UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF GENES ASSOCIATED WITH GAHARU FORMATION AND ANATOMICAL CHANGES IN STRESS-INDUCED *Aquilaria malaccensis* Lam.

WONG MUN THENG

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WONG MUN THENG MASTER IN SCIENCE 2010

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By

WONG MUN THENG

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

October 2010

SPECIALLY DEDICATED

TO MY LATE GRANDMOTHER,

Ng Yoon Hup

BELOVED PARENTS,

Wong Yeen Seong & Lor Chow Thye

BROTHER,

Wong Leong Kit

AND FRIENDS.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CHARACTERIZATION OF GENES ASSOCIATED WITH *GAHARU* FORMATION AND ANATOMICAL CHANGES IN STRESS-INDUCED *Aquilaria malaccensis* Lam.

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WONG MUN THENG

October 2010

Chair: Rozi Mohamed, PhD

Faculty: Faculty of Forestry

Aquilaria malaccensis (Karas) is a native tree that produces aromatic oleoresins (*gaharu* or agarwood) in response to external attack. Little is known about oleoresin synthesis in the wood. To understand this phenomenon, several candidate genes in oleoresin synthesis pathway were cloned and expression patterns determined at various time points after stress induction. Three genes were cloned in this study: two transcriptional factors from the WRKY family and a gene that encodes *terpene synthase*. A partial length cDNA of *AmWRKY1* was isolated through RACE-PCR. The cDNA fragment was 871 bp and the deduced polypeptide consisted of 194 amino acids. The deduced protein sequence exhibited high sequence similarity (63-72%) to WRKY proteins from group I. The second *WRKY* gene which was designated as *AmWRKY2* was 580 bp long. The translated sequence had poor similarity to other WRKY protein with only 36% similarity to Zinc-dependent activator protein-1 (Zap1) from *Arabidopsis thaliana*. The cloned *terpene synthase*

fragment had a length of 344 bp and was designated as *AmTPS1*. The deduced protein exhibited 62-80% sequence similarity to known acyltransferases proteins. The expression profile of the three transcripts including *phenylalanine ammonia-lyase* (*PAL*) gene from a previous study in a 30 days cycle were investigated using real-time RT-PCR (qPCR). Expression of all the four transcripts was regulated differently from 3 hours to 30 days. *AmWRKY1* and *AmTPS1* showed immediate-early expression at 3 hours while *AmWRKY2* and *AmPAL* were expressed later starting from 16 hours.

In addition, the anatomical structures of juvenile and mature resinous wood were compared, and changes in the woody tissues were determined following mechanical wounding and electrical stimulation. There was no difference between juvenile and mature wood except that the percentage of area covered by included phloem in juvenile wood was twice than that of mature wood. In juvenile wood, the content of starch grains decreased in inner sapwood when compared to outer sapwood. In resinous wood, brownish bodies were found in both ray and axial parenchyma, included phloems, xylem vessels and fibers. From unstained sections of 48 hours following wounding of juvenile tree, brownish substance was found in ray parenchyma cells, included phloem and fibers. Electrical stimulation on 3-year old trees was carried out by applying doses of high voltage currents. After 28 days of electrical stimulation, naked eye observation revealed that the outer sapwood was dehydrated, while the inner sapwood was carbonized. Included phloems were crushed and the vessels of the affected wood contained brownish bodies. In addition, fungal hyphae were observed inside the carbonized area.

In conclusion, the results of gene expression indicate that *AmWRKY1, AmWRKY2, AmTPS1* and *AmPAL* may be involved in 'gaharu' formation. It can be deduced that wounding, either by direct penetration into the stem or by indirect damage through electrical shock, is the primary cause to commence synthesizing of gaharu. Both juvenile and mature wood, have the ability to produce oleoresin as there was no major anatomical difference between them. In this study, juvenile tree as young as 3-year old can produce oleoresin when given proper treatment.

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

PENCIRIAN GEN YANG BERKAITAN DENGAN PEMBENTUKAN GAHARU SERTA PERUBAHAN ANATOMI DALAM STRES-TERINDUKSI *Aquilaria malaccensis* Lam.

Oleh

WONG MUN THENG

Oktober 2010

Pengerusi :Rozi Mohamed, PhD

Fakulti: Fakulti Perhutanan

Aquilaria malaccensis (pokok karas) adalah sejenis pokok yang menghasilkan damar aromatik atau gaharu akibat tindak balas dengan serangan luar. Tidak banyak yang diketahui tentang penghasilan resin oleh kayu dalam *Aquilaria*. Untuk memahami fenomena ini, beberapa gen dari karas yang terlibat dalam penghasilan resin dan pola ekspresi pada masa-masa tertentu dikenalpasti selepas stres-terinduksi. Tiga jenis gen telah diklon dalam kajian ini: dua faktor transkripsional daripada keluarga WRKY dan satu lagi gen yang mengekodkan '*terpene synthase*'. Sebahagian cDNA yang mengekodkan jujukan *AmWRKY1* telah dipencilkan dengan menggunakan kaedah RACE-PCR. Ia mempunyai kawasan pengkodan sebanyak 871 pb serta protein yang terdiri daripada 194 asid amino. Jujukan asid amino menunjukkan persamaan yang tinggi (63-72%) dengan protein WRKY daripada kumpulan I. Gen *WRKY* yang kedua yang dinamakan sebagai *AmWRKY2* mempunyai panjang sebanyak 580 pb. Jujukan yang diterjemahkan mempunyai kesamaan yang amat rendah dengan protein WRKY di mana hanya 36% kesamaan

