Faculty of Resource Science and Technology

In Vitro Propagation of *Aquilaria* Species and Induction of Gaharu in Calli and Cell Suspension Cultures

Zul Helmey Bin Mohamad Sabdin

Master of Science
2016

UNIVERSITI MALAYSIA SAWARAK

Grade: 2016

Please tick (√)

Final Year Project Report	
Masters	√
PhD	

DECLARATION OF ORIGINAL WORK

This declaration is made on the

Student's Declaration:

I Zul Helmey Bin Mohamad Sabdin, matric no. 10021661 from Faculty of Resource Sciences and Technology hereby declare that the work entitled, *In Vitro* Propagation of *Aquilaria* Species and Induction of Gaharu in Calli and Cell Suspension Cultures, is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

10/10/2016
Date submitted

Zul Helmey Bin Mohamad Sabdin, 10021661
Name of the student (Matric No.)

Supervisor's Declaration:

I, Prof. Dr. Sepiah Muid (SUPERVISOR'S NAME) hereby certifies that the work entitled, *In Vitro* Propagation of *Aquilaria* Species and Induction of Gaharu in Calli and Cell Suspension Cultures (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of Master of Science (Biotechnology) (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work

Received for examination by: **Prof. Dr. Sepiah Muid** Date: 30/12/2016
(Name of the supervisor)

I declare this Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*
☑ **OPEN ACCESS**

Validation of Project/Thesis

I therefore duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitise the content to for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student's signature _____ (Date) 10/10/2016

Supervisor's signature: Sepiah (Date) 10/1/2017

Current Address:

_____

_____

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument was duly prepared by The Centre for Academic Information Services]

In Vitro Propagation of *Aquilaria* Species and Induction of Gaharu in Calli and Cell Suspension Cultures

Zul Helmey Bin Mohamad Sabdin

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Biotechnology)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2016

DECLARATION

I, undersigned, hereby declare that this thesis is my own work and has not been presented in other universities, colleges or institutions, seeking for similar degree or other purposes. All sources of materials used for the thesis have been duly acknowledged.

(Zul Helmey Bin Mohamad Sadin)

Student No. 10021661

ACKNOWLEDGMENTS

First of all, I am grateful to Allah the most merciful for blessing me with good health, power and support throughout my studies.

I would like to express my appreciation to my supervisor, Prof. Dr. Sepiah Muid, my co-supervisors, Prof. Dr. Hamsawi Sani and Prof. Dr. Fasihuddin Badruddin Ahmad whose encouragement and support provided a strong foundation on which I was able to develop my thesis and continue my research. Their helpful opinions and dedications have enlightened me precious skills, experiences and knowledge to conduct this study efficiently and effectively.

Heartfelt thanks and appreciation are extended to UNIMAS ZAMALAH scholarships for providing me the monthly allowances, semester fees and examination fees throughout the study.

My honest and truthful gratitude extended to Mr. Benedict Samling (Assistant Science Officer) from Department of Chemistry, Faculty of Resource Science and Technology (FRST), for giving me the permission and guidance to use Gas Chromatography- Mass Spectrometry (GC-MS) for running samples.

I sincerely express my gratitude to my lab mates and friends, Mr. Chen Teck You, Ms. Anny Jong, Ms. Fong Yin Mei, Ms. Chen Mei Yin, Ms. Norhashimah Abdul Razak, Ms. Wan Nuur Fatiha Wan Zakaria, Ms. Nurafiza Aman, Ms. Sharifah Mazenah Wan Yusof

and Ms. Cherrie Lim for their friendships, kind assistance, invaluable comments and suggestions during the course of this study. Not forgetting my sincere thanks to Mr. Shafiq Sahat, Mr. Mohd Najib Firdous and Mdm. Fatimah Daud for samples collection and for running the laboratory smoothly.

To the one who are not only close to myself, but also are to my heart and mind, Ms. Atiqah Ismail. Thank for all the affection, support and encouragement during the good and hard times it took for me to finish this work. Your patience and understanding were essential for the completion of this thesis.

