



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF MYB-RELATED GENES
FROM OIL PALM (ELAEIS GUINEENSIS JACQ.)**

TEOH WAN CHIN

FSMB 2002 27

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By

TEOH WAN CHIN

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

October 2002



Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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By

TEOH WAN CHIN

October 2002

Chairman : Tan Siang Hee, Ph.D.

Faculty : Food Science and Biotechnology

Myb is a sequence specific DNA-binding protein that can activate or inactivate promoters containing its binding site. Plant Myb proteins represent a group of transcription factors which have a DNA-binding domain similar to that found in the products of the animal *myb* proto-oncogenes. Some of the functions, which have so far been assigned to plant *myb* genes, include regulation of phenylpropanoid biosynthesis, control of cellular differentiation, and contribution to control of plant responses to hormones and various stresses.

Two different *myb*-like genes were isolated. *OpMyb24*, which contains a full-length coding region, was obtained from an oil palm zygotic embryo (OPZE) cDNA library. Its Myb-domain shows extensive homology to the snapdragon proteins Myb308 and Myb330, and maize protein Zm38, and these *myb*-like genes have been found to be involved in regulating phenylpropanoid and lignin biosynthesis, and control of flavonoid biosynthesis. The second *myb*-like gene was



isolated from oil palm suspension culture (OPSC) cDNA library, *OpMyb15*, which lacks the 5' end, has greater similarity to MIXTA of *Antirrhinum*, which is essential for the development of the conical form of petal epidermal cells. These two predicted gene products showed high similarity within the myb domain with other *Myb* genes from other species; however, outside of this region virtually no similarity was found.

Expression of *OpMyb24* and *OpMyb15* was detected in all tissues tested, except *OpMyb24* transcript, which was not detected in three-month-old and young leaves. Both *OpMybs* were relatively more abundant in 28-cm male flowers and meristems, which showed distinct, tissue-specific expression patterns.

For the expression study in treated oil palm *in vitro* seedlings, *OpMyb15* and *OpMyb24* transcripts accumulated to high levels in response to gibberellic acids, while the levels of *OpMyb24* decreased significantly after wounding or heavy metals treatment. At the same time, *OpMyb24* mRNA increased slightly after UV light exposure. Much remains to be learned about the function of these oil palm *Myb* genes in their molecular responses in GA-regulated processes, wounding, heavy metal treatment and UV light exposure.

From the Southern blot analyses, these two *OpMybs* were determined to exist as a small gene family. As overexpression or downregulation of a gene is most frequently achieved by the production of transgenic plants carrying sense or antisense copy of the gene, the coding sequence of *OpMyb24* was successfully inserted in both sense and antisense orientations between the 35S CaMV promoter

and *nos* terminator of the expression vector p35S/NOS. The both constructs were consequently subcloned into a binary vector (pCAMBIA 1301) to be used to transform oil palm in the future. This is one of the approaches may provide important clues about the function of *OpMyb24* by studying the phenotype of the resulting transgenic oil palm.

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

**PENGASINGAN DAN PENCIRIAN GEN-GEN BERHUBUNGAN DENGAN
MYB DARIPADA KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)**

Oleh

TEOH WAN CHIN

Oktober 2002

Pengerusi : Tan Siang Hee, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Myb adalah sejenis protin pengikat DNA berjujukan spesifik yang dapat mengaktif atau menyahaktifkan promotor-promotornya. Protin Myb tumbuhan mewakili sekumpulan faktor transkripsi di mana kawasan pengikat DNA berjujukan spesifik mereka mempunyai persamaan terhadap produk *myb* “proto-oncogenes” haiwan. Beberapa fungsi yang selama ini ditugaskan kepada gen *myb* tumbuhan adalah termasuk pengaturan biosintesis “phenylpropanoid”, mengawal pembezaan sel, dan mengawal tindak balas tumbuhan terhadap hormon dan pelbagai tekanan.

