UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF GENES EXPRESSED IN EARLY FLOWERING TISSUES OF TEAK (*TECTONA GRANDIS* LINN. F)

NORLIA BASHERUDIN

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By

NORLIA BASHERUDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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ISOLATION AND CHARACTERIZATION OF GENES EXPRESSED IN EARLY FLOWERING TISSUES OF TEAK (*TECTONA GRANDIS* LINN. F)

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July 2007

Chairman : Associate Professor Norihan Mohd Saleh, PhD

Faculty : Biotechnology and Biomolecular Science

Teak is a highly sought-after timber species in the world, and therefore has been selected as one of the timber species for forest plantation in Malaysia. However, in Malaysia teak has been observed to flower as early as three years after planting. The early flowering leads to the forking phenomenon, which lowers the quality of the timber produced. This study was initiated in order to understand the genetic control of flower development in teak with the ultimate aim of being able to manipulate this process for improvement of the species.

In the observations of flower development in teak two different types of shoots were identified, flowering and vegetative shoots. The difference gave an opportunity to isolate the genes expressed in flowering shoots using the PCR-subtractive hybridization method. Based on 130 clones isolated, 22% were functionally unknown and 13% to 15% each were involved in cell structure, signal transduction and transcription. The other clones, 1% to 10% each, were involved in energy, protein synthesis, protein digestion and storage, disease and defense, intracellular traffic and metabolism.

Out of the 130 clones analyzed, two were chosen for further analysis. The clones were TFS3-B7, which is similar to Late Elongated Hypocotyls (LHY) gene and TFS3-B17, which is similar to Arabidopsis Shaggy kinase-11 (AtSK-11) gene. The full-length cDNA of TFS3-B7 was 2948 base pair (bp) and potentially encoded for 768 amino acids. It was named *Tectona grandis* LHY (Tg-LHY), as the gene was similar to the LHY gene of some species. The level of gene expression was found to be high four hours after dawn in flowering shoots and flower, which might indicate involvement of the circadian clock system in teak flower development. Temperature might be a potential environmental cue detected by the teak circadian clock system, as the temperature was found higher within three months before the flowering season occurred. The cDNA of Tg-LHY translated into a protein of about 110 kD in a prokaryotic expression system. The gene construct of Tg-LHY in GATEWAY expression vector was also transformed into *Arabidopsis*. GUS assay analysis indicated successful integration of reporter gene into the *Arabidopsis* genome. *Arabidopsis* transformation will be further investigated in the future.

The second clone, TFS3-B17, with its cDNA of 1705 bp in length, was potentially encoded for 410 amino acids. The gene was named *Tectona grandis* Shaggy kinase (Tg-SK), as it was similar to *Arabidopsis* Shaggy kinase-11 (AtSK-11). Analysis of the gene structure showed that it had 11 introns, similar to the number of introns found in AtSK-11. The high similarity between Tg-SK and AtSK-11 within their kinase region and structure might indicate their similar function. In *Arabidopsis,* AtSK-11 gene has been suggested to play a role in meristem identity fate. Higher transcription level of this gene was detected in early and later stage of flower development, which was similar with what has been reported in *Arabidopsis*. Gene

expression analysis in a prokaryotic system showed that Tg-SK cDNA translated into a protein of about 40 kD.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMENCILAN DAN PENCIRIAN GEN-GEN YANG DIEKPRESI DI DALAM TISU PEMBUNGAAN JATI (*TECTONA GRANDIS* LINN. F)

Oleh

NORLIA BASHERUDIN

Julai 2007

Pengerusi : Profesor Madya Norihan Mohd Saleh, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Jati adalah spesies balak yang mempunyai permintaan yang tinggi di dunia, oleh itu ia telah dipilih sebagai salah satu spesies untuk perladangan hutan di Malaysia. Walaubagaimanapun, jati yang ditanam di Malaysia didapati berbunga seawal 3 tahun selepas ditanam. Pembungaan awal ini mengakibatkan pembentukan cabang, dan seterusnya menurunkan kualiti balak yang dihasilkan. Kajian ini dijalankan dengan tujuan untuk memahami kawalan genetik ke atas pembentukan bunga di dalam jati, dengan matlamat agar proses tersebut dapat dikawal mengikut keperluan pembaikbakaan spesies ini.