Finally and most importantly, I must thank my beloved parents, Mr. Mohamad Sabdin Omar and Mdm. Sofiah Abd Razak. To my wonderful parents who give me the opportunity and provided unyielding support throughout my academic years. I would also like to thank my brother (Mr. Muzaffar Mohamad Sabdin) and my sister (Ms. Norihan Mohamad Sabdin) who have not only been my source of inspiration, but also given themselves in the most unselfish way.

ABSTRACT

Aquilaria species such as *A. malaccensis* and *A. microcarpa* are considered as a popular local tree species in Malaysia for production of gaharu. In addition to conserving the trees in their natural habitat and ensuring sustainable supply of the agarwood, *in vitro* techniques such as production of plantlets and induction for resin production in callus need to be developed. The present study started with an attempt to establish contamination-free culture for explants of the two species of *Aquilaria* using different concentration of Clorox and exposure time. Results showed that samples dipped in 10% Clorox for 15 minutes produced higher axenic leaf and shoot tips explants of the two species of *Aquilaria,* while treatment with 10% of Clorox and dipped for 20 minutes was the best to produce axenic explants for rachis explants. Lateral buds explants of the two species of *Aquilaria* produced higher number of axenic tissue after sterilization in 15% Clorox for 10 minutes. For results callus induction from leaf and rachis explants of the *Aquilaria* species, 100% leaf explants of the two species of *Aquilaria* produce callus after six weeks of culturing on MS medium supplemented with 2.0 and 3.0 mg/L 2,4-D alone or their combination with 0.5 mg/L BAP. MS medium supplemented with 1.0 and 2.0 mg/L 2,4-D generate rachis to produce callus explants while the MS medium supplemented with 2.0 mg/L 2,4-D alone or in combination with 0.5 mg/L BAP also succeed to produce callus for explants rachis for two species of *Aquilaria* after six weeks of culturing. The best protocol to establish fast growing cell suspension cultures of the *Aquilaria* species were cultured in flask containing liquid MS medium supplemented with 2.0 mg/L 2,4-D plus 0.5 mg/L BAP and 40 g/L of sucrose. For result shoot induction, BAP. The lower concentration of BAP (0.25 mg/L) was the best for bud

multiplication of explants for both species of the *Aquilaria*. For lateral buds explants of *A. malaccensis* and *A. microcarpa*, the higher number of shoot multiplication was on MS media supplemented with 0.25 mg/L BAP and 0.5 mg/L BAP. In indirect organogenesis using clumps of callus, the higher number of shoot multiplication for both species of the *Aquilaria* was on MS supplemented with BAP at 1.5 mg/L and Napthaleneacetic α- acid (NAA) at 0.25 mg/L. For results rooting of the regenerated shoots for both of the *Aquilaria* species, half strength MS medium supplemented with 0.5 mg/L of indole-3-butyric acid (IBA) was the most effective for rooting for both of the plant species. The regenerated plantlets both of the *Aquilaria* species may successfully adapt to media, such as Jiffy-7, and grew well after planting in mixed media in polybag containing peat, soil and sand in a ratio of 1: 1: 1. For resin production, elicitors P2 and P3 are expected enhancing the production of fragrant in the callus cultures and cell suspension cultures for both of the *Aquilaria* species. Brown colour or exudate from callus on solid media can be seen and sweet smell also produce in callus on solid and in liquid media for both species of *Aquilaria* treated with three elicitors after 28 days of incubation. Several important gaharu compounds that were detected in this study including alpha-guaiene, alpha-humulene, jinkoh-eremol, agarospirol and 6,7-dimethoxy-2-(2-pheneylethyl) chromone.