Dua gen berlainan yang mempunyai persamaan *myb* telah diasingkan. *OpMyb24* yang mengandungi kawasan pengkodan lengkap diperolehi daripada koleksi “cDNA” embrio zigotik kelapa sawit. Protin “snapdragon” Myb308 dan Myb330, serta protin jagung Zm38 yang terlibat dalam pengawalan biosintesis “phenylpropanoid” dan lignin, serta mengawal biosintesis “flavonoid” mempunyai

tahap persamaan yang tinggi terhadap lingkungan jujukan DNA *Myb* bagi *OpMyb24*. *OpMyb15* adalah gen kedua yang mempunyai persamaan *Myb* diasingkan daripada koleksi “cDNA” kultur ampaian kelapa sawit. Gen ini mengandungi kawasan pengkodan tidak lengkap iaitu kekurangan penghujung 5’, dan mempunyai tahap persamaan tinggi terhadap MIXTA daripada *Antirrhinum*, di mana ia adalah penting dalam pertumbuhan konikal bagi sel ranggi. Kedua-dua jenis gen ini mengandungi tahap persamaan yang tinggi dalam lingkungan jujukan DNA *Myb* dengan gen *Myb* daripada spesies yang lain tetapi tiada persamaan dijumpai langsung di luar lingkungan jujukan DNA *Myb* ini.

Ekspresi *OpMyb24* dan *OpMyb15* dikesan pada semua tisu yang diuji kecuali transkrip *OpMyb24* tidak dapat dikesan pada daun berumur tiga bulan dan daun muda. Kedua-dua *OpMybs* didapati berlebihan di dalam bunga jantan berukuran 28-cm dan “meristem”. Mereka telah menunjukkan corak ekspresi yang jelas terhadap tisu yang spesifik sahaja.

Dalam kajian “*in vitro* seedling”, kelapa sawit yang telah dirawat didapati bahawa transkrip *OpMyb15* dan *OpMyb24* terkumpul banyak sebagai suatu tindak balas terhadap asid gibberelik, manakala paras mRNA *OpMyb24* menurun selepas perlakuan dan rawatan “HgCl₂”. Pada masa yang sama, paras mRNA *OpMyb24* meningkat sedikit selepas terdedah kepada UV. Namun demikian, kajian lanjut diperlukan jika ingin lebih memahami fungsi *Myb* yang diasingkan daripada kelapa sawit terhadap tindak balas molekul dalam proses pengawalaturan GA₃, perlakuan, rawatan HgCl₂ dan pendedahan UV.

Daripada analisis “Southern Blot”, kedua-dua *OpMyb* telah disahkan wujud sebagai gen keluarga kecil. Ekspresi terlampau atau merosot bagi sesuatu gen kerap diperolehi daripada produksi tumbuhan ubahsuai yang mengandungi gen “sense” dan “antisense”. Oleh itu, gen *OpMyb24* yang berorientasi “sense” dan “antisense” telah berjaya dimasukkan di antara promotor “35S CaMV” dan penamat “nos” pada vektor ekspresi “p35S/NOS”. Kedua-dua jenis orientasi gen disubklonkan pada vektor binari (pCAMBIA 1301) untuk digunakan dalam pengubahsuaian kelapa sawit pada masa depan. Ini adalah salah satu pendekatan yang mungkin dapat memberi matlumat penting berkenaan fungsi gen *OpMyb24* melalui kajian fenotip kelapa sawit yang telah diubahsuaikan.

ACKNOWLEDGMENTS

The work presented in this Thesis could not have been possible without the guidance and support of many people.

In first place, I would like to thank my supervisor, Dr. Tan Siang Hee, for giving me the opportunity of pursue this work and directing my efforts to complete it. He has been an invaluable source of support and guidance all through my postgraduate program.

My thanks to Prof. Madya Dr. K. Harikrishna and Dr. Cheah Suan Choo of MPOB for agreeing to serve on my committee and taking a keen interest in my research. I am also grateful to Dr. Cheah Suan Choo for the financial support for this project.

My heartfelt thanks to Dr. Ho Chai Ling for her timely input and invaluable help in the experiments. I would also like to thank Dr. Jenni and Dr. Faridah for their support and valuable feedback to me during this project.

Thanks must also go to all members in the Genetic Lab of Faculty Food Science and Biotechnology, UPM. Hwang, Lee, Mei Chooi, Chin Ching, Au, Jason, Yang Peng, Peck Kuen, See, Choong, Chee How, Chuen Yi, Wai Har, Siew, Siti Suhaila, Siti Habsah, Rahsiah, Mr. Ong and Sequencing Officers who had shared many helpful hints, pointed out some pitfalls to avoid and spiced it all with true friendship.

