UNIVERSITI PUTRA MALAYSIA

AGARWOOD INDUCTION IN CELL SUSPENSION CULTURE OF Aquilaria malaccensis Lam

SHASHITA A/P JAYARAMAN

FH 2013 9
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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
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By

SHASHITA JAYARAMAN

November 2013

Chair : Associate Professor Rozi Mohamed, PhD
Faculty : Forestry

Most species of Aquilaria in the Thymelaeaceae family produce agarwood or 'gaharu'. In Malaysia, the primary producer of agarwood is Aquilaria malaccensis, a tree species locally known as 'karas'. 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 ampaian 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 semai 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-naftalenasetik (NAA), asid 2,4-dichlorophenoxyacetic (2,4-D), dan asid indole-3-butryc (IBA), telah diuji pada kepekatan yang berbeza (0.55, 1.1 dan 1.65 μM) . Kalus yang dihidupkan dalam 1.1 μM NAA mempunyai biomas berat kering yang tertinggi iaitu sebanyak 17.3 mg, walaupun bagaimanapun, kalus yang terhasil adalah jenis kompak. Pada kepekatan auksin ini, ia kemudiannya telah digabungkan dengan 6-benzil amino purina (BAP) atau kinetin, pada 0.55, 1.1, 2.2 dan 3.3 μM, untuk menghasilkan kalus rapuh. Gabungan 1.1 μM NAA + 2.2 μM BAP 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. Kesannya 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.
ACKNOWLEDGEMENTS

The success of any project depends largely on the encouragement and guidelines of many others. I take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this project.

First and foremost, I would like to extend my deepest gratitude to the chairman of my supervisory committee, Assoc. Prof. Dr. Rozi Mohamed, for her invaluable advice, excellence guidance, contribution, patient and criticism, not only in making the completion of this thesis a success, but also guided me to be a better person. Besides that, I sincerely thank to my co-supervisors of my supervisory committee, Dr. Rasminah Halis for her support, assistance, friendliness and as well as the suggestion throughout this research.

My sincere appreciation is extended to Dr. Ahmad bin Selamat for his statistical advices; Prof. Dr. Maziah Mahmood for her unmatched effort in guiding about plant tissue culture; Assoc.Prof.Dr.Faridah bt Abas for her generosity in providing GC-MS services; science officer of Chromatography Laboratory at Faculty of Food Science and Technology, Mr.Kamarul Ariffin Hadithon and to all my laboratory colleagues and friends; Nurul Hazwani bt Daud, Siah Chai Har, Liong Yan Yee, Jong Phai Lee, Nurul Irdayu bt Ismail, Wong Mun Theng, Lee Shiou Yih, Diau Ya Bing, Hazwani bt Oslan, Azzarina bt Anor Basoh for sharing their knowledge, ideas, as well as their life experiences during the course of this project.

Finally yet importantly, I would like to express my heartiest appreciation and thanks to my father, mother, brother, sisters and K.Danesh. Thanks for all their care, understanding and patience throughout the course of this project. Their love and care in many ways really kept me going and contributed to the accomplishment of my study.
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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