



UNIVERSITI PUTRA MALAYSIA

***CLONING AND CHARACTERIZATION OF
OLEOYL-COA DESATURASE GENE FROM OIL PALM
(ELAEIS GUINEENSIS L.)***

SYAHANIM SHAHWAN

FBSB 2006 31



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By

SYAHANIM SHAHWAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

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*To my father, Tuan Haji Shahwan Mansor
mother, Puan Hajah Siti Hayati Abas
and
those who believe*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**CLONING AND CHARACTERIZATION OF OLEOYL-COA
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By

SYAHANIM BINTI SHAHWAN

December 2006

Chairman : Ho Chai Ling, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Oil palm (*Elaeis guineensis*) is the main commodity crop in Malaysia. Oil palm is the second largest producer of vegetable oil in world vegetable oil markets with revenue of RM28.6 billion. Storage oil derived from oil palm contains 50% saturated, 40% unsaturated fatty acids and 10% polyunsaturated fatty acids. The oleoyl Co-A desaturase (FAD2; E.C 1.3.1.35) is involved in the production of polyunsaturated fatty acids. The enzyme utilizes oleic acid (C18:1) to produce linoleic acid (C18:2) by adding the second double bond at the 12th carbon of oleic acid. As high levels of oleic acid are often desirable for industrial applications, genetic manipulation *via* antisense technology and seed-specific suppression can be attempted to silence this gene, to manipulate the level of oleic acid to suit various downstream applications.

In this study, two specific primers (PD1As and PD2) were designed based on the conserved region of FAD2 sequences from other plant species. A partial gene of 350 bp in length was amplified and the partial region has a high percentage of sequence identities (91%) with other FAD2 genes from various plant species such as *Brassica campestris*, *Brassica rapa* and *Crepis palaestina*. The complete sequence, designated *EgFAD2*, which is 1510 bp in length, consisting of 391 amino acids in its open



reading frame was obtained *via* rapid-amplification of cDNA ends – polymerase chain reaction (RACE-PCR). The polypeptide carried three histidine clusters that were conserved among the desaturase genes. The deduced amino acid sequences showed significant identity to other plant FAD2 proteins such as *Oryza sativa* (75%), *Glycine max* (72%) and *Punica granatum* (71%). In addition it has the aromatic residues (-YNNTL) at the C-terminus similar to other gene targeted to be expressed in endoplasmic reticulum.

Northern blot was carried out to analyze the expression profile of this gene in various tissues of oil palm. The oil palm oleoyl-CoA desaturase gene was highly expressed at the later stages of mesocarp tissue development (15-, 17- and 20- week after anthesis) with the strongest signal at week-15. The expression of the transcript correlated with the levels of linoleic acid deposited in the mesocarp as the fatty acids begin to accumulate in mesocarp tissues at week-15. The results also showed that the gene plays an important role for oil storage as the oil deposition starts at week-15 in the oil-bearing tissue. Southern analysis indicated the existence of at least two to four copies of oleoyl-CoA desaturase gene in the oil palm genome. The open reading frame of the oil palm oleoyl-CoA desaturase was expressed as a fusion protein in *E. coli*. The expected size of 44 kDa was obtained. However, the expression construct had to be further confirmed. In a conclusion, the findings of this study could aid the development of high oleic trait in oil palm by manipulating the full-length gene of oleoyl-CoA desaturase and will serve as an initial step for future biochemical characterization of the encoded product of this gene.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Master Sains

**PENGGKLONAN DAN PENCIRIAN GEN OLEOIL-KOA DESATURASE
DARIPADA POKOK KELAPA SAWIT (*ELAEIS GUINEENSIS* L.)**

Oleh

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Kelapa sawit (*Elaeis guineensis*) adalah tanaman komoditi utama di Malaysia. Sawit merupakan pengeluar minyak sayuran kedua terbesar dalam pasaran minyak tumbuhan dunia dan telah menyumbang sebanyak RM 28.6 bilion kepada ekonomi negara. Minyak sawit terdiri daripada 50% minyak tepu, 40% minyak monotaktepu dan 10% minyak politaktepu. Oleoil-KoA desaturase (FAD2; EC 1.3.1.35) terlibat di dalam tindakbalas penghasilan minyak tak tepu di mana ia mengkatalisis tindakbalas penghasilan asid linoleik (C18:2) daripada asid oleik (C18:1) melalui penambahan ikatan ganda dua yang kedua pada rantai karbon yang ke-12 asid oleik. Oleh kerana permintaan yang tinggi terhadap asid oleik di dalam industri oleokimia, manipulasi genetik melalui teknologi ‘antisense’ dan ‘seed-specific supression’ boleh dilaksanakan bagi menghalang aktiviti enzim ini.

