UNIVERSITI PUTRA MALAYSIA

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

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By

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

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October 2002

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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 kepade 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

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Myb adalah sejenis protin pengikat DNA berjujukan specifik 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 spesis 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 menunjukan 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 perlukaan dan rawatan "$HgCl_2$". 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 GA_3, perlukaan, rawatan $HgCl_2$ dan pendedahan UV.

Daripada analisis "Southern Blot", kedua-dua *OpMyb* telah disahkan wujuk 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.

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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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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_2O	-	distilled water
DNA	-	deoxyribonucleic acid
dNTPs	-	deoxynucleotide triphosphate
DTT	-	dithiothreitol
EDTA	-	ethylenediamine tetraacetic acid
EGTA	-	ethylene glycol-bis (β-aminoethylether)-*N,N,N',N*-
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
$MgSO_4.7H_2O$	-	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