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PROPAGATION OF *AQUILARIA MALACCENSIS* SEEDLINGS THROUGH TISSUE CULTURE TECHNIQUES

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Abstract

Aquilaria malaccensis or karas is the principal source of gaharu resin, which is used in many cultures for incense, perfumes and traditional medicines. The species is mainly propagated conventionally through seeds, cuttings and graftings. Propagation by seeds is usually a reliable method for other forest species, but for karas, this technique is inadequate to meet the current demand of seedling supplies. This is principally due to its low seed viability, low germination rate, delayed rooting of seedlings, long life-cycle and rare seed production. Tissue culture has several advantages over conventional propagation, especially for obtaining large number of uniform and high-yielding plantlets or clones. This paper presents the current progress on mass-propagation of *Aquilaria malaccensis* seedlings through tissue culture technique at Malaysian Nuclear Agency.

Keywords: *Aquilaria malaccencis*, gaharu, propagation, tissue culture

Abstrak

Aquilaria malaccensis atau karas ialah sumber utama resin gaharu yang digunakan dalam kebanyakan budaya untuk aroma, minyak wangi dan ubat-ubatan tradisional. Secara tradisionalnya spesis ini boleh dipropagasikan melalui kaedah bijibenih, keratan dan cantuman tunas. Pembiakan anak benih biasanya adalah satu kaedah yang boleh dipercayai untuk spesies hutan lain tetapi untuk karas, teknik ini tidak memadai bagi memenuhi permintaan semasa anak benih. Ini disebabkan oleh kebolehhidupan biji benih gaharu adalah rendah, kadar percambahan rendah, lambat berakar, kitaran hayat lama dan pengeluaran benih yang tidak seragam. Kultur tisu mempunyai beberapa kelebihan dibandingkan dengan pembiakan secara konvensional, terutamanya bagi mendapatkan jumlah anak benih yang besar lagi seragam. Kertas ini membentangkan perkembangan semasa di dalam propagasi anak benih *Aquilaria malaccensis* melalui kaedah teknik kultur di Agensi Nuklear Malaysia.

Keywords: *Aquilaria malaccencis*, gaharu, propagasi, kultur tisu

INTRODUCTION

Aquillaria malaccensis has a wide distribution across the region, from India, Burma, Malaysia, Sumatera, Kalimantan Timur, Bangladesh, Indonesia, Myanmar, Philippines and Thailand. In Malaysian, it is locally known as Karas, Tengkaras and Gaharu. A member of the family Thymelaceae, *Aquillaria* is a large evergreen tree growing up 40 meter with diameters of trunk approximately 1.5 to 2.5 meter in diameter. It is found typically in mixed forest habitat at altitudes below 1000 meter above sea level. *A. malaccensis* and other species in the genus *Aquilaria* are also known as agarwood due to their resinous characteristics. Pure resin in distilled form is used as perfume and as a perfume component in traditional Chinese medicine and in Buddhist, Islamic and Christian religious beliefs - hence it is also regarded as 'Wood of the Gods' to some cultures (Jensen, 2007).

Aquillaria malaccensis is mainly propagated conventionally through seeds, cuttings and graftings. Propagation by seeds is usually a reliable method for other forest species, but for karas, this technique is inadequate to meet the current demand of seedling supplies. This is principally due to its low seed viability, low germination rate, delayed rooting of seedlings, long life-cycle and rare seed production. Therefore micropropagation is an alternative, as it has a number of advantages over conventional methods of propagation such us grafting and own rooting: (i) the production of genetically uniform and pathogen-free plant material in a short times; (ii) the possibility of propagating cultivars difficult to obtain through own-rooted cutting: (iii) the possibility of exporting in vitro material more rapidly with no obligation to have a long quarantine period; (iv) the possibility of scheduling plantlet production closer to the market demand. Tissue culture has several advantages over conventional propagation, especially for obtaining large number of uniform and high-yielding plantlets or clones (Briccoli Bati et al., 2006). Multiple shoots produced from axillary buds and explants can be stored *in-vitro* in a less space, and it requires minimal attention between subcultures; means they are no extra intention and materials for watering, weeding, spraying, etc are needed.