Di dalam kajian yang dijalankan, dua pencetus spesifik (PD1As dan PD2) telah direka berdasarkan kepada jujukan oleoil-KoA desaturase yang terpuhara daripada tumbuhan lain. Jujukan separa lengkap sepanjang 350 bp, telah diamplifikasi melalui tindakbalas rantai polimerase dengan menggunakan cDNA tisu mesokarpa sebagai templat. Jujukan separa tersebut mempunyai peratusan homologi yang tinggi (91%)

dengan jujukan gen oleoil-KoA desaturase tumbuhan yang lain seperti *Brassica campestris*, *Brassica rapa* dan *Crepis palaestina*. Melalui analisis amplifikasi hujung cDNA dan tindakbalas rantai polimerase (RACE-PCR), jujukan lengkap oleoil-KoA desaturase telah berjaya dipencilkan daripada kelapa sawit. Jujukan ini mengandungi 1510 bp jujukan nukleotida dan mengkodkan 391 asid amino pada rangka bacaan terbuka. Polipeptida bagi gen ini mengandungi tiga kotak histidin yang terpuhara antara gen desaturase daripada tumbuhan lain. Analisis jujukan asid amino *EgFAD2* melalui pangkalan data BLAST menunjukkan identiti yang signifikan diperoleh berbanding gen oleoil-KoA desaturase tumbuhan yang lain seperti *Oryza sativa* (75%), *Glycine max* (72%) dan *Punica granatum* (71%). Tambahan pula, jujukan asid amino oleoil-KoA desaturase mengandungi residu-residu aromatik pada terminal-C (-YXXTL) dan mempunyai persamaan dengan gen-gen sasaran yang diekspreskan di retikulum endoplasma.

Analisa dekapan Northern terhadap pelbagai tisu kelapa sawit menunjukkan pengekspresan yang tinggi dapat dikesan pada tisu mesokarpa pada usia yang lewat iaitu pada minggu ke-15, -17 dan -20 minggu selepas pendebungaan. Oleh kerana itu, dapat disimpulkan bahawa kadar pengekspresan *EgFAD2* berkait rapat dengan pengumpulan asid lemak di dalam mesokarpa kerana pengumpulan asid lemak juga bermula pada minggu ke-15 tisu mesokarpa. Analisa dekapan Southern pula mendapati terdapat sekurang-kurangnya dua hingga empat salinan gen oleoil-KoA desaturase kelapa sawit hadir di dalam genom sawit. Rangka bacaan terbuka gen oleoil-KoA desaturase sawit telah di ekspreskan dengan banyak dalam bentuk protein gabungan di dalam *E. coli*. Saiz protein gabungan yang dijangka ialah 44kDa berjaya diperolehi. Melalui manipulasi jujukan lengkap oleoil-KoA desaturase yang diperolehi, kelapa sawit yang mengandungi peratusan asid oleik yang lebih tinggi

daripada nilai biasa dapat dihasilkan dan ia dapat dijadikan sebagai rujukan untuk langkah-langkah awal bagi pencirian biokimia produk yang dikodkan oleh gen yang dikaji.

ACKNOWLEDGEMENTS

The author would like to thank ALLAH Subhanahuwataala for giving an opportunity to meet a bunch of people who means a lot to me. The author would like to give acknowledgments to a group of people who had inspired, helped and been supportive through out the years. First of all, to Prof Madya Datin Dr Siti Nor Akmar Abdullah for her guidance and inspiration, Dr Ho Chai Ling, for supervising and being understanding all the way, Dr Abrizah Othman (MPOB), for her help and support, Prof Madya Dr Suhaimi Napis, for his supervision, Dr Arif Abd Manaf (MPOB) who was very supportive and helpful and Dr Tan Siang Hee who guided me for the first year of my study. Also, my appreciation goes to gene expression group members of MPOB (Pn Aminah, Pn Zaini, Pn Anita, Pn Nurniwalis, Pn Zubaidah, En Khairul, En Mahadzir, Cik Safiza,, Cik Nurhafizah, Cik Roslinda, Cik Zetty, En. Shafiq) for all the technical help, support they gave me, guidance and assistance, for the friendship and for the ideas. Besides that, the authors would like to thank Malaysian Palm Oil Board for their full support and technical help throughout this research and MPOB for provision of studentship programme. Last but not least, abah (Haji Shahwan B Mansor), mak (Pn Hjh Siti Hayati bt Abas) and also Along and family, Angah and family, Ari and family and Eisyah who gave their full-support in whatever I do, financially, emotionally and spiritually.



