



**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**

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*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

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UNIVERSITI MALAYSIA SARAWAK

2016

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## 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  $\alpha$ - 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-phenylethyl) 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 mengandung 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 diperbanyak 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 memperbanyak tunas eksplan bagi kedua-dua spesies Aquilaria. Untuk eksplan tunas sisi bagi dua spesies Aquilaria bilangan pucuk diperbanyak 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 ampai 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-phenylethyl) chromone.

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

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