LICENSING OF A MESOCARP-SPECIFIC PROMOTER

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Genetic engineering provides the opportunity to diversify the use and to increase the economic value of palm oil. Production of speciality oils for industrial applications will be a very attractive proposition for the oil palm, since it is the most productive oil crop. This is considered a useful strategy to maximize income in the midst of problems of labour shortage, high labour cost and lack of arable land for expansion. At MPOB, the tools and techniques for genetic manipulation to produce high oleate and high stearate transgenic palms are now in place, and these are also applicable to other target products of interest. A schematic representation for producing a novel product in oil palm mesocarp by genetic engineering is given in *Figure 1.*

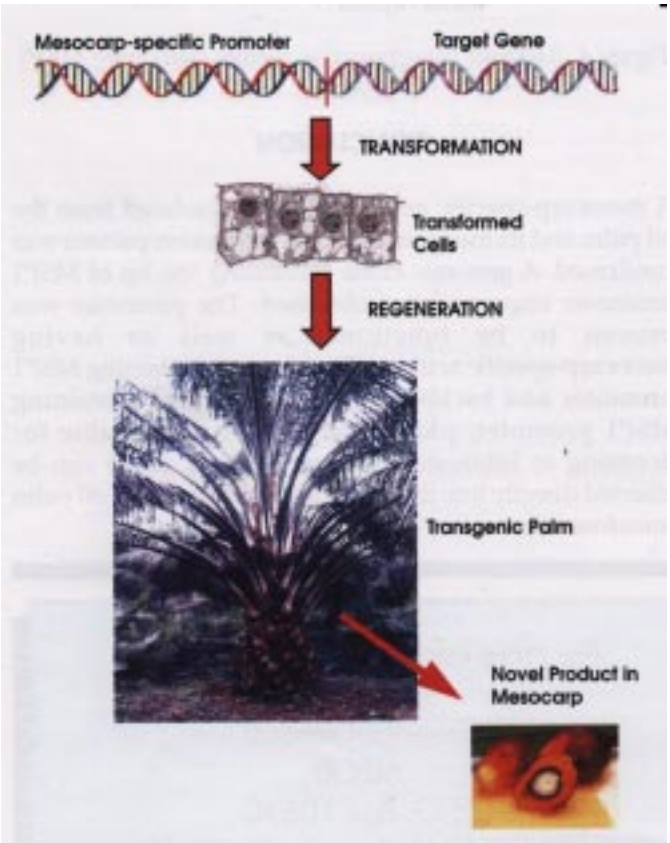

Figure 1.Production of novel product in oil palm mesocarp

The success of any genetic engineering efforts relies heavily on the availability of the following four main components:

i. Genes to produce the desired trait
ii. Regulatory sequence or promoter for controlling expression of introduced genes
iii. Reliable transformation technique
iv. Plant regeneration system

THE IMPORTANCE OF MESOCARP-SPECIFIC PROMOTER

The availability of temporal and tissue-specific gene promoters is essential for the effort to modify mesocarp oil composition by genetic engineering. Such promoters must be able to drive specific expression of introduced genes during the period of oil synthesis (15-20 weeks after anthesis) and preferentially produce an expression pattern similar to that of a fatty acid biosynthetic gene in the mesocarp. This promoter will ensure that most of the effects on lipid metabolism is confined to storage lipids without significantly affecting lipid metabolism in leaves or other tissues which can otherwise leads to deleterious effects in the transgenic plant. It will also limit the production of other novel products to the mesocarp, a storage organ and a non-essential tissue for normal plant growth and development.

CHARACTERISTICS OF A MESOCARP-SPECIFIC GENE, MSP1

A cDNA clone corresponding to a mesocarp-specific gene (MSP1) was isolated from an oil palm mesocarp cDNA library. Confirmation of temporal and mesocarp-specific expression of MSP1 was made by Northern blot analysis. It was shown that MSP1 is highly and specifically expressed in the mesocarp tissue of *Elaeis guineensis*, the high yielding commercial species, suggesting that its promoter would be valuable for expressing foreign genes aimed at effecting oil modification (*Figure 2*). Furthermore, expression pattern of MSP1 is very similar to that of a gene encoding stearoyl-ACP desaturase (SAD1), an enzyme with direct involvement in fatty acid biosynthesis in oil palm mesocarp.

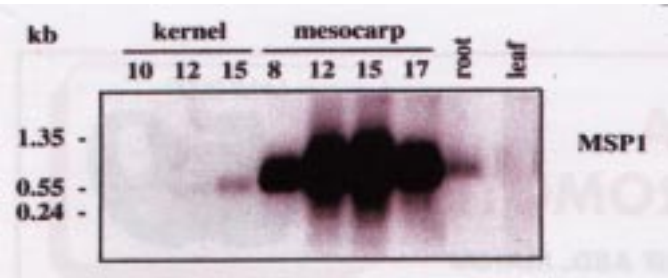

Figure 2. Mesocarp-specific expression of MSP1.

CHARACTERISTICS OF MSP1 PROMOTER

A genomic fragment containing MSP1 was cloned and sequenced. Sequence analysis revealed that the genomic fragment contains 986 base pairs (bp) of promoter sequence. The transcription start site, TATA box and a putative ethylene responsive element were identified in the promoter sequence.

