UNIVERSITI PUTRA MALAYSIA

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM-BASED DETECTION OF DEOXYRIBONUCLEIC ACID METHYLATION AND THE ANALYSIS OF THREE MADS-BOX GENES IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

ADELENE AU YONG SHU MEI

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By

ADELENE AU YONG SHU MEI

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

March 2006

Dedicated to
the Auyongs'
and the Eohs'

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

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March 2006

Chairman : Associate Professor Raha Abdul Rahim, PhD

Institute : Bioscience

Tissue culture propagation can induce genetic and epigenetic changes in regenerated plants. In oil palm and other plants, these changes could result in floral and vegetative abnormalities, also termed as 'somaclonal variations'. DNA methylation is considered to be a potential mechanism by which somaclonal variants experience changes in their gene expression pattern. Most genes controlling the flower architecture is largely determined by homeotic genes containing a conserved MADS-box domain. Compared to *Arabidopsis thaliana* and *Antirrhinum majus*, little is known about MADS-box genes controlling flower development in oil palm. To detect the changes in methylation of random genomic sequences in the vicinity of MADS-box genes among the normal, abnormal and reverted oil palm regenerants, a novel DNA fingerprinting technique called MADS-box directed profiling which is an AFLP-based technology is described. The technique involves three steps: (i) digestion of the genomic DNA with methylation-sensitive enzymes and ligation of adapter, (ii)

amplification of the selective fragments using a specific primer (MADS5'R) and (iii) gel analysis of the amplified fragments. One-hundred twenty five polymorphisms were identified throughout the study. The 20 most interesting polymorphisms were selected for analysis of which 16 were successfully cloned and sequenced from a total of 126 individual DNA samples sourced from 17 oil palm clones. Most altered methylation especially in the abnormal palms is of the hypo type. Analysis from the cloned polymorphic fragments revealed that they are constantly present in an overlapping manner and therefore could be classified into three groups based on their common consensus. In the latter effort to obtain the full-length DNA sequences, 3'RACE extension was done. Three new full-length MADS-box genes, namely *OPMADS14, OPMADS15* and *OPMADS16* were identified. The sequence constituting these MADS-box fragments are thought to be novel as they are not found in the oil palm MADS-box collection. They showed similarity to the *Arabidopsis thaliana AGL6*, *FUL* and *SEP1,* respectively. Their genomic organizations related to methylation were studied through Southern analysis. Cytosine methylation polymorphism indeed exists at the CCGG sites of some MADS-box sequence among the oil palm clones. Expression patterns were studied by RT-PCR. *OPMADS15* showed no differences in the abundance of transcript level between the normal or mantled palms when compared between similar tissues. Thus, it is concluded that *OPMADS15* is a flower-specific MADS-box gene that is involved in flower development and its expression is not affected by the differences in mantling condition, or at least in the oil palm clones used for this study. The fact

that *OPMADS14* and *OPMADS16* transcripts were also found in non-floral organs reflect that these genes may not be necessarily involved in flower development per se but may play wider roles in different aspects of plant development.

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

PENGESANAN METILASI ASID DEOKSIRIBONUKLEIK MELALUI TEKNIK BERDASARKAN AMPLIFIED FRAGMENT LENGTH POLYMORPHISM DAN PENGANALISAAN TIGA GEN KEKOTAK MADS DALAM KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

ADELENE AU YONG SHU MEI

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Propagasi kultura tisu boleh merangsang perubahan keadaan genetik dan epigenetik di dalam pertumbuhan semula pokok. Di dalam pokok kelapa sawit dan tumbuhan lain, perubahan jenis ini boleh mengakibatkan perubahan pada perkembangan normal bunga dan vegetatif yang juga dikenali sebagai variasi somaklonal. Metilasi DNA dianggap sebagai mekanisma yang berpotensi mengubah corak pengekspresan gen di dalam variasi-variasi somaclonal tersebut. Kebanyakan gen yang mengawal struktur bunga dikendalikan oleh gen-gen homeotik yang mengandungi domain kekotak MADS. Pengetahuan mengenai kekotak MADS yang mengawal pertumbuhan bunga di dalam pokok kelapa sawit agak terhad berbanding dengan *Arabidopsis thaliana* dan *Antirrhinum majus*. Bagi mengkaji perubahan metilasi jujukan-jujukan genomik secara rawak di persekitaran gen kekotak MADS di antara pokok normal, abnormal dan berbalik (reverted), sejenis teknik baru yang disebut sebagai

'MADS-box directed profiling' yang merupakan teknologi berdasarkan AFLP dinyatakan. Teknik ini melibatkan tiga langkah: (i) pencernaan DNA genomik dengan enzim-enzim sensitif metilasi dan ligasi adaptor, (ii) pengamplifikasi fragmen-fragmen terpilih dengan menggunakan primer spesifik (MADS5'R) dan (iii) penganalisaan fragmen-fragmen teramplifikasi dalam gel. Seratus dua puluh lima polimorfisme telah dipastikan melalui kajian ini. Daripada jumlah ini, 20 polimorfisme yang paling unik telah dipilih untuk penganalisaan di mana 16 daripadanya telah berjaya diklon dan dijujuk daripada sejumlah 126 individu DNA yang disumberkan daripada 17 klon-klon kelapa sawit. Kebanyakan perubahan metilasi terutama dalam pokok kelapa sawit abnormal adalah jenis hypo. Analisa daripada fragmen polimorfisme yang telah diklon menjelaskan bahawa fragmen-fragmen ini hadir dalam keadaan bertindih dan seterusnya boleh diklasifikasikan kepada tiga kumpulan berdasarkan konsensus yang serupa. Seterusnya, 3'RACE digunakan dalam usaha untuk mendapatkan jujukan DNA lengkap. Tiga gen kekotak MADS yang mengandungi jujukan DNA lengkap, *OPMADS14, OPMADS15* dan *OPMADS16* telah dikenalpasti. Kesemua fragmen yang mengandungi jujukan kekotak MADS ini dianggap baru kerana jujukan-jujukan tersebut tidak terkandung dalam koleksi kekotak MADS kelapa sawit yang ada sehingga kini. Walau bagaimanapun, jujukan-jujukan tersebut menunjukkan kesamaan dengan *AGL6*, *FUL* dan *SEP1 Arabidopsis thaliana*. Organisasi genomik yang berkait dengan metilasi dikaji melalui analisis Southern. Sememangnya, polimorfisme metilasi sitosin wujud di sesetengah bahagian CCGG kekotak MADS. Corak pengekspresan juga dikaji

