UNIVERSITI PUTRA MALAYSIA

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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 DESATURASE GENE FROM OIL PALM *(ELAEIS GUINEENSIS* L.)

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

SYAHANIM BINTI SHAHWAN

Disember 2006

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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 terpulihara 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 camprestis, 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 terpulihara 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.

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I certify that an examination committee has met on 26 December 2006 to conduct the final examination of Syahanim 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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Date: 27 APRIL 2007

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date: 10 MAY 2007

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.

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SYAHANIM SHAHWAN

Date: 17 MARCH 2007

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LIST OF ABBREVIATIONS

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

GFP	Glyoxal/ Formamide/ Phosphate
HCl	Hydrochloric acid
His	Histidine
H_2O	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
O. sativa	*Oryza sativa*
OD	Optical density
ORF	Open reading frame
P. americana	*Persea americana*
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
Poly A^+ RNA	Polyadenylated RNA
PVP	Polyvinyl-pyrolydone
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	Triacyglycerol
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
°C	degree celcius