MATERIAL AND METHOD

Plant material and growth medium

The *Aquillaria malaccensis* axillary buds used as the explants were collected from unirrigated agricultural field at Jenderam Hilir, Dengkil, Selangor. A medium consisting MS (Murashige & Skoog 1962) supplemented with 10% CW, 0.25% *phytagel*™ and 0.1% vitamin at pH 5.7 were used. Plant hormones, 6-Benzyaminopurine (BAP) and Indole-3-butyric Acid (IBA) together with Naphthaleneacetic acid (NAA) were used as growth regulators.

Surface sterilization

For surface sterilization, the explants were rinsed under running water for half an hour. After that the explants were immersed completely in 20% sodium hypochlorite containing 0.05% Tween 20 for 20 minutes and followed by 10% sodium hypochlorite containing 0.05% Tween 20 for 10 minutes. These sterilized explants were then rinsed four to five times with sterile distilled water. The stems were excised and were cut into 10 mm upper and lower part of the nodes, and placed in the test tubes filled with 5ml initial medium, as described by Rugini (1984), to form shoots.

Growth conditions

The adventitious shoots and roots developed were grown under 16 hours light regime at 25°C±2 for a period of 6 – 8 weeks. Sub-culturings the explants into fresh proliferation mediums were made monthly. After 3 months in proliferation medium, shoots with two to three nodes were transferred into root inducing medium that was half-strength proliferation medium. Rooting usually took place after 4 weeks.

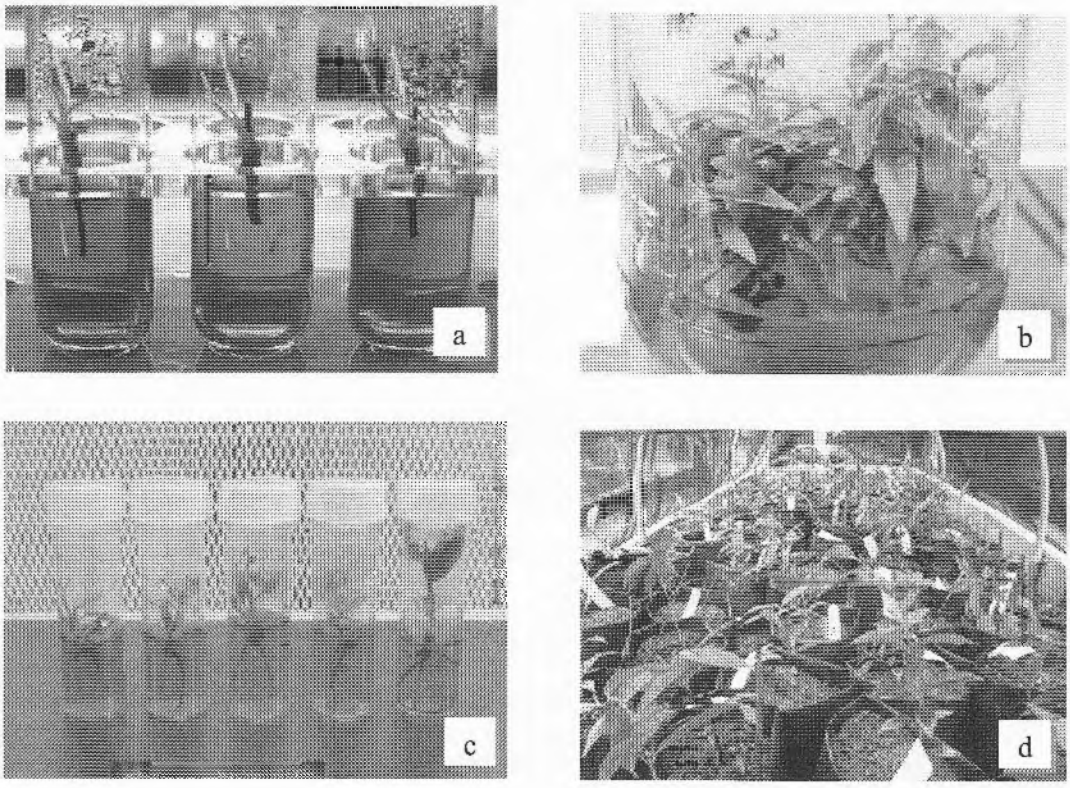
Acclimatization of rooted plantlets

Rooted plantlets were acclimatized on sand and peat moss (2:1, v/v), and placed in the hardening chamber in greenhouse. The second transplant on sand, peat moss and clay soil (1:1:2, v/v) in medium size poly-bag of 2 liter took placed 3 months later. Field trials of tissue culture propagated plants were carried out.

