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M – 00273 | Review Article

A Review of *Aquilaria malaccensis* Propagation and Production of the Secondary Metabolite from Callus

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Abstract

Aquilaria is an evergreen non-timber agarwood obtained from the 15 species of *Aquilaria belonging* to the family *Thymelecea*. There are two species endemic to Northeast India, *A. malaccensis* and *A. khasiana*. *A. malaccensis* generate a high-grade degree of resin as compared to the other *Aquilaria* species, and it contributes to the economy of the Northeast states of India and the country as a whole. Due to its profoundly valuable sources, it is overexploited, which impacted its availability in genetic environments. The cultivation of the tree is challenging due to some environmental factors like the sensitivity of the seeds to desiccation, high light intensity, low shelf life, slow growth rate, and the effect of insects and microorganisms. Therefore, conservation and proliferation are urgently required for environmental sustainability and prevention from the stage of extinction. The objective of this paper is to compile the major research works on the conservation, production of the secondary metabolite from callus of *A. malaccensis* and updated information on its development and approaches that are rapidly taking place in recent years so that further novel research can be envisaged.

Keywords

Aquilaria malaccensis; Conservation and propagation; Callus; Secondary metabolite production

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Introduction

Aquilaria malaccenis is one of the precarious non-timber woods obtained from 15 species of genera *Gyrinorops* and *Aquilaria,* belonging to the family *Thymelecea,* which produces a high-grade quality of agarwood. *Aquilaria* is commonly known as agarwood, aloeswood, eaglewood, Sashi, or Agaru (Saikia and Khan, 2014). Agarwood is referred to as 'the wood of God' because of its religious uses. It is a great scented medicinal and fragrance tree of Southeast Asia and is mostly grown in the evergreen rainforest. It is a dominant species, chiefly distributed over several countries including India, Bangladesh, Malaysia, Myanmar, China, Singapore, Bhutan, Vietnam, Indonesia, and Thailand (Oldfield, Lusty and MacKinven, 1998). In India, there are three endemic species viz. *Aquilaria khasiana* Hallier, *A. macrophylla* Miq. and *A. malaccenis*. *A. khasiana* is found only in the East Khasi Hills of Meghalaya, and *A. macrophylla* is found only in the Nicobar Islands. *A. malaccensis* naturally grows at an altitude of 1,000 meters above sea level in the foothills of Assam, Meghalaya, Manipur, Nagaland, Mizoram, Tripura, Arunachal Pradesh, and West Bengal (Borpuzari and Kachari, 2018a). In upper Assam, Northeast India Sashi (*A. malaccensis*) is comprehensively planted in the home garden for propagation and production of agarwood and exceptionally boost the economy of the states (Saikia and Khan, 2011). Sashi farming was initiated in the 1970s in Assam followed by Thailand and Cambodia in the 1980s (Elias, Ibrahim and Mahamod, 2017). Development of new leaves and branches occurs during the pre-monsoon (March-April) and persists up to an actual monsoon season (July-August). *A. malaccensis* flowers at the onset of monsoon (April-June) and yields fruits during the following months till September (Plate 1). The actual reproductive phase of the plant stays for 5 months only (April to September), which is facilitated by monsoon. *A. malaccensis* organs development is significantly affected by temperature and rainfall (Borogayary, Das and Nath, 2018). Therefore, it favors a mean annual rainfall of 1,500 to 6,500 mm, the maximum temperature in the range of 22ºC – 28º C and minimum temperature in the range of 14ºC-21ºC (Beniwal, 1989). The height of the tree is 18-20 m with 1.5-2 m in diameter. *Aquilaria* species are well acclimatized in diverse habitats including rocky, sandy, well-drained slopes, ridges, and swamps (Akter and Neelam, 2008).

