UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF GENOME SIZE OF *Aquilaria* SPECIES AND CHARACTERIZATION OF TERPENE SYNTHASE TRANSCRIPTS OF *Aquilaria malaccensis* Lam.

FARAH HANANI BINTI AZMAN

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By

FARAH HANANI BINTI AZMAN

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

November 2018

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

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November 2018

Chairman : Professor Rozi Mohamed, PhD
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Aquilaria species (Thymelaeaceae family) are an important botanical resources for the fragrant agarwood. Agarwood is mainly composed of terpenoids that are produced by a family of enzyme called terpene synthases (TPS). The species have been studied for the decades, however, the size of its genome which are important for breeding effort is yet to be analyzed. In addition, identification of terpenoid biosynthesis gene in *Aquilaria spp*. is critical for a deeper understanding of the mechanisms involved in agarwood formation. In this study, the genome size of five Aquilaria species which include *A. hirta, A. malaccensis, A. subintegra, A. sinensis,* and *A. microcarpa.* were estimated by using flow cytometry. The results showed no large variation in the 2C-values of the five species (1.35 to 2.23 pg). The TPS genes was analyzed in one the five species, *A. malaccensis* based on the previously developed in-house transriptome database. Putative TPS transcripts in *A. malaccensis* encoding monoterpene, sesquiterpene and diterpene were found clustered within known TPS families. A transcript encoding linalool synthase, an acyclic monoterpene common to floral scents of numerous plant was selected for protein expression analysis in *E. coli* where the protein accumulated in insoluble form. Collectively, the information obtained on the 2C-values can help elucidate the underlying mechanism in the evolutionary forces driving genome size variation in Aquilaria species while identification of putative TPS transcripts in *A. malaccensis* provides a clue for the role of TPS in agarwood formation in that particular species.

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

ANALISIS SAIZ GENOM SPESIS *Aquilaria* DAN PENCIRIAN TERPENE SYNTHASE TRANSKRIP *Aquilaria malaccensis* Lam.

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Spesis *Aquilaria* (keluarga Thymelaeaceae) adalah spesies terancam yang kini dilindungi oleh undang-undang antarabangsa kerana perdagangan gaharu yang merupakan sumber botani penting bagi wangian gaharu. Kajian ini merangkumi tiga objektif utama iaitu menganggarkan saiz genom lima spesies Aquilaria termasuk *A. hirta, A. malaccensis, A. subintegra, A. sinensis,* dan *A. microcarpa.* Sedikit diketahui mengenai kepelbagaian spesies Aquilaria pada tahap saiz genom. Dalam kajian ini, saiz genom lima spesies Aquilaria dianggarkan dengan menggunakan cytometry aliran. Tidak terdapat variasi besar dalam nilai 2C dari lima spesies (1.35 hingga 2.23 pg). Objektif kedua dalam kajian ini adalah untuk mengenalpasti dan menganalisis urutan terpene sintase daripada *A. malaccensis*. Gaharu terdiri daripada banyak metabolit sekunder yang penting khususnya terpenoids, yang dikenali sebagai terpene sintase (TPS) yang melibatkan pengeluarannya juga disiasat. Menggunakan transkriptom dalaman yang dikumpul daripada *A. malaccensis* penjujukan, jujukan homolog kepada TPS yang diambil dan pokok filogenetik telah dibina untuk menunjukkan fungsi transkrip TPS. Transkrip TPS (monoterpene, sesquiterpene dan diterpene) didapati berkumpul dalam keluarga terpene sintase masing-masing. Selain itu, salah satu transkrip yang mengodkan sintesis linalool (*AMTPS*), berdasarkan jujukan analisis, telah dipilih untuk expresi protein kerana ia adalah monoterpene aciklik yang biasa untuk kebanyakan aroma bunga dalam tumbuhan. Tujuan kajian ini adalah untuk mencirikan sifat enzimatik *AMTPS* yang diletakkan dari *A. malaccensis* dalam *E. coli*. Transkrip ini dinyatakan dalam *E. coli* Rosetta untuk ekspresi heterologus dan digambarkan oleh gel SDS-PAGE. Protein mengekpreskan tanpa tag histidin (50 kDa) dalam bakteria menghasilkan protein yang terkumpul dalam bentuk tidak larut. Sebagai kesimpulan, kumpulan TPS pengekodan transkrip dari pangkalan data dalaman *A. malaccensis* telah dikenalpasti. Salah satu pengekodan transkrip yang dikenal pasti untuk synthase monoterpene berjaya dinyatakan dalam perumah bakteria. Kajian ini dapat membantu menjelaskan mekanisme asas dalam kuasa evolusi yang memacu variasi saiz genom dalam spesies Aquilaria dan menyediakan asas untuk menguraikan lagi peranan gen TPS gaharu dalam *A. malaccensis*.