RESULTS AND DISCUSSION

When the axillary buds were cultured on MS medium supplemented with selected concentrations of BAP, shoot growth was initiated in about 7 days for majority of the cultures. BAP is a cytokinin, and therefore it promotes cell division, shoot multiplication and axillary bud formation while inhibiting root development (Sutter, 1996).

In shoot initiation cultures, multiple shoots were generated from the nodes in 8 weeks on MS medium supplemented with 1.5 µmol/L BAP + 0.5 µmol/L IBA and NAA medium. The development of adventitious shoots and roots derived from the axillary buds depends on the conditions of tissues and experimental treatments. Roots were produced from the explants after subsequent 4 weeks in proliferation media. MS medium was suitable for shoot initiation, shoot proliferation, shoot elongation and rooting. Figure 1 shows the tissue culture propagation process for *Aquillaria malaccensis*.



Fiq..1 In vitro propagation of *Aquilaria malaccencis* (a) Buds induction on MS+1.5 µmol/L in the first 2 weeks;(b) Bud elongation on MS + 1.5 µmol/L Bap + 0.5 µmol/L IBA and NAA medium in subsequent 4 weeks; (c) Shoot with roots on ½ MS medium; (d) well grown plants (after 16 weeks)

Suitable culture media composition of Plant Growth Regulators (PGRs) was essential for the proliferation of new plantlets. Figure 2 shows average numbers of shoots and roots developed on proliferation medium. If the micro shoots were transferred onto 1/2 MS medium or supplemented with low concentrations of NAA directly, they developed roots slowly. The method of root induction here was relatively more effective and had been used in some Temperate Zone fruit trees (Dodds, 1983).

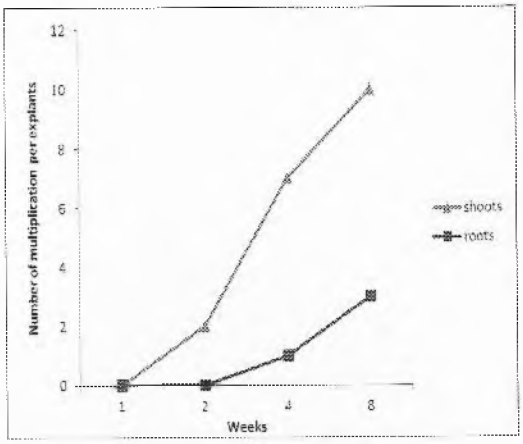


Fig. 2 : Shoot and roots proliferation rates in the MS medium

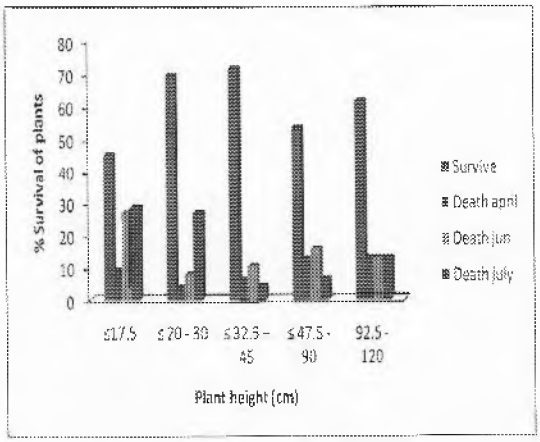


Fig. 3 : Total number survival of plants (%)

Figure 3 shows the relationship between the survival percentage of plants in Nuclear Malaysia's field plots. The data taken were survival rates from April to July 2010 based on the initial height of the plants. It was observed that plants with 32.5 cm to 45 cm in height have the highest survival rate as compared to the others, while plants with 17.5 cm and lower height have the lowest survival rate. Jensen (2002) found that *Aquilaria* trees grow well under optimal conditions and annual growth of over 1 m in height per year is common.

CONCLUSION

The most optimum medium for shoot proliferation is MS medium supplemented with 1.5 µmol/L BAP + 0.5 µmol/L IBA. Shoots were formed in 8 weeks whilst roots formed after subsequent 4 weeks. Samples of tissue culture plants have been planted in Nuclear Malaysia field plot, and plants are currently under monitoring for growth performance.

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