STEAROYL-ACP DESATURASE GENES FROM OIL PALM

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Stearoyl-ACP desaturase (SAD) 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 Δ_9 position to produce mono-unsaturated oleoyl-ACP (18:1-ACP) (*Figure 1*). It has an important housekeeping role for producing unsaturated fatty acids for membrane lipid biosynthesis. In oil accumulating tissues like anthers, seeds and mesocarp, it is involved in the developmentally regulated process of storage lipid biosynthesis.

Oleic acid is a valuable feedstock for the oleochemical industry. An important objective of the oil palm genetic engineering programme is to increase the level of oleic acid in palm oil at the expense of palmitic acid (16:0). The strategy is to antisense palmitoyl-ACP thioesterase and to increase expression of β-ketoacyl-ACP synthetase II (KAS II). Manipulation of the stearoyl-ACP desaturase gene may also be required to cope with possible accumulation of stearic acid when palmitoyl-ACP thioesterase is reduced and KAS II activity is increased. There is also an interest in producing high stearate palm oil for use as a cocoa butter substitute. This

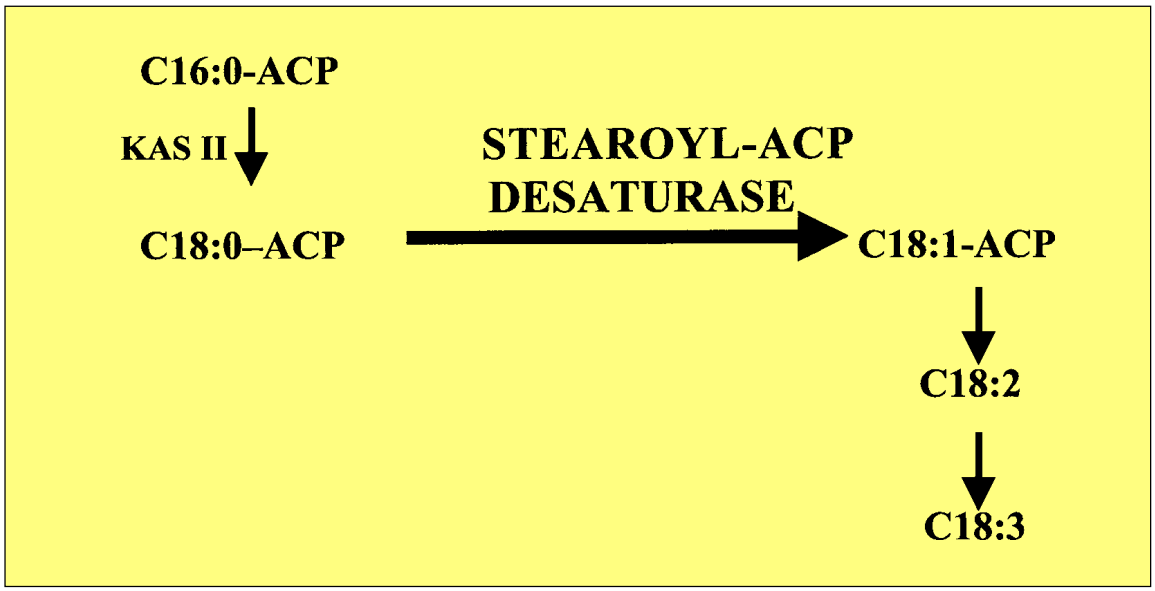

Figure 1. Stearoyl-ACP desaturase reaction.

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may be achieved by down regulating expression of the stearoyl-ACP desaturase gene.

STEAROYL-ACP DESATURASE GENES ISOLATED FROM OIL PALM

Two different stearoyl-ACP desaturase genes, *SAD1* and *SAD2* have been isolated from mesocarp cDNA library of the oil palm (*E. guineensis*) fruits (Siti Nor Akmar *et al*., 1999). The presence of two stearoyl-ACP desaturase genes was further confirmed by Southern blot analysis. *SAD1* cDNA clones of about 1.7 kb in size were sequenced and found to encode the complete sequence of the chloroplast transit peptide and mature protein. The deduced amino acid sequence of *SAD1* has two occurrences of the conserved D/E EX_2H motif found in plant SADs (*Figure 2*). The deduced amino acid sequence of *SAD1* was strongly homologous to sequences of known SADs such as from castor (Shanklin and Somerville, 1991), rice (Akagi *et al*., 1995) and rape (Slocombe *et al*., 1992) with greater than 80% sequence identities. *SAD1* was used to screen an *E. oleifera* 17 w.a.a cDNA library. The nucleotide sequence of the *E. oleifera* clone showed remarkable homology with *SAD1* not only within the coding region but also within 3′ and 5′-UTRs with identities exceeding 99%.