melalui RT-PCR. Paras transkrip *OPMADS15* di antara pokok normal dan abnormal tidak menunjukkan perbezaan apabila tisu yang sama dibandingkan. Oleh itu, kesimpulannya ialah *OPMADS15* merupakan gen kekotak MADS pengawalan bunga yang spesifik; terlibat dalam pertumbuhan bunga dan pengekspresannya tidak dipengaruhi oleh syarat perubahan keabnormalan, ataupun dalam klon-klon yang digunakan dalam kajian ini. Fakta menyatakan transkrip-transkrip *OPMADS14* dan *OPMADS16* juga ditemui di dalam organ bukan bunga menunjukkan gen-gen ini tidak semestinya terlibat dalam pertumbuhan bunga seperti yang dijangkakan tetapi mungkin mempunyai fungsi yang lebih luas dalam aspek lain pertumbuhan pokok.

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I certify that an Examination Committee has met on 17th March 2006 to conduct the final examination of Adelene Au Yong Shu Mei on her degree in Master of Science thesis entitled "Amplified Fragment Length Polymorphism-Based Detection of Deoxyribonucleic Acid Methylation and the Analysis of Three MADS-Box Genes in Oil Palm (*Elaeis guineensis* Jacq.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia 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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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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ADELENE AU YONG SHU MEI

Date:

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LIST OF ABBREVIATIONS

%	Percentage
β	beta
γ	gamma
μg	microgram
μl	microlitre
μm	micrometer
^{o}C	degree centigrade
aa	Amino acid
AAR	Applied Agricultural Research Sdn. Bhd.
Ab	Abnormal ramet
AFLP	Amplified Fragment Length Polymorphism
AG	AGAMOUS
AGL	AGAMOUS-LIKE
AP1	APETALA-1
APS	Ammonium Persulfate
BAH	Bromo adjacent homology
bp	base-pair
BSA	Bovine Serum Albumin
cDNA	copy Deoxyribonucleic Acid
C	Cysteine

Ci	Curie
cm	centimeter
CHR	Chromodomain
CMT	Chromomethylase
dATP	2'-Deoxy-adenosine-5'-triphosphate
dCTP	2'-Deoxy-cytidine-5'-triphosphate
DEF	DEFICIENS
DEPC	Diethyl Pyrocarbonate
dGTP	2'-Deoxy-guanosine-5'-triphosphate
dH_2O	distilled water
DMAP	DNMT-associated protein
DNA	Deoxyribonucleic Acid
DNMT	DNA (cytosine-5-)-methyltransferase
DTT	Dithiothreithol
dTTP	2'-Deoxy-thymidine-5'-triphosphate
DRM	Domain rearranged methyltransferase
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
EtOH	Ethanol
G	Gram
FUL	FRUITFULL
GLO	GLOBOSA

HCl	Hydrochloric Acid
hr	hour
Jacq.	Jacquin
ISSR	Inter-Simple Sequence Repeat
kb	kilo base-pair
KCl	Potassium Chloride
L	Litre
LB	Luria-Bertani
LFY	LEAFY
LiCl	Lithium Chloride
M	Molar
MADS	MCM1-AGAMOUS-DEFICIENS-SRF
$MgCl_2$	Magnesium Chloride
mg	milligram
min	minute
ml	mililitre
mm	millimeter
mM	milimolar
MPOB	Malaysian Palm Oil Board
mRNA	Messenger Ribonucleic Acid
NCBI	National Center for Biotechnology Information
NaCl	Sodium Chloride
NaOAc	Sodium Acetate

NaOH	Sodium Hydroxide
ng	nanogram
nm	nanometer
N	Normal ramet
nt	nucleotide
OD	Optical density
Or	Ortet
P	Phosphate
PAGE	Polyacrylamide Agarose Gel Electrophoresis
PCR	Polymerase Chain Reaction
PI	PISTILLATA
pmol	picomole
PVP	Polyvinylpyrrolidone
PWWP	Pro-Trp-Trp-Pro
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RNase	Ribonuclease
rpm	Revolution per minute
Rv	Reverted ramet
SEP1	SEPALLATA-1
SDS	Sodium Dodecyl Sulfate or Sodium Lauryl Sulfate
sec	seconds

smqt	semi-quantitative
SSC	Sodium Chloride-Sodium Citrate Buffer
SQUA	SQUAMOSA
TAE	Tris-Acetate-EDTA
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
TEMED	N, N, N', N'-Tetramethylethylenediamine
Tm	Annealing temperature
tRNA	Transfer Ribonucleic Acid
U	Unit
UBA	Ubiquitin associated
UPM	Universiti Putra Malaysia
UV	Ultraviolet
V	Voltage
v/v	volume per volume
W	Watt
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
ZF	Zinc finger