Short communication

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

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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.
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 ExCel 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

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**Figure 1.** Nucleotide sequence (GenBank accession number AF110462) and amino acid translation of pKTT1 (*Tenera* kernel oleoyl-ACP thioesterase) clone.
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 EcoRI, HindIII or BamHI sites (Dormann *et al.*, 1995), but it has an internal PstI site at position 365 as well as an XmnI 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.

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