dengan ‘Zinc-dependent activator protein-1’ (Zap1) daripada *Arabidopsis thaliana*. Fragmen ‘terpene synthase’ yang diklonkan mempunyai panjang sebanyak 344 pb dan dinamakan *AmTPS1*. Protein yang disimpulkan mempamerkan 62-80% kesamaan sukuan tahap asid amino. Profil ekspresi tiga transkrip ini dengan gen *phenylalanine ammonia-lyase (PAL)* daripada penyelidikan sebelum ini dalam pusingan 30 hari dikaji dengan menggunakan tindak balas rantai polimerase berbalik transkriptase masa-nyata (qPCR). Keempat-empat transkrip ini mempunyai pola ekspresi yang berbeza dari 3 jam sehingga 30 hari. *AmWRKY1* dan *AmTPS1* menunjukkan ekspresi awal segera pada jam ketiga sementara *AmWRKY2* dan *AmPAL* diekspres kemudian pada jam enam belas.

Selain itu, penelitian ke atas struktur anatomi di antara kayu muda dan kayu matang serta pencirian terhadap perubahan anatomi berikutan luka mekanik serta stimulasi elektrik juga dijalankan. Tiada perbezaan ketara di antara kayu muda dengan kayu matang selain daripada peratus litupan floem terkandung dalam kayu muda adalah dua kali ganda lebih daripada kayu matang. Dalam kayu gubal, kandungan kanji kelihatan berkurangan di bahagian dalam dibandingkan dengan bahagian luar kayu gubal. Dalam kayu gaharu, bahan-bahan kecoklatan ditemui di dalam sel parenkima, floem terkandung, vesel xilem serta fiber. Daripada sampel kayu juvenil 48 jam selepas dilukakan, bahan-bahan kecoklatan mula kelihatan dalam ruji parenkima, floem terkandung dan fiber. Stimulasi elektrik dijalankan ke atas pokok juvenil yang berumur tiga tahun dengan mengenakan beberapa dos voltan arus tinggi. Selepas 28 hari dirangsang kejutan elektrik, penelitian dengan mata kasar mendapati bahagian luar kayu gubal dinyahhidratkan manakala bahagian dalam kayu gubal terkarbon.

Floem-floem terkandung musnah dan di dalam vesel kayu yang terjejas mengandungi bahan-bahan kecoklatan. Tambahan lagi, hifa kulat kelihatan di dalan kawasan terkarbon.

Kesimpulannya, keputusan untuk ekspresi gen-gen *AmWRKY1, AmWRKY2, AmTPS1* dan *AmPAL* berkemungkinan terlibat dalam penghasilan gaharu. Ini boleh dikatakan luka samada secara penembusan terus ke dalam batang pokok atau stimulasi elektrik, merupakan sebab utama mulanya penghasilan gaharu. Kedua-dua kayu juvenil dan kayu matang berupaya menghasilkan resin dalam kayu disebabkan tiada perbezaan ketara dalam struktur anatomi. Dalam penyelidikan ini, kayu juvenil seawal umur tiga tahun berupaya menghasilkan resin apabila diberikan rawatan yang sepatutnya.

ACKNOWLEDGEMENTS

I want to extend my warmest gratitude to everyone who, in one way or another, made the completion of my Master's thesis possible: to Dr. Rozi Mohamed, my supervisor, for her patience in guiding me throughout the duration of project and for her perseverance in proofreading my manuscript; to Prof. Nobuchi Tadashi and Assoc. Prof. Dr. Faridah Qamaruz Zaman, my co-supervisors, for their technical proficiency and valuable feedback throughout my project; Cik Rasmina Halis for her generosity in helping; to Dr. Mohd. Roslan Mohamed Kassim for his statistical advice; to Dr. Teo Guan Young and Dr. Tan Sheau Wei, for their unmatched effort in guiding and advice about setting up the quantitative real-time RT-PCR; to Nancy Liew Woan Charn, science officer of Molecular Biomedicine Laboratury at Institute Bioscience, UPM, for her kindness, to all my laboratory colleagues and friends; Lee Shiou Yih, How Chee Wun, Liong Yan Yee, Jong Phai Lee, Siah Chai Har, Jency Jenuai Anak Tiun, Tan Hui Rus, Amir A'ffan Abdul A'zim, Lim Kian Lum, Chong Yi Way, Ng Wei Keat, Goh Su Hua, Foo Jhi Biao, Nurul Hidayah binti Abdullah Zawawi, for their help and advice directly or indirectly in my research even though they were working on other projects; to En. Jelani Alias, my faculty's driver, for bringing me to field trip and to UPM, for giving me the privilege of university education through the Graduate Research Fellowship.

My heartiest appreciation goes to my parents and brother for their emotional support, encouragement and understanding throughout my entire study period especially during my writing of thesis. Last but not least, a special thanks to my

uncle, aunt and cousin brother, Wong Leong Kin who has kindly provided their transport and forgive me for the damage I brought to their car.

These words are not enough to describe my admiration and appreciation for everyone's help. Research indeed is a collaborative work. No one does it by oneself.

I certify that a Thesis Examination Committee has met on 22nd October 2010 to conduct the final examination of Wong Mun Theng on her Master thesis entitled "Characterization of genes associated with *gaharu* formation and anatomical changes in stress-induced *Aquilaria malaccensis* Lam." in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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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 Master of Science. The 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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WONG MUN THENG

Date: 22 Oktober 2010

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