeds. Plant Physiol. 114: 669-677.