A special thanks to the staff at the MPOB especially Dr. Meilina Ong, Dr. Sharifah, Siew Eng, Zaidah, Ayu, Kak Azizah, Kak Feshah and many others for providing the technical support, and their continuous and invaluable assistance.

Lastly, I would like to thank my family for their encouragement and support of my work.

DECLARATION

I hereby declare that the thesis is based on my original work except for equations 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.

TEOH WAN CHIN

Date:

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4.26 DNA Gel Blot Analysis of the *OpMyb* Genes in Oil Palm Genomic DNA. *EcoRI* was used to digest 20 μ g (lane 2), 40 μ g (lane 3) and 60 μ g (lane 4) genomic DNA; *BamHI* was used to digest 20 μ g (lane 5), 40 μ g (lane 6) and 60 μ g (lane 7) genomic DNA; *KpnI* was used to digest 20 μ g (lane 8), 40 μ g (lane 9) and 60 μ g (lane 10) genomic DNA. The blot was hybridized with (A) *OpMyb15* cDNA probe or (B) a full-length *OpMyb24* cDNA probe 78

4.27 The cDNA sequence that encodes for *OpMyb24*. The sequence in bold on top showed the start codon, whereas the sequence in bold below showed the stop codons. The sequences underlined indicate the restriction site for *SpeI* 80

4.28 Restriction enzyme analysis of p35S/NOS-Mybs [1.5% (w/v) agarose gel]. Clones p35S/NOS-Mybs were digested with *KpnI* and *SacI*. Sense constructs showed DNA fragment of 0.95 kb, while antisense constructs were digested into two DNA fragments consisting of 0.53 kb and 1.25 kb. Lane 1: Clone p35S/NOS-Myb8AS; lane 2: Clone p35S/NOS-Myb10AS; lane 3: Clone p35S/NOS-Myb12S; lane 4: Clone p35S/NOS-Myb20S; lane 5: Clone p35S/NOS-Myb22S; lane 6: Clone p35S/NOS-Myb23AS and lane 7: 1 kb DNA marker..... 82



ABBREVIATIONS

μg	-	microgram
μl	-	microliter
2-BE	-	ethylene glycol monobutyl ether
BLAST	-	Basic Local Alignment Research Tool
bp	-	base pair
cDNA	-	complementary DNA
CsCl	-	Cesium chloride
CTAB	-	Hexadecyltrimethylammonium bromide
DEPC	-	diethyl pyrocarbonate
dH ₂ O	-	distilled water
DNA	-	deoxyribonucleic acid
dNTPs	-	deoxynucleotide triphosphate
DTT	-	dithiothreitol
EDTA	-	ethylenediamine tetraacetic acid
EGTA	-	ethylene glycol-bis (β-aminoethylether)- <i>N,N,N',N'</i> -
GSH	-	glutathione
GTE	-	Glucose-Tris-EDTA
GUS	-	β-glucononidase
HCl	-	hydrochloride acid
Jacq.	-	Jacquin
kb	-	kilobase pair
KCl	-	potassium chloride
LB	-	Luria-Bertani
LiCl	-	lithium chloride
M	-	Molar

MgCl ₂	-	magnesium chloride
MgSO ₄ .7H ₂ O	-	magnesium sulfate heptahydrat
Min	-	minute(s)
Mm	-	millimolar
MOPS	-	3-(N-morpholino) propanesulfonic acid
mRNA	-	messenger RNA
MYB	-	myblastosis
N	-	Normal
NaCl	-	sodium chloride
NaOH	-	sodium hydroxide
NCBI	-	National; Center for Biotechnology Information
ng	-	nanogram
NMR	-	Nuclear Magnetic Resonance
OD _{600nm}	-	Optical density at 600 nanometer
Oligo(dT)	-	oligodeoxythymidylic acid
ORF	-	open reading frame
PAL	-	phenylalanine ammonia-lyase
PCI	-	phenol-chloroform-isoamyl alcohol
PCR	-	polymerase chain reaction
pfu	-	plaque-forming units
Poly(A) ⁺	-	polyadenylated (mRNA)
PVPP	-	polyvinylpolypyrrolidone
QTL	-	Quantitative Trait Locus
RNA	-	ribonucleic acid
RNase	-	ribonuclease
Rpm	-	revolution per minute
RT	-	reverse transcriptase