Melalui pemerhatian terhadap pembentukan bunga di dalam jati, dua jenis pucuk yang berbeza telah dikenalpasti, iaitu pucuk pembungaan dan vegetatif. Perbezaan tersebut membolehkan gen-gen yang diekspresi di dalam pucuk pembungaan dipencilkan menggunakan kaedah hibridisasi subtraktif-PCR. Daripada 130 klon yang telah dipencilkan, 22% tidak diketahui fungsinya dan antara 13 hingga 15% setiap satu, adalah yang terlibat di dalam struktur sel, isyarat transduksi dan transkripsi. Klon-klon lain, di mana di antara 1–10% setiap satu, terlibat di dalam pembentukan tenaga; sintesis proten; penghadaman dan penyimpanan proten; melawan penyakit dan ketahanan; pergerakan antara sel dan metabolisma.

Daripada 130 klon yang dianalisa, 2 klon telah dipilih untuk dianalisa dengan lebih lanjut. Klon-klon tersebut ialah TFS3-B7, yang menyamai gen 'Long Hypocotyl Elongated' (LHY) dan TFS3-B17, yang menyamai gen 'shaggy kinase–11' (AtSK-II) daripada *Arabidopsis*. cDNA TFS3-B7 yang lengkap adalah sepanjang 2948 bp dan berpontensi untuk mengekod 768 asid amino. Ia telah dinamakan *Tectona grandis* LHY (Tg-LHY), memandangkan gen in mempunyai persamaan dengan gen LHY dari beberapa spesis. Pengekspresan gen ini dikesan dengan banyak di pucuk pembungaan dan bunga 4 jam selepas waktu subuh, di mana ini mungkin menandakan penglibatan sistem 'circadian clock' dalam proses pembentukan bunga jati. Suhu berkemungkinan merupakan faktor persekitaran yang dikesan oleh sistem tersebut di dalam pokok jati, memandangkan suhu yang tinggi telah dikesan sepanjang 3 bulan sebelum bermulanya musim berbunga. cDNA Tg-LHY mentranslasi kepada protein yang bersaiz lebih kurang 110 kD di dalam sistem ekspresi prokariot. Konstruk gen Tg-LHY di dalam vector ekspresi GATEWAY juga telah dipindahkan ke dalam *Arabidopsis*. Analisa asai GUS menunjukkan kejayaan mengintegrasi gen pelapor tersebut ke dalam genom *Arabidopsis*. Kajian selanjutnya ke atas transformasi *Arabidopsis* akan dijalankan di masa hadapan.

Klon kedua ialah TFS3-B17, cDNAnya adalah sepanjang 1705 bp berpotensi mengekod 410 asid amino. Gen ini dinamakan *Tectona grandis* 'shaggy kinase' (Tg-SK), memandangkan ianya mempunyai persamaan dengan 'shaggy protein kinase'-11 (AtSK-11) daripada *Arabidopsis*. Analisis ke atas struktur gen ini menunjukkan ianya mempunyai 11 intron, seperti juga bilangan intron yang terdapat pada AtSK-11. Persamaan yang tinggi antara Tg-SK dan AtSK-11 pada bahagian kinase dan struktur gennya mungkin menandakan persamaanan fungsi kedua-dua gen. Di dalam *Arabidopsis*, AtSK-11 dicadangkan berperanan dalam menentukan

pembentukan identiti sesuatu meristem. Paras transkripsi gen ini dikesan lebih tinggi pada peringkat awal dan akhir pembungaan, di mana pemerhatian yang serupa telah dilaporkan di dalam *Arabidopsis*. Analisa pengekspresi gen di dalam sistem prokariot mendapati Tg-SK telah mentranslasi kepada protein bersaiz lebih kurang 40 kD.