In order to confirm that the MSP1 promoter can direct mesocarp-specific gene expression, a transient assay analysis was performed. In this analysis, a promoter: reporter gene construct was produced. The chimeric gene construct was used to bombard mesocarp and leaf (control) tissue slices. The result using β-glucuronidase (GUS) as the reporter gene is shown in *Figure 3*. It was found that MSP1 promoter is a functional promoter and that it has mesocarp-specific activity because GUS expression (blue spots) can only be detected on bombarded mesocarp slices and not on bombarded leaf tissue. In contrast, GUS expression was detected in both mesocarp and leaf tissues when a constitutive promoter was used in the bombardment. The MSP1 promoter has been filed for patent application.

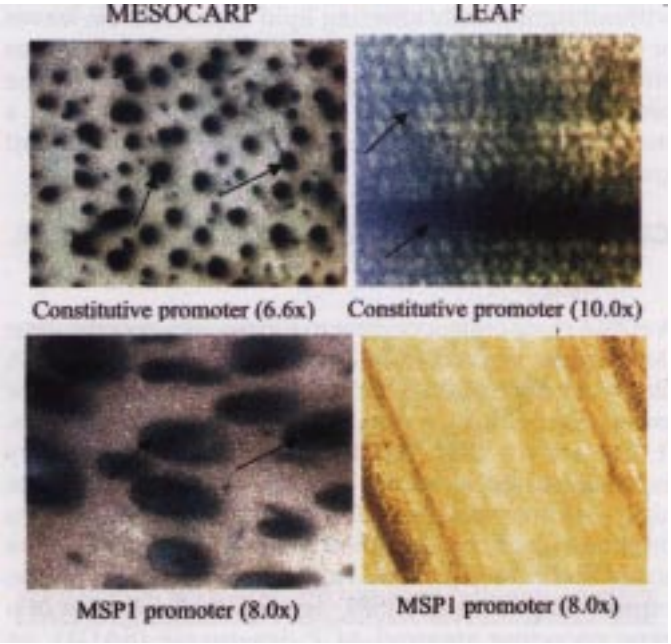

Figure 3. Mesocarp-specific activity of MSP1 promoter.

VECTOR CONSTRUCTS CONTAINING MSP1 PROMOTER

Two basic backbone vector constructs containing the MSP1 promoter were produced and are designated as pMP1 and pMP2. Schematic representation of pMP1 and pMP2 is shown in *Figure 4*. The pMP1 consists of MSP1 promoter and nopaline synthase (NOS) terminator. An *Asc* I (rare cutter) site was inserted in between MSP1 sequence and NOS sequence to serve as the site for insertion of target gene. While pMP2 is similar to pMP1 with the exception that it has a plastid targeting sequence before the *Asc* I site. The pMP1 can be used for inserting any fatty acid biosynthetic gene which carries its own plastid targeting sequence. The pMP2 is specially designed for introducing fatty acid biosynthetic gene without plastid targeting sequence or for inserting desired gene in an antisense orientation. Genes from other metabolic pathways can also be introduced into pMP1 and pMP2.

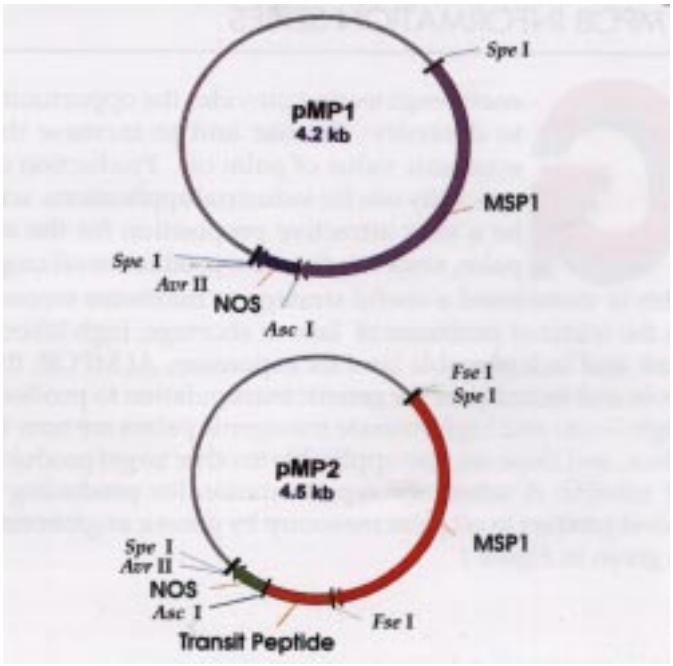

Figure 4. Backbone transformation vectors containing MSP1 promoter.

CONCLUSION

A mesocarp-specific gene (MSP1) was isolated from the oil palm and its mesocarp-specific expression pattern was confirmed. A genomic clone containing 986 bp of MSP1 promoter sequence was obtained. The promoter was proven to be functional as well as having mesocarp-specific activity. The plasmid containing MSP1 promoter and backbone vector constructs containing MSP1 promoter, pMP1 and pMP2 are available for licensing to interested parties. Desired genes can be inserted directly into the vector constructs prior to oil palm transformation.

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