The sequence of the longest *SAD2* clone of about 1.1 kb was also determined. The nucleotide sequences of *SAD1* and *SAD2* share 93% and 76% homologies within the coding and 3' – untranslated regions (UTRs), respectively. The identity at the amino acid level is 95%.

EXPRESSION ANALYSIS

Gene-specific probes of approximately 300 bp were designed based on the 3' untranslated regions of *SAD1* and *SAD2*. The probes produced were used to screen northern blots containing mRNA from six different stages of mesocarp (8-20 w.a.a), three different stages of kernel development (10-14 w.a.a) and from vegetative tissues using high stringency conditions. It was shown that the two probes each hybridized specifically to transcripts of about 1.7 kb. *SAD1* and *SAD2* were shown to be differentially regulated. Constitutive expression of *SAD2* suggests a possible housekeeping role in membrane lipid biosynthesis. *SAD1*, 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 (*Figure 3*).

Western blot analysis using the polyclonal antibodies raised against SAD1 protein showed that enzyme level is high in the mesocarp at the late stages of ripening and remains high in ripe fruits. The leaf form of the enzyme appeared to be about 2 kDa larger. High levels of enzyme

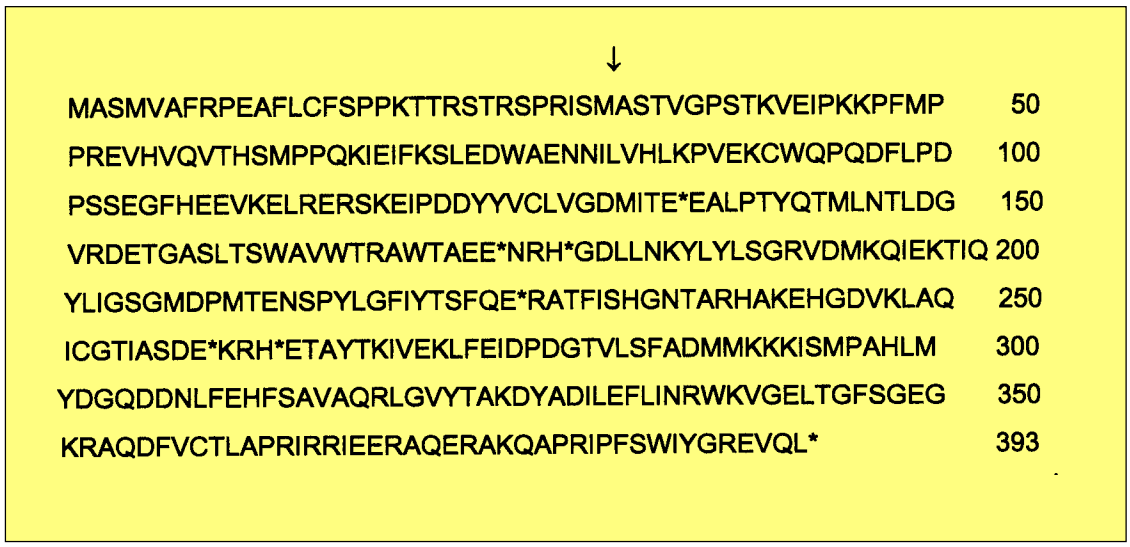

Figure 2. Deduced amino acid sequences of E. guineensis *SAD1. ↓ indicates proposed cleavage for transit peptide. The conserved E and H residues are indicated by *.*