The tree produces a unique fragrance oil and compound, which causes the demand in the international trade for cosmetics, pharmaceuticals, religious practices scents, and perfume production. Overexploitation lessens its availability in the natural habitat and also has a great impact on the biodiversity. Therefore, for mass production of scent, the tree is overexploited and put on the verge of extinction. All the *Aqualaria* species that produce agarwood were registered in Appendix I and II of CITES in 2004 (CITES, 2004). Therefore, urgent need to improvise the conservation and production of agarwood trees, biological techniques are applied to protect from extinction, and hence to improve the economy. Therefore, the present review compiles and analyzes the existing research data on the propagation of *A. malaccensis*, and recommends further extensive and instant efforts to be employed to cultivate the plants using biotechnology.

Scientific Classification of Agarwood (Ibrahim *et al*, 2019)

Kingdom**:** Plantae
Phylum: Tracheophyta
Class: Angiosperm
Order: Malvales
Family: Thymelaeacea
Genus: *Aquilaria*
Species: *malaccensis*.

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Plate 1: *Aquilaria malaccenis* (A) Tree, (B) Flowers (C) Fruits, (D) Immature and mature seeds

Economical Values of Agarwood

The principal uses of Sashi are exceedingly demanded under three categories of products i.e., perfume products, incense sticks, and pharmaceuticals.

Perfume

Agarwood perfume has a unique smell obtained from fragrant essential oil and aromatic compound. Agarwood is existing as a scent for a thousand years, whereas modern perfumery began in the 19th century. Traditionally in the Middle East, agarwood smoke and oil are used as a scent (Chakrabarty, Kumar and Menon, 1994), and Minyak attar (water-based) is a distilled agarwood used by the Muslims on clothes (Yaacob, 1999). Agarwood fragrance is also being employed as an aromatic gradient in detergent, viz soap, and shampoo (Kadir *et al*., 1997). Several researchers tried to synthesize agarwood aromatic compounds by copying the chemical structure of the ordinary oil, but they could do it having low quality (Beek and Philips, 1999).

Incense sticks

Agarwood incense has an important role in prayers and religious rituals, or as an insect repellent. The aromatic compounds are the main chemical components in agarwood smoke and create an atmosphere of peace and serenity. It scents heavenly, woody nuance, balsamic and warm aura of bittersweet when the chromones break into low molecular weight at high temperature. In Taiwan, the agarwood stick is used in traditional festivals or ceremonies to bring safety and good luck to the believer. The agarwood incense stick is used in the bathroom as a customary sense, and in the paddy field to chase the local spirits by the Malay tribe in Malaysia (Chakrabarty, Kumar and Menon, 1994), and Puja celebration by the Hindus. The quality of Indian and Chinese agarwood incense sticks has to drop down, as reported the agarwood oil concentration is less or is replaced by synthetic oil (Chakrabarty, Kumar and Menon, 1994). The use of agarwood incense sticks has reduced these days because of the hike in the cost.

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Pharmaceutical use

Agarwood plays a vital role in the field of medicine, as it contains various chemical components, including alkaloids, terpenoids, phenolic acid, fatty acids, flavonoids, etc. having medicinal properties to include anti-cancer, anti-inflammatory, antioxidant, antibacterial, antifungal, antidiabetic, and other properties. Traditionally, agarwood is prescribed to treat pleurisy[1] by the Sahih Muslim (Islamic religious sect), to relieve from pain, and to arrest vomiting and asthma (Anon, 1995). The high-grade quality agarwood is utilized in Chinese drugs for treating various diseases and producing pharmaceutical tinctures (Yaacob, 1999; Beek and Philip, 1999). The uninfected wood is used for the treatment of jaundice and body pain (Chakrabarty, Kumar and Menon, 1994). *A. malaccensis* products are an essential source in the field of Ayurveda for treating various diseases while acting as appetizer, analgesic, antipyretic, antihistaminic, styptic, carminative, cytotoxic, insecticidal, general tonic, etc. (Sarma *et al*., 2015).

Propagation of *A. malaccensis*

Propagation is a technique to clone the species to ensure its availability in natural habits. The principal techniques of propagation are described as below.