ACKNOWLEDGEMENTS

First and foremost, I thank The Almighty Allah S.W.T for His blessing and guidance throughout my graduate studies. Without His guidance, this thesis will not have been possible. On the other hand, I must express my deepest gratitude to my supervisor, Prof. Dr. Rozi Mohamed, for the patience, guidance, encouragement and advice she has provided throughout my time as her student. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly. In particular, I would like to extend my sincere gratitude to my co-supervisor, Dr. Razak Terhem for his contributions in my thesis checking and valuable comments in making this research a success.

Completing this work would have been all the more difficult without assistance by Mr Thomas Perrot from IAM, Université de Lorraine and Mr Razik from Malaysian Palm Oil Board for their guidance and informative description on my project. Special thanks go to my Forest Biotechnology Laboratory's members especially Dr Lee Shiou Yih and Mohammad Syahmi Bin Hishamuddin for friendships, support, help and advices directly or indirectly for past few years.

My heartiest appreciation goes to my parents Azman Abd Rahim and Zarimah Zakaria, brothers and sisters for their financial support, encouragement and understanding throughout my entire studies. Last but not least, thank you to my lovely husband Iyad Iqbal who always be at my side through thick and thin and always support me during my studies time.

This thesis master is especially dedicated to our late son,
Ramandhan Iyad Iqbal, mother love you so much.
Thank you…

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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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BLAST	Basic Local Alignment Research tool
BAP	benzylaminopurine
CITES	Convention on International Trade In Endangered Species of Wild Flora and Fauna
CMK	4-(Cytidine 5_-diphospho)-2-C-methyl-D-erythritol kinase
CV	coefficient of variation
DMAPP	dimethylallyl diphosphate
DNA	deoxyribonucleic acid
DXP	deoxyxylulose 5-phosphate
DXR	1-deoxy-D-xylulose- 5-phosphate reductoisomerase
DXS	1-deoxy-D-xylulose-5-phosphate synthase
ER	endoplasmic reticulum
FPP	farnesyl diphosphate
GPP	geranyl diphosphate
GGPP	geranylgeranyl diphosphate
GAP	glyceraldehyde 3-phosphate
HDR	4-hydroxy-3- methylbut-2-enyl-diphosphate reductase
HDS	4-hydroxy-3- methylbut-2-enyl-diphosphate synthase
HMBDP	hydroxymethylbutenyl diphosphate
HMG	3-hydroxy-3-methylglutaryl
IDI	isopentenyl diphosphate isomerase
IPP	Isopentenyl diphosphate
IPTG	Isoprophyl β-D-1-thiogalactopyranoside
JTT	Jones-Taylor-Thornton model

LB	Luria-Bertani
LPP	linalyl diphosphate
MCS	2-C-methyl-Derythritol-2,4-cyclodiphosphate synthase
MCT	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase
MDS	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
MEcDP	methylerythritol 2,4-cyclodiphosphate
MEGA	Molecular Evolutionary Genetics Analysis
MEP	methylerythritol phosphate pathway
ML	Maximum likelihood
MPOB	Malaysian Palm Oil Board
MVA	Mevalonic acid pathway
NCBI	National Centre for Biotechnology Information
NAA	naphthaleneacetic acid
ORF	open reading frames
PCR	Polymerase chain reaction
pg	picogram
PI	propidium iodide
qRT-PCR	quantitative real-time PCR
RNA	Ribonucleic acid
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TPS	Terpene synthase
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 General

