UNIVERSITI PUTRA MALAYSIA

HISTOLOGICAL AND MOLECULAR INVESTIGATIONS OF THE SHOOT APICAL MERISTEM OF TEAK (TECTONA GRANDIS LINN. F.)

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By

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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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HISTOLOGICAL AND MOLECULAR INVESTIGATIONS OF THE SHOOT APICAL MERISTEM OF TEAK (*TECTONA GRANDIS* LINN. F.)

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March 2011

Chairman : Associate Professor Norihan Mohd Saleh, PhD

Faculty : Biotechnology and Biomolecular Sciences

Early flowering in plantation grown teak trees was reported to be controlled by both genetic and environmental factors. The first flowering occurs at the terminal of the main axis, which causes the formation of branches and forking of the main axis. The forking of the main axis at a very early stage could reduce the length of the clear bole for quality timber. Thus, the introduction of genes that turn off or delay early flowering, through molecular biology techniques and genetic engineering, can boost vegetative growth with improved tree form. This thesis reports the studies on the flowering of teak which can be divided into two major parts. The first part is on the observations made on teak flowering behavior, followed by morphological and histological observations made on several developmental stages of terminal shoots from vegetative to inflorescence. From these observations, terminal shoots from selected developmental stage were used for further molecular investigations. The second part is on the molecular investigations carried out on terminal shoots that were observed to bear floral meristem. Genes expressed in these tissues were investigated through the construction

of cDNA library followed by the generation of Expressed Sequence Tags (ESTs), and Rapid Amplification of cDNA Ends-Polymerase Chain Reactions (RACE-PCR) to clone selected floral gene. Gene expression analysis using real-time PCR was carried out on selected ESTs and the cloned floral gene. Based on observations made on teak trees that grow in different plantation conditions, particularly with respect to light intensity, it was found that flowering only occurs on branches that are exposed to bright sunlight. It was also observed that flowering starts from the top branches progressing downwards to lower branches. Morphological changes of terminal shoots from vegetative to inflorescence stages were photographed and described. These morphological changes were classified into several stages named as TIS1-TIS4 (Teak Inflorescence Shoot Stages 1-4). Histological observations showed that floral meristems were only observed in TIS4 shoots. Floral meristem bearing TIS4 shoots were selected to construct two cDNA libraries with different insert size ranges; one library with 500-1500 bp and the other with 1500-5000 bp. From the cDNA library with the insert size range of 1500-5000 bp, a total of 1125 ESTs were generated. After filtering and clustering, the total of 674 nonredundants (69 consensus sequences and 605 singletons) were generated and searched for their identities through BLASTX. Five sequences were found to be related to flowering and light induction. Gene expression analysis using real-time PCR was carried out on these five selected ESTs. All the 567 nonredundants identified through BLASTX were then classified into their functional categories and their protein signatures. Simple sequence repeats (SSRs) identification was also carried on the 674 nonredundants where 21 SSRs were identified in 46 ESTs. In addition, a partial *ZEITLUPE* (*ZTL*) gene sized 1.3 kb was isolated from teak using RACE-PCR. This cloned fragment was named *TgZTL* (*Tectona grandis ZEITLUPE)* and contained a 675 bp coding region for a 225 aa residue. Gene expression analysis using real-time PCR was carried out on this partial gene.

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

KAJIAN HISTOLOGI DAN MOLEKUL KE ATAS MERISTEM APEKS PUCUK JATI (*TECTONA GRANDIS* LINN. F.)

Oleh

MOHD ROSLI BIN HARON

Mac 2011

Pengerusi : Profesor Madya Norihan Mohd Saleh, PhD

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Pembungaan awal pokok jati yang ditanam secara perladangan telah dilaporkan dikawal oleh faktor genetik dan persekitaran. Pembungaan pertama berlaku di terminal batang utama menyebabkan pembentukan dahan dan percabangan. Percabangan batang utama yang terlalu awal mengurangkan panjang batang pokok untuk balak berkualiti. Oleh itu, penggunaan gen-gen yang boleh menyekat atau melewatkan pembungaan awal melalui biologi molekul dan kejuruteraan genetik boleh mempertingkatkan tumbesaran vegetatif dan memperbaiki bentuk pokok. Tesis ini melaporkan kajian-kajian ke atas pembungaan pokok jati yang terbahagi kepada dua bahagian utama. Bahagian pertama adalah mengenai pemerhatian ke atas perlakuan pembungaan pokok jati, diikuti pemerhatian morfologi dan histologi ke atas beberapa peringkat perkembangan pucuk-pucuk terminal daripada vegetatif kepada pembungaan. Daripada pemerhatian ini, pucuk terminal pada peringkat perkembangan terpilih telah digunakan untuk kajian molekul. Bahagian kedua adalah mengenai kajian molekul ke atas pucuk yang membawa tisu meristem pembungaan. Gen-gen yang diekspresikan di dalam tisu ini telah dikaji melalui penghasilan koleksi cDNA diikuti penghasilan 'Expressed

Sequence Tags' (EST), dan 'Rapid Amplification of cDNA Ends-Polymerase Chain Reactions' (RACE-PCR) untuk mengklon gen pembungaan terpilih. Analisa pengekspressan gen telah dilakukan secara 'real-time' PCR ke atas EST terpilih dan gen pembungaan yang berjaya diklonkan. Berdasarkan pemerhatian ke atas pokok-pokok jati di berbagai keadaan perladangan terutamanya berkaitan keamatan cahaya, didapati pembungaan hanya berlaku pada dahan-dahan yang terdedah kepada cahaya yang terang. Didapati juga pembungaan bermula dari dahan-dahan di atas membawa ke dahan-dahan di bawah. Perubahan morfologi pucuk-pucuk terminal daripada peringkat vegetatif kepada pembungaan telah difotografikan dan diperincikan. Perubahan morfologi ini dibahagikan kepada beberapa peringkat yang dinamakan TIS1-TIS4 ('Teak Inflorescence Shoot Stage' 1-4). Pemerhatian histologi menunjukkan tisu meristem pembungaan hanya ditemui di dalam pucuk TIS4. Pucuk TIS4 telah dipilih untuk menghasilkan dua koleksi cDNA dengan julat saiz selitan yang berbeza; iaitu selitan 500-1500 bp dan satu lagi 1500-5000 bp. Daripada koleksi cDNA dengan selitan 1500-5000 bp, sejumlah 1125 EST telah dihasilkan. Selepas dikelompokkan, sejumlah 674 jujukan tidak bertindan (69 jujukan konsensus dan 605 jujukan tunggal) dihasilkan dan ditentukan identiti melalui BLASTX. Lima jujukan menunjukkan identiti yang berkaitan dengan pembungaan dan induksi cahaya. Analisa pengekspressan gen telah dilakukan ke atas lima EST terpilih ini. Kesemua 567 jujukan yang mempunyai identiti dikelaskan mengikut kategori fungsi dan ciri protein mereka. Identifikasi 'simple sequence repeats' (SSRs) ke atas 674 jujukan tidak bertindan telah menemui 21 SSRs di dalam 46 EST. Seterusnya, satu serpihan gen *ZEITLUPE* (*ZTL*) bersaiz 1.3 kb telah berjaya dipencilkan secara RACE-PCR. Klon ini dinamakan sebagai *TgZTL* (*Tectona grandis ZEITLUPE)* dan membawa 675 bp kawasan mengkod untuk 225 aa. Analisa pengekspresan gen telah dilakukan ke atas serpihan gen ini secara 'real-time' PCR.

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I certify that an Examination Committee has met on the 11th of March 2011 to conduct the final examination of Mohd Rosli bin Haron on his Doctor of Philosophy thesis entitled "Histological and Molecular Investigations of the Shoot Apical Meristem 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 student be awarded the degree of Doctor of Philosophy.

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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MOHD ROSLI BIN HARON

Date: 11 March 2011

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