Traditional method

The traditional practice of the techniques is supplemented based on the kind of the environment. Mass production of *Aquilaria* can be undertaken by grafting, stem cutting, and seedling (Figure 1) because of the short life span of the seed viability, low germination rate. The insect attack is the principal impediment to the propagation of plants and agarwood production. A seed viability test showed that heavier seeds having higher viability demonstrate greater germination rate and seedling growth rate compared to the lighter seeds (Shankar, 2012). The seed is recalcitrant, and its shelf life ranges from 15 to 40 days at room temperature (Shankar, 2012; Tabin and Srivastava, 2014). Therefore, propagation and conservation are needed urgently to avoid extinction (Saikia and Khan, 2013). To enhance the traditional method of stem cutting was developed by treating the injured stems with various concentrations of Indole Butyric Acid (IBA) (Figure 2) (Borpuzari and Kachari, 2018b). Cryopreservation is a recently developed method to understand its viability and reliable method for the long-term storage of *A. malaccensis* recalcitrant seeds (Devi, Kumaria and Das, 2019). Further studies can be done to understand the viability of the seed using the tetrazolium test[2], and using culture of the seeds in sterile soil to check the effect of growth hormones.

Biotechnological technique to improve the traditional method

The applied technique is cost-effective and helps maintain/conserve the endangered species and the commercial lucrative enterprise based on it. It is a rapid, aseptic, and environmentally controlled technique to improve plant growth, germplasm conservation, and secondary metabolite production. Therefore, this technique meets the demand of *A. malaccensis* conservation, propagation, and agarwood production. The Sashi trees meet an extensive economic demand for both national and international trade. Hence, to uplift the economy and maintain its availability, cloning is urgently needed. Saikia, Shrivastava and Singh (2012) experimented and produced an efficient callus from the leaf tissues of *A. malaccensis* in Murashige and Skoog (MS) medium[3] supplemented with 6-Benzylaminopurine (BAP) 0.5 mg/l + Naphthalene acetic acid

[1] Pleurisy is inflammation of the tissues that line the lungs and chest cavity.
[2] Tetrazolium test is a rapid test method to evaluate the seed viability. The living cells stain red in color, dark red color indicates deteriorate cells and no color indicates death cells.
[3] MS medium is an artificial medium used in the laboratory for culturing's all types of plant species.

(NAA) 3mg/l and 4% of sucrose, after 45-60 days of incubation. Saikia *et al.*, (2013) also reported that the MS medium is more appropriate for callus induction and maintenance rather than Woody Plant Medium (WPM)[4] supplemented with different concentrations of 2, 4-dichlorophenoxyacetic acid kinetin. According to Jayaraman, Hazwani-Daud and Mohamed (2014), the auxin hormones (NAA at 1.1 µM) produce a compact callus, whereas the combination of auxin (NAA 1.1 µM), cytokinin (BAP 2.2 µM), and sucrose 15 g/l at pH 5.7 produces friable callus with the highest biomass rate. Salam, Awal and Abdullah (2019) obtained a high rate of embryogenic callus from leaves of *A. malaccenis* and *A. subintegra* that was observed in the MS medium having 2.0 mg/l BAP and 0.5 mg/l 2, 4-D.