Aquilaria malaccensis is one a member of the Thymelaeaceae family. Agarwood-producing tree species are endangered and listed as such in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2014) since 2005 due to the overexploitation and deforestation of tropical forest. This tropical tree has significant economic potential due to its agarwood-producing ability which is used commercially in traditional medicines such as antiemetic drugs, sedatives, and digestives. It is also widely used as perfume and incense especially in South Asia, China, Japan, and the Middle East. In Asia, interior decoration from agarwood sculpturing is a source of significant income. International markets claim that high-quality agarwood is more expensive than gold (Xu et al., 2013).

Agarwood formation in *Aquilaria* wood stem is a type of defence response against abiotic and biotic factors whether singly or in combination including mechanical wounding and infection by fungus, or in natural conditions from wind damage or lightning strikes can cause wounds. However, these processes in nature develop slowly over years (Xu et al., 2013; Azzarina et al., 2016; Siah et al., 2016). In *Aquilaria,* agarwood compound is synthesised by many chemical substances resulting in a defence response and changes in plant secondary metabolites (Gao and Wei, 2016). The formation of agarwood is closely related to terpene metabolite that are extracted for many different purposes such as fragrances, pharmaceutical agents and insecticides. Terpene synthases play an important role in the various interactions between plants and the environment (Tholl, 2006).

In generating the carbon skeletons of terpenoid in natural products, terpene synthases makes use of prenyl diphosphate substrates geranyl diphosphate (GPP) (C_{10}), farnesyl diphosphate (FPP) (C_{15}), and geranylgeranyl diphosphate (GGPP) (C_{20}). The *TPS* gene family consists of monoterpene (C_{10}), sesquiterpene (C_{15}), and diterpene (C_{20}) synthases in secondary metabolism (Bohlmann et al., 1999). Monoterpenes contain low molecular weight volatile compounds which play a role in plant biology. They are present in the floral volatiles to attract insects and other pollinators. Other than that, this metabolite is often released from vegetative tissues to function as toxic agents in direct defence against microbes or animals. It also protects the plant from predators by attacking the herbivores by attracting the predators (Tholl et al., 2004). In modern medicines, some of the important terpenoids are synthesised such as the Indian ginseng withanolides in *Withania somnifera* (L.) Dunal and the antimalarial agent artemisin in *Artemisia annua L.* (Rasool and Mohamed, 2016).

1.2 Problem Statements

This research is critically important as the uses agarwood from Aquilaria species has increased dramatically with economic growth. The *Aquilaria* has being studied for the decades, however, the size of its genome which are important for breeding effort is yet to be discovered. Furthermore, the terpene groups contribute towards the fragrance smell in agarwood. Since terpenes constitute the largest class of plant specialised (secondary) metabolites, understanding the biosynthesis and regulation of terpenes synthase in *Aquilaria spp*. is critical for determining the mechanism of agarwood formation. Genes encoding sesquiterpene synthase, were successfully cloned from *Aquilaria malaccensis* (Azzarina et al., 2016). However, there is still no study done onmonoterpene synthase in *A. malaccensis*.. It was hypothesized that there is significant different between five Aquilaria species and the terpene group are clustered under respective group.

1.3 Objectives

The main objectives of this research are:

i. To estimate the genome size of *A. hirta, A. malaccensis, A. subintegra, A. sinensis,* and *A. microcarpa,*
ii. To identify and analyse the sequence of terpene synthases from *A. malaccensis,*
iii. To characterize the enzymatic property of putative monoterpene synthase from *A. malaccensis* in *E. coli.*

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PUBLICATION

Farah, A. H., Lee, S. Y., Gao, Z., Yao, T. L., Madon, M., & Mohamed, R. (2018). Genome Size, Molecular Phylogeny, and Evolutionary History of the Tribe Aquilarieae (Thymelaeaceae), the Natural Source of Agarwood. *Frontiers in plant science*, *9*.

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