Keywords: *A. malaccensis*, *A. microcarpa*, gaharu, *in vitro*, elicitor, chemical component

Pembiakan Spesies *Aquilaria* Secara In Vitro dan Aruhan Gaharu pada Kultur Kalus dan Kultur Ampaian Sel

ABSTRAK

Spesies Aquilaria seperti A. malaccensis dan A. microcarpa dianggap sebagai spesies pokok tempatan yang popular di Malaysia bagi penghasilan gaharu. Selain memelihara pokok-pokok ini dalam habitat semulajadi dan memastikan bekalan gaharu berterusan, teknik in vitro seperti penghasilan anak pokok dan aruhan untuk penghasilan resin dalam kalus perlu dibangunkan. Kajian ini bermula dengan mewujudkan keadaan yang steril bagi eksplan untuk dua spesies Aquilaria dengan menggunakan kepekatan Clorox dan masa rendaman yang berbeza. Hasil kajian menunjukkan bahawa 10% Clorox selama 15 minit menghasilkan eksplan daun dan pucuk axenik yang lebih tinggi bagi dua spesies Aquilaria. Sementara itu, 10% Clorox selama 20 minit adalah yang terbaik untuk menghasilkan rachis yang steril. Eksplan tunas sisi bagi dua spesies Aquilaria menghasilkan jumlah steril tisu yang lebih tinggi selepas pensterilan dalam 15% Clorox selama 10 minit. Keputusan bagi induksi kalus dari eksplan daun dan rachis bagi spesies Aquilaria, 100% eksplan daun bagi kedua-dua spesies Aquilaria menghasilkan kalus selepas enam minggu pengkulturan dalam medium MS yang ditambah dengan 2.0 dan 3.0 mg/L 2,4-D sahaja atau gabungan dengan 0.5 mg/L BAP. Medium MS yang ditambah dengan 1.0 dan 2.0 mg/L 2,4-D menjana eksplan rachis untuk menghasilkan kalus manakala medium MS yang ditambah dengan 2.0 mg/L 2,4-D sahaja atau gabungan dengan 0.5 mg/L BAP juga berjaya menghasilkan kalus bagi eksplan rachis bagi kedua-dua spesies Aquilaria selepas enam minggu pengkulturan. Kaedah terbaik untuk menghasilkan kultur ampaian sel daripada spesies Aquilaria adalah dikulturkan

dalam kelalang yang mengandungi medium MS cecair ditambah dengan 2.0 mg/L 2,4-D dan digabung dengan 0.5 mg/L BAP dan 40 g/L sukrosa. Pucuk diperbanyakkan melalui organogenesis langsung dan tidak langsung dengan menggunakan kepekatan BAP yang berbeza. Kepekatan BAP (0.25 mg/L) yang lebih rendah adalah yang terbaik untuk memperbanyakkan tunas eksplan bagi kedua-dua spesies Aquilaria. Untuk eksplan tunas sisi bagi dua spesies Aquilara bilangan pucuk diperbanyakkan lebih tinggi dalam medium MS ditambah dengan 0.25 mg/L BAP dan 0.5 mg/L BAP. Dalam organogenesis tidak langsung menggunakan gumpalan kalus, bilangan pucuk dibanyakkan adalah lebih tinggi untuk dua spesies Aquilaria dalam medium MS ditambah dengan BAP pada 1.5 mg/L dan NAA pada 0.25 mg/L. Untuk pengakaran dua spesies Aquilaria, separuh kepekatan medium MS ditambah dengan kepekatan IBA yang rendah pada 0.5 mg/L adalah yang paling berkesan untuk pengakaran untuk dua spesies Aquilaria. Anak pokok bagi dua spesies Aquilaria berjaya menyesuaikan diri dengan media seperti Jiffi-7 dan ditanam dalam polibeg yang mengandungi media seperti tanah gambut, organik dan pasir dengan nisbah 1: 1: 1. Untuk penghasilan resin, elisitor P2 dan P3 dapat meningkatkan penghasilan resin dalam kultur kalus pada medium pepejal dan kultur ampaian sel untuk dua spesies Aquilaria. Warna coklat atau eksudat dari kalus pada kultur boleh dilihat dan menghasilkan bau manis bagi dua spesies Aquilaria yang dirawat dengan tiga elisitor selepas 28 hari inkubasi. Beberapa sebatian kimia gaharu penting yang dikesan dalam kajian ini termasuklah alpha-guaiene, alpha-humulene, jinkoh-eremol, agarospirol dan 6,7-dimethoxy-2- (2-pheneylethyl) chromone.

Kata Kunci: *A. malaccensis, A. microcarpa, gaharu, in vitro, elisitor, komponen kimia*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES