A review on *Aquilaria malaccensis* propagation and production of secondary metabolite from callus.

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Abstract

Aquilaria malaccensis is an evergreen non-timber wood of the fifteen species of *Aquilaria* and family *Thymelecea* that yield agarwood. There are two species endemic to northeast India *A. malaccensis* and *A. khasiana*. *A. malaccensis* generate a high-grade degree of resin as correlated to the other *Aquilaria* species, and it gives a high economy to the Northeast state of India and the country as a whole since they are fairly valuable. Due to its profoundly valuable sources, it became overexploited, and it impacted their availability in their genetic environments. Since the cultivation of the tree throughout the year becomes challenging due to some environmental factors like the sensitivity of the seeds to desiccation, high light intensity, low shelf life, slow growth rate, and 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 secondary metabolite from callus of *A. malaccensis* and updates information on its developments and approaches that have been rapidly taking place in recent years so that further novel research can be envisaged.

Keywords: *Aquilaria malaccensis*, *Aquilaria Khasiana*, Northeast, conservation, seed desiccation, secondary metabolite production.

Introduction

Aquilaria malaccenis is one of the precarious non-timber wood of the fifteen species, of genera *Gyrinorops* and *Aquilaria*, and belongs 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 religious practices. It is a great scented medicinal and fragrance tree of South East Asia and is mostly grown in the evergreen rainforest. It is a dominant species, which is chiefly distributed over several countries, India, Bangladesh, Malaysia, Myanmar, China, Singapore, Bhutan, Vietnam, Indonesia, and Thailand (Oldfield & Mackinven 1998). In India, there are three endemic species viz *A. khasiana* Hallier, *A. macrophylla* Miq. and *A. malaccenis*. *A. khasiana* is ascertained only in the East Khasi Hills of Meghalaya and *A. macrophylla* found only in Nicobar Islands. *A. malaccensis*, naturally grow at an altitude of 1000 meters above the sea level in the foothills of Assam, Meghalaya, Manipur, Nagaland, Mizoram, Tripura, Arunachal Pradesh, and West Bengal (Borpuzari and Kachari 2018). 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 & Khan 2011). Sashi farming was initiated in the '70s in Assam followed by Thailand and Cambodia in the '80s (Elias et al, 2017). Development of new leaf and branches occurs during the pre-monsoon (March-April) persist up to an actual monsoon season (July-August). *A. malaccensis* give flowers at the onset of monsoon (April-June) and gives fruits for the following months till September (Figure 1). The actual reproductive phase of the plant is confined 5 months only (April to September) which is facilitated by monsoon. *A. malaccensis* organs development is significantly effect by temperature and rainfall (Borogayary et al. 2018). Therefore, it favors a mean annual rainfall of 1500 to 6500 mm, utmost temperature from 22^{o}C – 28^{o}C and minimum temperature from 14^{o}C-21^{o}C (Baniwal, 1989). The height of the tree is 18-20m and 1.5-2m in diameter. *Aquilaria* species are well acclimatized in diverse habitats including rocky, sandy, well-drained slopes, ridges, and swamp (Akter & Neelam 2008).

The tree produces a unique fragrance oil and compound, which causes the demand in the international trade of cosmetic, pharmaceutical, religious practice, and perfume production. It causes a great impact on the loss of biodiversity and lessens their availability in the environmental atmosphere. Therefore for its mass production, the tree became over-exploited and put it on the verge of extinction. All the *Aqualaria* species that produce agarwood are 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 improve the economy. Therefore, the present review compiles and analyze the existing research data on the propagation of *A. malaccensis* and gives recommendation for further extensive and instant effort in progress of the cultivation through the tools of biotechnology.

Scientific Classification

Kingdom**:** Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade Eudicots

Clade: Rosids

Order: Malvales

Family: Thymelaeacea

Genus: *Aquilaria*

Species: *A. malaccensis*

Figure 1: *Aquilaria malaccenis* (A) Tree (B) Flower (C) Fruits (D) Immature and matured seeds

Economical value

The principal uses of Sashi are exceedingly demanding in three categories i.e. perfume production, incense stick, and pharmaceutical.

Perfume

Agarwood perfume has a unique smell obtained from fragrance essential oil and aromatic compound. Agarwood is exerting as the scent a thousand years back where modern perfumery began in the 19th century. Traditionally in the Middle East, agarwood smoke and oil are used as a scent (Chakrabarty et al. 1994), and Minyak attar (water-based) is a distilled agarwood oil use by the Muslims to prayer clothes (Yacoob 1999). Agarwood fragrance is also being employed as an aromatic gradient in detergent, viz soap, and shampoo (Kadir et al. 1997). Several researchers were synthesizing agarwood aromatic compounds to match with the chemical structure of the ordinary oil but in low quality (Beek and Philips 1999).

Incense stick

Agarwood incense has a magnificent account in prayers and religious ritual purposes 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 scent 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, during Ramdan prayer by the Muslim, in the paddy field to chase the local spirits by the Malay tribe in Malaysia (Chakrabarty et al. 1994), and Puja celebration by the Hindu and other religious practice.

The quality of Indian and Chinese agarwood incense stick has to drop down, as reported the agarwood oil concentration is less or they might have replaced with synthetic oil (Chakrabarty et al. 1994). The use of agarwood agarbattis is sporadical because of the hike in the cost.

Pharmaceutical use

Agarwood plays a vital role in the field of medicine, contains various chemical components, including alkaloids, terpenoids, phenolic acid, fatty acids, flavonoids, etc. is a trace of its medicinal property include anticancer, anti-inflammatory, antioxidant, antibacterial, antifungal, antidiabetic, and other activities. Traditionally agarwood is prescribed to treat pleurisy by the Sahih Muslim, relieve pain, arrest vomiting, and asthma (Anon, 1995). According to the research quality of agarwood play a great role in term of medicinal properties; the high-grade quality is authorized in Chinese drug for the treatment of various diseases and production of pharmaceutical tinctures (Yacoob 1999, Beek and Philip 1999), uninfected wood use for the treatment of jaundice and body pain (Chakrabarty 1994). *A. malaccensis* products are an essential source in the field of Ayurveda for treating various diseases such 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 meet there availability in their natural habit. The principle techniques are illustrated below.

Traditional method

Traditional practice the ancient techniques, supplemented based on the role of the environment played. Mass production of *Aquilaria* can produce by grafting, stem cutting, and seedling due to the short life span of the seed viability, low germination delayed rooting, and insect attacked is the principal impediment for the propagation and agarwood production. A prediction test on seed weight was observed the heavier seeds have higher supremacy for a great germination and seedling growth in contrast to the lighter seeds (Shankar 2012). The seed is recalcitrant and its shelf life ranging from 15 to 40 days at room temperature (Shankar 2012, Tabin and Srivastava 2014). Therefore, urgent need for propagation and conservation are needed to avoid the stage of extinction (Saikia and Khan 2013). To enhance the traditional method stem cutting was developed by treating the injured stems with various concentrations of Indole Butyric Acid (IBA) for rooting development (Borpuzari & Kachari 2018). Cryopreservation is a recently developed method to understand its viability and reliable method for the long-term storage of *A. malaccensis* recalcitrant seed (Devi et al. 2019). Further studies can be done to understand the viability of the seed using the tetrazolium test, culture the seed in sterile soil to check the effect of growth hormones.

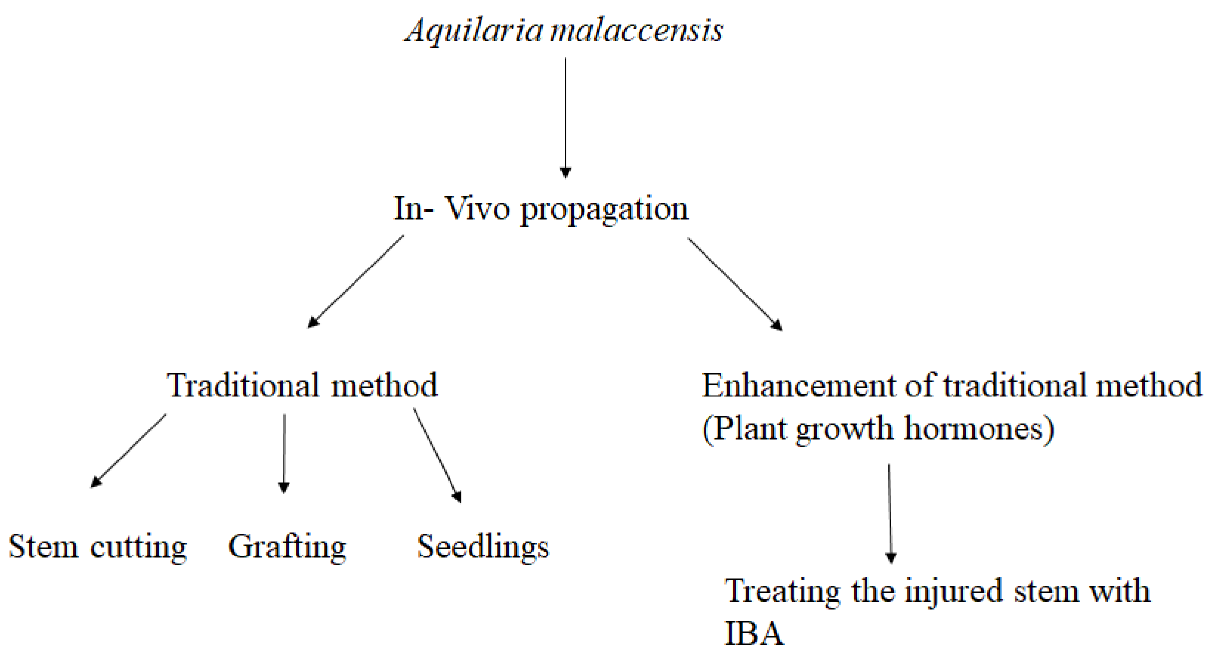

Figure 2: Schematic diagram of *A. malaccensis* propagation using traditional methods and enhancement of traditional methods by using plant growth regulators.

Biotechnological technique to improve traditional method

The applied technique is cost-effective and helps to maintain, conserved the endangered species and commercial lucrative enterprise. It is a rapid, aseptic, and under control environmental status to improve plant growth, germplasm conservation, and secondary metabolite production. Therefore these tools meet the demand of *A. malaccensis* conservation, propagation, and agarwood production of human needs in a different aspect. The Sashi trees are meeting an extensive demand for both national and international trade. Therefore to uplift the economy and maintain its availability in the environment, cloning is urgently needed. Saikia *et al.* (2012), experimented and produced an efficient callus from the leaf tissues of *A. malaccensis* in murashige and Skoog (MS) medium supplemented with BAP (0.5mg/l) + NAA (3mg/l) and 4% of sucrose, after 45-60 days of incubation. In 2013 Saikia and his co-worker also reported MS medium is more appropriate for callus induction and maintenance rather than Woody Plant Medium (WPM) supplemented with different concentration of 2,4-dichlorophenoxyacetic acid + kinetin. According to Jayaraman et al. (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 and Abdullah (2019) obtained a high rate of embryogenic callus through leaves of *A. malaccenis* and *A. subintegra* was observed in MS medium with 2.0 mg/l BAP and 0.5 mg/l 2, 4-D.

Sabdin *et al.* (2011) 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 and roots development observed in a ½ strength MS supplementing with IBA 1mg/l. Direct organogenesis was determined from the leaf at a high concentration of cytokinin (BAP 2mg/l) and a low concentration of auxin (0.1 mg/l NAA) were as root development was observed best in a ½ strength MS medium supplemented with 1 mg/l NAA (Saikia & Shrivastava 2015). Esyanti et al, (2019) developed a method to improve shoot multiplication using bubble column reactor and the immersion time on the temporary immersion system (TIS)-RITA. The shoot culturing was first cultured, propagate, and maintain 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 + 0.5mg/l NAA and 20mg/l glutamine without callus production and the roots are well developed in media obtaining 1.5mg/l of IBA (Borpuzari and Kachari (2018).