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I certify that an Examination Committee has met on 4th July 2007 to conduct the final examination of Norlia Basherudin on her Doctor of Philosophy thesis entitle " Isolation and Characterization of Genes Expressed in Early Flowering Tissues of Teak (*Tectona grandis* LINN. F)" 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:

Raha Abdul Rahim, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd. Puad Abdullah, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Siti Nor Akmar Abdullah, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Ismanizan Ismail, PhD
Associate Professor
Centre of Science and Biotechnology
Universiti Kebangsaan Malaysia
(External Examiner)

_____
HASANAH MOHD GHAZALI,PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 September 2007

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Norihan Mohd Saleh, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Suhaimi Napis, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Norwati Muhammad, PhD
Forest Research Institute Malaysia (FRIM)
(Member)

_____
AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 15 November 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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NORLIA BASHERUDIN

Date: 3 September 2007

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LIST OF ABBREVIATIONS

%	percentage
^{0}C	degree Celsius
µg	microgram
µl	microliter
2-BE	ethyleneglycolmonobutylether
AP1	APETALA 1
APS	ammonium persulphate
BLAST	Basic Local Alignment Search Tool
bp	base pair
BSA	bovine serum albumin
CAB1	chlorophyll A/B binding protein 1
CCA1	circadian clock-associated 1
cDNA	copy DNA
CO	CONSTANS
CTAB	Hexadecyltrimethylammonium bromide
dATP	2'-deoxy-adenosine-5'-triphosphate
DEPC	diethyl pyrocarbonate
DH_2O	distilled water
DNA	deoxyribonucleic acid
Dnase I	deoxyribonuclease I
dNTPs	deoxynucleotides
DTT	dithiotrietol
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis- (β-aminoethyle ether)

EtBr	ethidium bromide
FT	FLOWERING LOCUS T
FLC	FLOWERING LOCUS C
FRI	FRIGIDA
g	gram
HCL	hydrochloric acid
Hr	hour(s)
k	kilo
kb	kilobase
KCl	potassium chloride
kD	kilo dalton
L	liter
LD	long day plant
LFY	LEAFY
LHY	late elongated hypocotyls
LiCl	lithium chloride
M	molar
mg	milligram
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulfate
min	minutes
ml	milliliter
mm	millimeter
mM	milimolar
MOPS	3-(N-morpholino) propane-sulphonic acid
mRNA	messenger RNA

MW	molecular weight
N	normal
NaCl	sodium chloride
NaI	sodium iodide
NaOAc	sodium acetate
Ng	nanogram
N-terminal	amino terminal
OD	optical density
ORF	open reading frame
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PHYB	PHYTOCHROME B
PIF3	PHYTOCHROME INTERACTING FACTOR 3
Poly A^+ RNA	polyadenylated RNA
PVP	polyvinylpyrrolidone
PVPP	polyvinylpolypyrrolidone
RNA	ribonucleic acid
Rnase	ribonuclease
Rpm	revolution per minute
rRNA	ribosomal RNA
RT	reverse transcriptase
SAM	shoot apical meristem
SD	short day plant
SDS	sodium dodecyl sulfate
SOC1	SUPPRESSOR OF CONSTANS OVEREXPRESSION
SSC	sodium chloride0sodium citrate buffer

TAE	tris acetate EDTA
TBE	tris borate EDTA
TE	tris-HCl-EDTA
TEMED	N,N,N',N'- Tetra-methylethylenediamine
TFL1	TERMINAL FLOWERING 1
TOC1	Timing of CAB1
tRNA	transfer RNA
U	unit
UTR	untranslated region
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galatopyranose

CHAPTER 1

INTRODUCTION

The wood of teak (*Tectona grandis)* is well known to the world. It is much sought after timber and famous for its beauty, strength and resistance to termites. However, the tree itself in nature is declining in number at an alarming rate. Most countries where teak is native have started to plant this tree in plantations. Teak was introduced to Malaysia in 1800 and the first plantation was developed in Langkawi in 1915 (Thai, 2000). Since then, the area of teak plantation has been increasing.

Teak is a deciduous tree species. It flowers yearly, in a huge panicle with thousands of tiny and whitish flowers. The panicle occurs at the main axis of the tree (Syarach-Larsen, 1966). Once flowering is over, the shoot will partly die back, and lateral buds immediately below it will compete with each other to develop into big branches. This will then lead the tree becoming forked.

Generally, teak starts to flower five to six years after planting. However, teak planted in Malaysia under plantation condition has been observed to flower as early as three years. The early flowering will indirectly reduce the timber quality due to the shorter clear bole produced. Flowering has also been known to reduce vegetative growth due to energy utilization. These two phenomena related to flowering in teak have become two major issues that affect the performance and management of teak plantation, which are challenges to the relatively low growth rates achieved and problem of maximizing the length of the clear bole (Khrishnapillay, 2000).