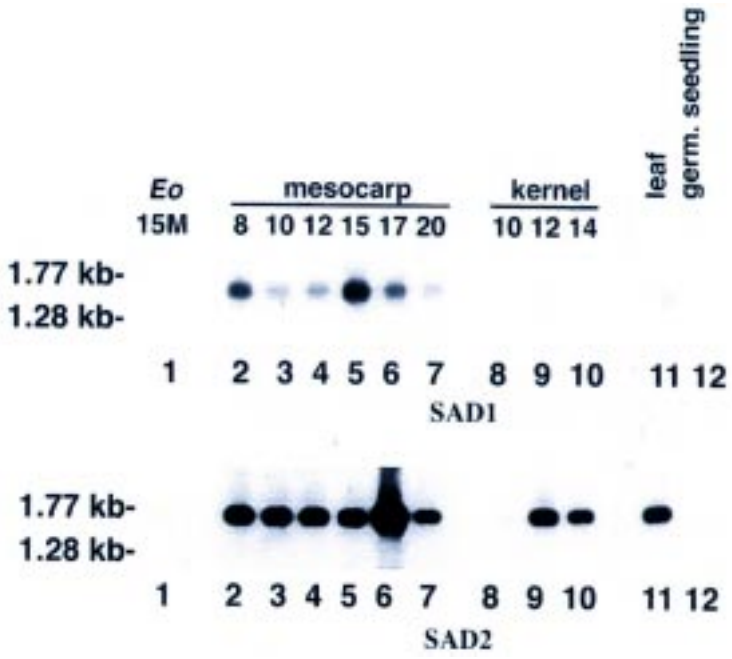

Figure 3. Expression profile indicating that expression of SAD1 *is induced in lipid-rich mesocarp and kernel tissues while* SAD2 *is constitutively expressed in different tissues.*

and gene expression were detected in young mesocarp tissue consistent with the requirement for high levels of unsaturated fatty acids for membrane lipid biosynthesis (*Figure 4*).

CONCLUSION

Two stearoyl-ACP desaturase genes (*SAD1* and *SAD2*) were identified in oil palm. The sequence of *SAD1* is highly conserved in *E. guineensis* and *E. oleifera*. Constitutive expression of *SAD2* suggests housekeeping role in membrane lipid biosynthesis. *SAD1* which is induced in mesocarp and kernel in phase with oil synthesis indicates direct involvement with storage oil synthesis. Regulation at transcriptional level is important in controlling levels of stearoyl-ACP desaturase in oil accumulating tissues. Thus, it is possible to manipulate level of SAD1 in mesocarp and kernel without interfering with membrane lipid biosynthesis by genetic manipulation to produce oil with the desired composition.

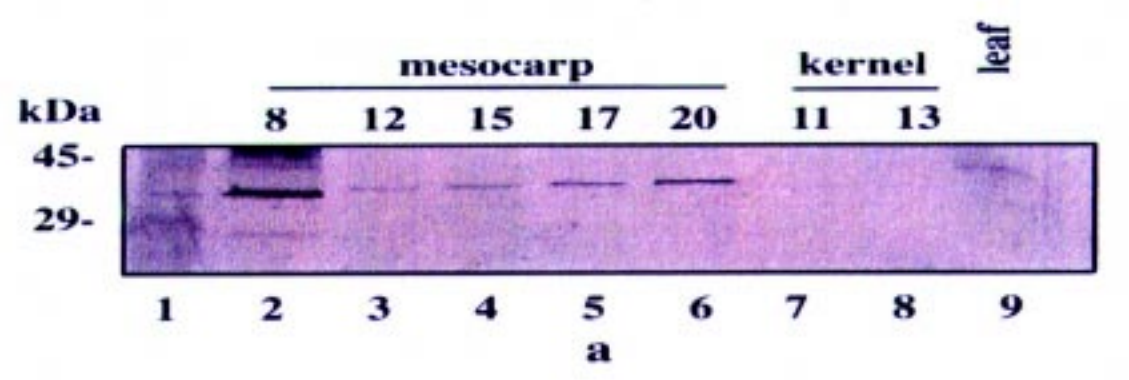

Figure 4. Western blot analysis using antibodies specific for SAD1 protein showing the increase in enzyme levels following the increase in gene expression in the mesocarp tissue.

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