



UNIVERSITI PUTRA MALAYSIA

AGARWOOD INDUCTION IN CELL SUSPENSION CULTURE OF *Aquilaria malaccensis* Lam

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**AGARWOOD INDUCTION IN CELL SUSPENSION CULTURE OF
Aquilaria malaccensis Lam.**

By

SHASHITA A/P JAYARAMAN

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

November 2013

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DEDICATED TO:

FATHER, MOTHER, BROTHER, SISTERS

&

K.DANESH

WHO ALWAYS HAVE FAITH IN ME

AND

THEIR SUPPORTS HAVE GUIDED ME TO GONE THROUGH

ALL THE OBSTACLES IN LIFE

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

**AGARWOOD INDUCTION IN CELL SUSPENSION CULTURE OF
Aquilaria malaccensis Lam.**

By

SHASHITA JAYARAMAN

November 2013

Chair : Associate Professor Rozi Mohamed, PhD

Faculty : Forestry

Most species of *Aquilaria* LQ WKH 7K\PHODHDFHDH IDPLO\ SURGXFH I
In Malaysia, the primary producer of agarwood is *Aquilaria malaccensis*, a tree species ORFDOONQRZQDVμNADWV. Agarwood is one of the precious woods on earth and prized for its rich and wonderful fragrance. This study aimed at inducing agarwood production in cell suspension culture of *Aquilaria malaccensis*, by investigating the optimal growth medium, optimal inocula size and finding suitable elicitors. To establish cell suspension culture, callus was first induced from nursery-derived leaf explants incubated on basic Murashige and Skoog (MS) solid medium containing 1.5% sucrose, at pH 5.7. Different auxin types including 1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and indole-3-butyric acid (IBA), were tested at various concentrations (0.55, 1.1 and 1.65 μM). Compact type callus was induced by 1.1 μM NAA with highest biomass dry weight (DW) of 17.3 mg. This auxin concentration was then combined with either 6-benzylaminopurine (BAP) or kinetin, at 0.55, 1.1, 2.2 and 3.3 μM, to induce growth of friable callus. The 1.1μM NAA + 2.2μM BAP combination produced friable callus with the highest biomass (93.3mg DW). Microscopic observations revealed the arrangement of the friable callus as loosely packed with relatively large cells. However, for the compact callus, the cells were small and densely packed. Further investigation on effects of elicitor in agarwood production were studied using cell suspension culture with initial an inocula of 4% (fresh weight, FW) of callus. The culture was challenged with fungal elicitor prepared from two species of fungi, *Trichoderma* and *Lasiodiplodia*. Fungal elicitor in the form of mycelial crude extract was added to the medium to a final

concentration of 2, 4, 6, 8, and 10 mg/L, respectively. A light scent of agarwood was detected from the culture after it had been challenged for 20 days with 8mg/L *Trichoderma* extract. To increase scent intensity, cell suspension cultures were initiated from 4%, 8%, 16% and 32% (FW) of calli and elicited with 8 and 16 mg/L *Trichoderma* extract, respectively. The combination of 16% of inocula and 8 mg/L *Trichoderma* extract produced the most intense fragrance comparable to agarwood scent. Several important agarwood compounds were detected using GC-MS including 8-epi-.gama.-eudesmol, á-guaiene, alloaromadendrene oxide-1 and chromone,5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl. *Trichoderma* appeared to be a suitable elicitor for agarwood production. In conclusion, the presence of these compounds was evidence that agarwood had been induced in the fungal inoculated cell suspension culture.

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

PENGHASILAN GAHARU MELALUI AMPAIAN SEL
Aquilaria malaccensis Lam.

Oleh

SHASHITA JAYARAMAN

November 2013

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Kebanyakan spesies *Aquilaria* di dalam keluarga Thymelaeaceae menghasilkan gaharu. Di Malaysia, pengeluar utama gaharu adalah *Aquilaria malaccensis*, iaitu spesies pokok tempatan yang dikenali sebagai karas. Gaharu adalah salah satu kayu yang berharga di dalam dunia dan kaya dengan aroma yang indah. Kajian ini bertujuan untuk mengalakkan penghasilan gaharu dalam amapaian sel *Aquilaria malaccensis*, dengan menyiasat media pertumbuhan yang optimum, saiz inocula yang optimum dan mencari elisitor yang sesuai. Untuk mewujudkan ampaian sel, kalus dihasilkan daripada eksplant daun yang diperolehi daripada tapak semeaian dan dikulturkan di dalam Murashige dan Skoog media gel yang mengandungi 1.5% sukrosa, pada pH 5.7. Jenis auksin yang berbeza termasuk asid 1-naftalenaasetik (NAA), asid 2,4-dichlorophenoxyacetic (2,4-D), dan asid indole-3-butyric (IBA), telah diuji pada kepekatan yang berbeza (0.55, 1.1 dan 0.1 mg/L). Kepekatan auksin yang tertinggi iaitu sebanyak 17.3 mg/L, walaupun bagaimanapun, kalus yang terhasil adalah jenis kompak. Pada kepekatan auksin ini, ia kemudiannya telah digabungkan dengan 6-aminocaproic acid (6-ACA) menghasilkan kalus rapuh. Gabungan 1.1 mg/L NAA dan 0.1 mg/L 6-ACA menghasilkan kalus rapuh dengan biomas tertinggi (93.3 mg). Pemerhatian mikroskopik mendedahkan susunan kalus yang rapuh adalah longgar dengan sel-sel yang agak besar. Manakala bagi kompak kalus, sel-selnya adalah kecil dan padat. Kesan elisitor dalam pengeluaran gaharu telah dikaji dengan menggunakan ampaian sel dengan saiz inokulum awal 4% (berat segar) kalus. Kultur itu telah dicampur dengan elisitor disediakan daripada dua spesies kulat, *Trichoderma* dan *Lasiodiplodia*. Elisitor kulat dalam bentuk ekstrak mycelial telah ditambah ke dalam media masing-masing pada kepekatan akhir sebanyak

2, 4, 6, 8, dan 10 mg/L. Sedikit aroma gaharu dikesan daripada kultur selepas ia telah dicabar dengan 8 mg/L ekstrak *Trichoderma* selama 20 hari. Untuk meningkatkan intensiti bau, ampaian sel telah dimulakan dari 4%, 8%, 16% dan 32% kalus dan masing-masing dicampurkan dengan 8 dan 16 mg/L ekstrak *Trichoderma*. Gabungan 16% inokulum dan 8 mg/L *Trichoderma* menghasilkan aroma yang setanding dengan bau gaharu. Beberapa komponen gaharu yang penting telah dikesan melalui analisis menggunakan kromatografi gas ±spesifikasi jisim (GC-MS) iaitu 8-epi-gama-eudesmol, á-guaiene, alloaromadendrene oksida-1 dan chromone,5-hydroxy-6,7,8-trimethoxy-2,3-dimetil. *Trichoderma* muncul sebagai elisitor yang paling sesuai untuk pengeluaran gaharu. Kesimpulannya, kehadiran komponen ini adalah bukti bahawa gaharu dapat dihasilkan dalam ampaian sel yang mengandungi elisitor kulat.

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I certify that a Thesis Examination Committee has met on 26 November 2013 to conduct the final examination of Shashita a/p Jayaraman on her thesis entitled "Agarwood Induction in Cell Suspension Culture of *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.

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