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 synthesize 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 4o C 23±2oC 23 ± 2°C respectively, 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.

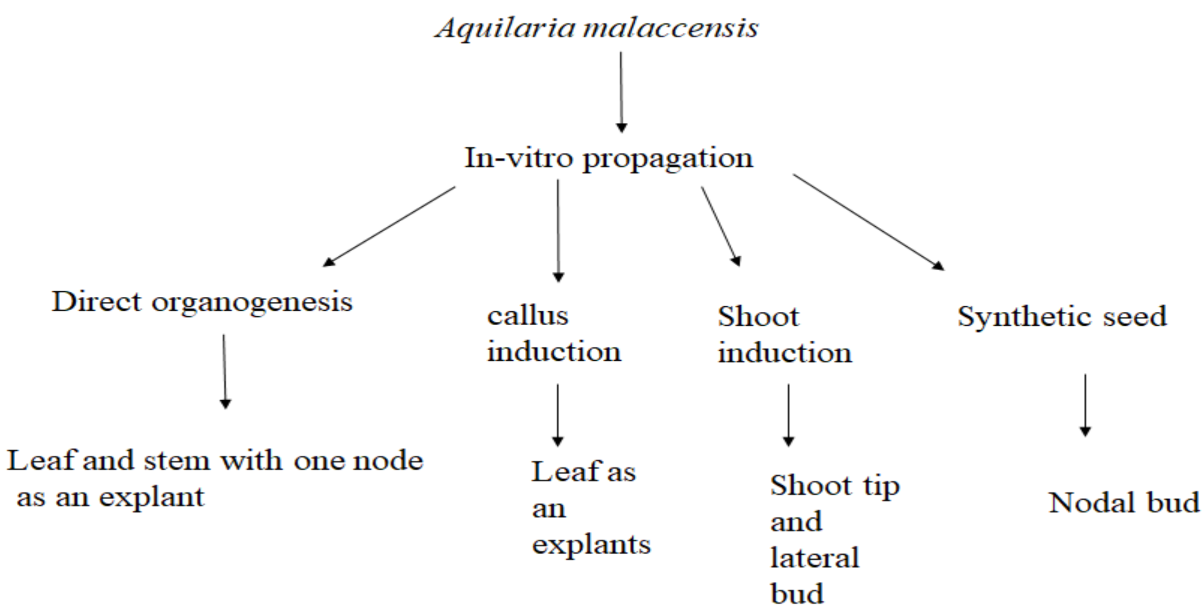

Figure 3: Schematic diagram of *A. malaccensis* propagation using biotechnological technique for organogenesis, callus induction, shoot induction and synthetic seed production using various explant.

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 et al. (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 et al. (2016) reveal that the genes obtain from senescence callus actuate the identical response as infected agarwood in 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 interaction on three categories, viz. callus, juvenile plants, and resinous wood chips infected with *Fusarium*. They observed the callus-fungus communication, produces an 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 and gene amplification which is a radical step for genetic conservation associated to somatic embryogenesis research.

Future Prospects

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 mass 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 phase are also 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, in vivo* method for the mass production of callus to meet the demand 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 and which is also a turning point to study the communication of plants and microbes 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 plants which is not clear till date.

References

1. AKTER N AND NEELIM AZ. 2008 Agarwood Plantation at BRAC Tea Estate: Introduction, Environmental Factors and Financial Analysis. BRAC Research Report.
2. BEEK HVH, & PHILLIPS D. 1999. Agarwood: Trade and CITES Implementation in Southeast Asia. Unpublished report prepared for TRAFFIC Southeast Asia, Malaysia.
3. BENIWAL BS. 1989. Silvical characteristics of *Aquilaria agallocha* Roxb. Indian Forester 115: 17–21.
4. BOROGAYARY B, DAS AK, & NATH AJ. 2018. Vegetative and reproductive phenology of *Aquilaria malaccensis* Lam. (Agarwood) in Cachar District, Assam, India. Journal of Threatened Taxa. 10(8): 12064–12072; http://doi.org/10.11609/jott.3825.10.8.12064-12072.

5. BORPUZARI PP, KACHARI J. 2018. Effect of glutamine for high frequency In-vitro regeneration of *Aquilaria malaccensis* Lam. through nodal culture. Journal of medicinal Plants Studies. 6(2): 9-16.
6. BORPUZARI PP, KACHARI J. 2018. Roots stimulation of selected genotypes of *Aquilaria malaccensis* Lamk., through Indole-butyric acid (IBA): A most economically important species of northeastern region. International Journal of Botany Studies. 3(2): 16-20.
7. CITES. 2004. Amendments to appendices I and II of CITES. In: Proceedings of Thirteenth Meeting of the Conference of the Parties, Bangkok, Thailand.
8. DEVI SD, KHARSAHNOH B, KUMARIA S, & DAS MC. 2018. Artificial seed for short-term storage: using nodal buds in *Aquilaria malaccensis* Lam. Current science. 115(11): 2103-2109.
9. DEVI SD, KUMARIA S, & DAS MC. 2019. Development of cryopreservation protocol for aquilaria malaccensis lam., a recalcitrant seeded tropical tree species. CryoLetters . 40 (1): 18-27.
10. ELIAS MF, IBRAHIM H & MAHAMOD WRW. 2017. A Review on the Malaysian *Aquilaria* species in Karas Plantation and Agarwood Production. International Journal of Academic Research in Business and Social Science. 7(4): 1021-1029.
11. ESYANTI RR, FADHOLI M, RIZKI RM, & FAIZAL A. 2019. Shoot multiplication and growth rates of *Aquilaria malaccensis* lamk. shoot cultures in temporary immersion system (tis)-rita® and bubble column bioreactors. Pak. J. Bot., 51(4): 1317-1321. DOI: http://dx.doi.org/10.30848/PJB2019-4(36).

12. HAMDAN MSM, AMINAN AW, SHAARANI SHN, & TAJUDDIN SN. 2020. Characterization of *Aquilaria malaccensis* callus cells using SEM and somatic embryogenesis associated genes identification. IOP Conf. Series: Earth and Environmental Science 549: 012070. doi:10.1088/1755-1315/549/1/012070.
13. JAYARAMAN S, HAZWANI-DAUD N, & MOHAMED RHR. 2014. Effects of plant growth regulators, carbon sources and pH values on callus induction in *Aquilaria malaccensis* leaf explants and characteristics of the resultant calli. Journal of Forestry Research. 25(3): 535−540. doi:10.1088/1755-1315/549/1/012070.
14. LATA A. 2007 . Investigation of seed longevity and viability and cutting propagation for *Aquilaria crassna*. PGDip Thesis, James Cook University.
15. NOORDIN N, IBRAHIM I, KHALID N, & ABD. RAHMAN N. 2010. Establishment of *Aquilaria malaccensis* Callus, Cell Suspension and Adventitious Root Systems. Applied life science. 43(17).
16. OKUDERA Y, & ITO M. 2009. Production of agarwood fragrant constituents in *Aquilaria* calli and cell suspension cultures. Plant Biotechnology. 25(3): 307-315. https:// doi.org/F10.5511/plantbiotechnology.26.307.
17. OLDFIELD S, LUSTY C. & MACKINVEN A. 1998. The Word List of Threatened Trees, World Conservation Press, Cambridge, UK. 650pp.
18. SAIKIA M & SHRIVASTAVA K. 2015. Direct shoot organogenesis from leaf explants of *Aquilaria malaccensis* Lam. Indian Journal of Research in Pharmacy and Biotechnology. 3(2): 164-170.

19. SAIKIA M, SHRIVASTAVA K, & SINGH SS. 2012. An Efficient Protocol for Callus Induction in *Aquilaria malaccensis* Lam. Using Leaf Explants at Varied Concentrations of Sucrose. International Journal of Plant Research. 2(6): 188-194.
20. SAIKIA M, SHRIVASTAVA K, & SINGH SS. 2013. Effect of culture media and growth hormones on callus induction in *Aquilaria malaccensis* Lam., medicinally and commercially important trees species of North East India. