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

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By

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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

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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

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Sequence Tags’ (EST), dan ‘Rapid Amplification of cDNA Ends-Polymerase Chain Reactions’ (RACE-PCR) untuk mengklon gen pembungaan terpilih. Analisa pengekspresan 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 difotografi 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 pengekspresan 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 (ZIL) bersaiz 1.3 kb telah berjaya dipencilkan secara RACE-PCR. Klon ini dinamakan sebagai TgZIL (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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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.

MOHD ROSLI BIN HARON

Date: 11 March 2011
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td></td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td></td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td></td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td></td>
<td>xvi</td>
</tr>
</tbody>
</table>

## CHAPTER 1

1 INTRODUCTION 1

## CHAPTER 2

2 LITERATURE REVIEW 4

2.1 Introduction to Teak (*Tectona grandis* Linn.f.) 4

- 2.1.1 Distribution 4
- 2.1.2 Morphology 7
- 2.1.3 Climatic and Ecological Conditions 8
- 2.1.4 Teak Races and Provenances 9
- 2.1.5 Silvicultural Characters 9
- 2.1.6 Phenology 12

2.2 Anatomy and Histology of Shoot Apical Meristem 19

2.3 Molecular Mechanisms of Flowering 23

- 2.3.1 Flowering Time Genes 23
- 2.3.2 Meristem Identity Genes 28
- 2.3.3 Organ Identity Genes 30

2.4 Circadian Rhythms in Plants 33

2.5 *ZTL* Gene Family 40

- 2.5.1 *ZEITLUPE* (*ZTL*) 40
- 2.5.2 *FLAVIN binding KELCH REPEAT F-BOX1* (*FKF1*) 44
- 2.5.3 *LOV KELCH PROTEIN2* (*LKP2*) 45
- 2.5.4 Mode of Action of *ZTL* Gene Family 46

2.6 Application of ESTs Generation 48

2.7 Microsatellites (SSRs) 50

## CHAPTER 3

3 MATERIALS AND METHODS 53

3.1 Materials 53

- 3.1.1 Plant Materials 53
- 3.1.2 Buffers and Solutions 54
- 3.1.3 Kits 56
- 3.1.4 Culture Media 57
- 3.1.5 Infiltration and Embedding Media for Histology 57
3.1.6 Antibiotics 58
3.1.7 PCR Primers 58
3.1.8 Computer Software 58
3.2 Methods 59
3.2.1 General Observations on Teak Flowering 59
3.2.2 Observations on Teak Shoot Development 59
3.2.3 Histology 61
3.2.5 Construction of cDNA Library 65
3.2.5 Generation and Analysis of Expressed Sequence Tags (ESTs) 76
3.2.6 Rapid Amplification of cDNA Ends-Polymerase Chain Reaction (RACE-PCR) 81
3.2.7 Real-time PCR 85

4 RESULTS AND DISCUSSION 90
4.1 Morphological Observations on Flowering and Shoot Development
  4.1.1 General Flowering Characteristics 90
  4.1.2 Morphological Development of Terminal Shoots 95
4.2 Histological Observations 104
4.3 Sampling of Shoots for cDNA Library Construction 110
4.4 Total RNA Extraction 111
4.5 Construction and Quality Assessment of cDNA Library 113
4.6 Generation of Quality EST Data 115
4.7 Clustering and Consensus Generation 117
4.8 Identifying and Indexing Genes 119
4.9 SSRs Identification in Teak ESTs 125
4.10 Gene Expression of the Selected EST Clones 129
4.11 RACE-PCR of TgZTL Gene 141
4.12 Gene Expression of TgZTL 146

5 CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 150

REFERENCES 154
APPENDICES 169
BIODATA OF STUDENT 181
LIST OF PUBLICATIONS 182