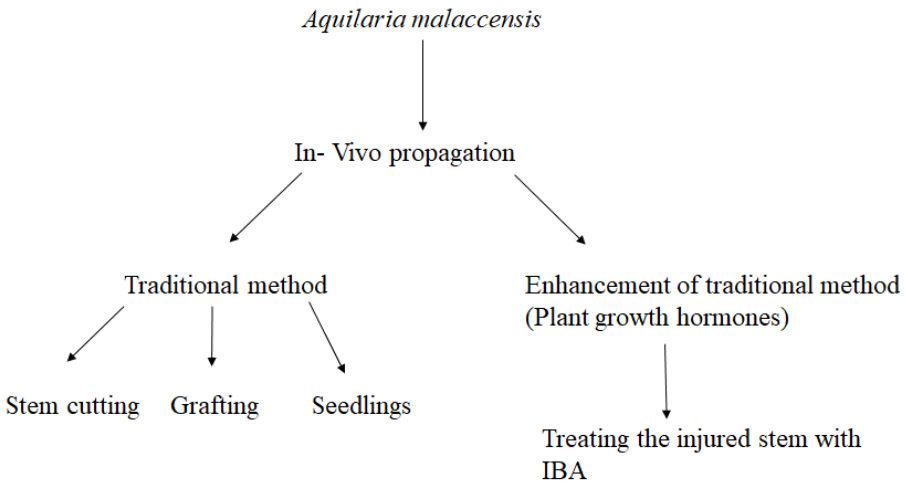

Figure 1: Schematic diagram of *A. malaccensis* propagation using traditional methods and enhancement of traditional methods by using plant growth regulators.

Noordin *et al.* (2010) reported an optimal shoot production obtained from shoot tip and lateral bud in modified MS media containing 0.5 mg/l BAP and 0.25 mg/l TDZ (Thidizuron), and roots development was observed in a ½ strength MS supplemented with IBA 1 mg/l. Direct organogenesis (Figure 3) was determined from the leaf at a high concentration of cytokinin (BAP 2 mg/l) and a low concentration of auxin (0.1 mg/l NAA) root development was observed best in a ½ strength MS medium supplemented with 1 mg/l NAA (Saikia and Shrivastava, 2015). Esyanti *et al.* (2019) developed a method to improve shoot multiplication using a bubble column reactor and the immersion time on the temporary immersion system (TIS)-RITA. The shoot was first cultured, propagated, and maintained in MS medium before performing the bioreactor cultivation. They observed that the immersion gives a better insight compared to the bubble column reactor for *A. malaccensis* shoot propagation. The finest shoot regeneration was procured from the stem with one node in MS medium containing 0.5 mg/l BAP, 0.5 mg/l NAA and 20 mg/l glutamine without callus production. The roots are well developed in media obtaining 1.5 mg/l of IBA (Figure 3) (Borpuzari and Kachari, 2018a).

Synthetic seed is a new applicable method in the field of biotechnology for seed storage of *Aquilaria* spp. and other plants. Devi *et al.* (2018) reported that an artificial seed of *A. malaccenis* was synthesized using nodal bud as the explants. The optimum regeneration was obtained at 2.5% sodium alginate and 100 mM calcium chloride, about 83.3% and 75.0% from encapsulated bud stored at 4°C and 23 ± 2°C, respectively,

[4] WP medium is the formulation of this medium is for culturing woody plant species.

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for 10 days. Storage was possible for 60 days at 4°C and 50 days at 23 ± 2°C with an average regeneration rate of 8.3% and 16.7%, respectively (Figure 2).

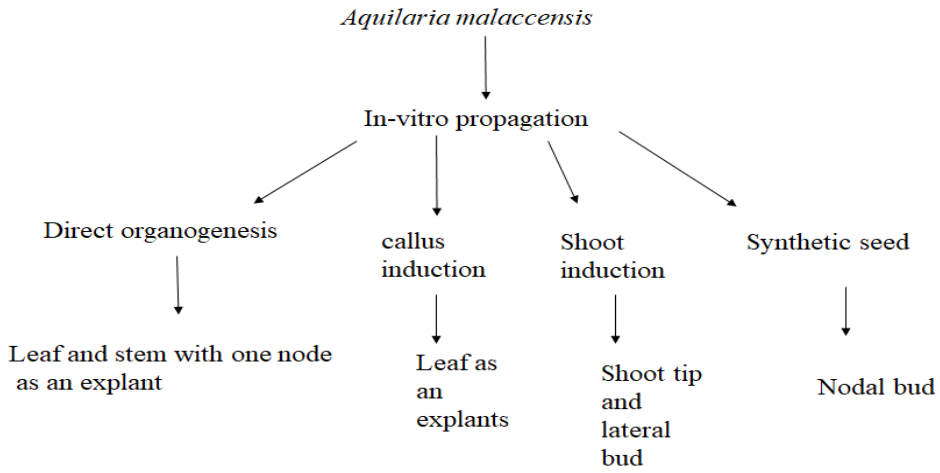

Figure 2: Schematic diagram of *A. malaccensis* propagation using the biotechnological technique for organogenesis, callus induction, shoot induction, and synthetic seed production using various explants.