I certify that an examination committee has met on 26 December 2006 to conduct the final examination of Syhanim binti Shahwan on her Master of Science thesis entitled ‘Cloning and Characterization of Oleoyl-CoA Desaturase Gene from Oil Palm (*Elaeis guineensis* L.)’ in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SYAHANIM SHAHWAN

Date: 17 MARCH 2007



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LIST OF ABBREVIATIONS

| | |
|----------------------|---|
| AA | Amino acid |
| ACP | Acyl-carrier protein |
| <i>A. thaliana</i> | <i>Arabidopsis thaliana</i> |
| BLAST | Basic Local Alignment Search Tool |
| Bp | Base pair |
| <i>B. napus</i> | <i>Brassica napus</i> |
| <i>B. rapa</i> | <i>Brassica rapa</i> |
| <i>C. palaestina</i> | <i>Crepis palaestina</i> |
| cDNA | Complementary deoxyribonucleic acid |
| CTAB | Cetyltrimethyl ammonium bromide |
| DIECA | Diethyldithiocarbamic acid |
| DNA | Deoxyribonucleic acid |
| dNTPs | Deoxynucleotides |
| DTT | Dithiotreitol |
| EDTA | Ethylenediaminetetraacetic acid |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| <i>E. guineensis</i> | <i>Elaeis guineensis</i> |
| <i>E. oleifera</i> | <i>Elaeis oleifera</i> |
| <i>EgFAD2</i> | <i>Elaeis guineensis</i> oleoyl-CoA desaturase |
| <i>EgFAD2-O</i> | ORF region of <i>Elaeis guineensis</i> oleoyl-CoA desaturase |
| EtBr | Ethidium bromide |
| <i>G. hirsutum</i> | <i>Gossypium hirsutum</i> |
| <i>G. max</i> | <i>Glycine max</i> |



| | |
|-------------------|--|
| GFP | Glyoxal/ Formamide/ Phosphate |
| HCl | Hydrochloric acid |
| His | Histidine |
| H ₂ O | Water |
| i.e. | Such as |
| IPTG | Isopropyl-β-D-thiogalactopyranoside |
| Kb | Kilobase |
| KOH | Calium hydroxide |
| L | Litre |
| LB | Luria-bertani |
| LD-PCR | Long Distance- Polymerase Chain Reaction |
| LiCl | Lithium Chloride |
| MOPS | (3-[N-morpholino] propanesulfonic acid) |
| MPOB | Malaysian Palm Oil Board |
| mRNA | messenger RNA |
| M | Molar |
| mM | millimolar |
| m | Metre |
| μg | Microgram |
| μl | Microlitre |
| MgCl ₂ | Magnesium chloride |
| MgSO ₄ | Magnesium sulphate |
| MMLV | Murime Moloney Leukemia Virus |
| NaCl | Natrium chloride |
| NaOH | Natrium hydroxide |

| | |
|-------------------------|--|
| NCBI | National Centre for Biotechnology Information |
| ng | nanogram |
| nm | nanometre |
| <i>O. sativa</i> | <i>Oryza sativa</i> |
| OD | Optical density |
| ORF | Open reading frame |
| <i>P. americana</i> | <i>Persea americana</i> |
| PAGE | Polyacrylamide gel electrophoresis |
| PCR | Polymerase Chain Reaction |
| Poly A ⁺ RNA | Polyadenylated RNA |
| PVP | Polyvinyl-pyrrolidone |
| RACE | Rapid Amplification of cDNA End |
| RNA | Ribonucleic acid |
| rpm | reverse per minute |
| RT-PCR | Reverse transcriptase- Polymerase Chain Reaction |
| SDS | Sodium dodecyl sulphate |
| SMART | Switching Mechanisms At 5' End of RNA Transcript |
| TAE | Tris acetate EDTA |
| TAG | Triacylglycerol |
| TE | Tris- Ethylenediaminetetracetic acid |
| UKM | Universiti Kebangsaan Malaysia |
| UPM | Universiti Putra Malaysia |
| UPM | Universal Primer Mix |
| USA | United States of America |
| UTR | Untranslated region |



| | |
|--------------|---|
| UV | Ultraviolet |
| V | Voltage |
| WAA | week after anthesis |
| X-gal | 5-bromo-4-chloro-3-indoyl- β -D-galactopyranoside |
| w/v | weight per volume |
| v/v | volume per volume |
| 3'-UTR | 3'-untranslated region |
| 5'-UTR | 5'-untranslated region |
| % | Percentage |
| $^{\circ}$ C | degree celcius |