Secondary metabolite production

A. malaccensis is an attractive species for the production of aromatic metabolites rather than biologically active compounds. The callus induction technique assists in the production of the unique secondary compound by modifying the culturing media and understanding the plant and insect or microbes' interaction of *Aquilaria* species. Okudera and Ito (2009) observed the production of the two major compounds of *Aquilaria* spp., sesquiterpenoids and chromone. The study shows that sesquiterpenoids are produced in living cells and chromones might generate from the debris of dead cells. Siah, Parameswari and Mohamed (2016) reveal that the genes obtained from senescence callus actuate the identical response in the form of aromatic compound synthesis and defense response pathways, which are essential pathways to understand the compound formation. Sen *et al*. (2017) conducted a study to enhance the mechanism of plant and microbes interacting on three levels, viz., callus, juvenile plants, and resinous wood chips infected by *Fusarium*. They observed the callus-fungus communication, producing essential aromatic compounds pentatriacontane {fold change (log2FC) =3.47)}, 17-pentatriacontene (log2FC=2.95), tetradecane, 2-methyl- (log2FC=1.10). The fungal interaction in juvenile plants and resinous wood chips signal the development of terpenoid precursors (e.g., farnesol, geranylgeraniol acetate) and agarwood sesquiterpenes (e.g., agarospirol, γ-eudesmol). The discovery of the unknown aromatic compound to agarwood is a spotlight for further research. Hamdan *et al*. (2020) reported an innovative method to study the embryogenic formation of callus by using SEM (Scan Electron Microscope), and the genes (SERK, BBM, LEC1, and WOX) associated with somatic embryogenesis were studied and the data were used for primer designing. Gene amplification is a radical step for genetic conservation associated to somatic embryogenesis research.

Conclusion

The current review on propagation summarizes the traditional method and new *in-vitro* based studies. The *in-vitro* techniques determine an alternative source for agarwood production from mast cell/callus of *Aquilaria* species. Although the callus induction using leaf, nodal, and shoot explants are reported by

various researchers, other parts from the vegetative and reproductive phases might be the best explants. No work has been reported so far for the production of plantlets from the callus. Further research is highly recommended to improve the *in vitro* and *in vivo* methods for the mass production of callus to meet the demands of agarwood in world trade and plantlet production to meet the demand of nature as well. As reported by various researchers, callus impersonates a significant character in the production of unique secondary metabolites. It is also important to study the communication in plant and microbe relationship, as it is more efficient and more specific in contrast to direct tree artificial infection. For further studies, a comparative study could be done for the isolation, identification, and classification of microorganisms and compounds obtained between post and pre-artificial infection on the tree and callus, which might give a better understanding of various microbes and plant.

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Authors' Declarations and Essential Ethical Compliances

Authors' Contributions (in accordance with ICMJE criteria for authorship)

Contribution	Author 1	Author 2
Conceived and designed the research or analysis	Yes	Yes
Collected the data	Yes	No
Contributed to data analysis & interpretation	Yes	Yes
Wrote the article/paper	Yes	No
Critical revision of the article/paper	Yes	Yes
Editing of the article/paper	Yes	No
Supervision	No	Yes
Project Administration	No	No
Funding Acquisition	No	No
Overall Contribution Proportion (%)	70	30

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Research involving human bodies (Helsinki Declaration)
Has this research used human subjects for experimentation? No

Research involving animals (ARRIVE Checklist)
Has this research involved animal subjects for experimentation? No

Research involving Plants
During the research, the authors followed the principles of the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. Yes

Research on Indigenous Peoples and/or Traditional Knowledge
Has this research involved Indigenous Peoples as participants or respondents? No

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)
Have authors complied with PRISMA standards? Yes

Competing Interests/Conflict of Interest
Authors have no competing financial, professional, or personal interests from other parties or in publishing this manuscript.

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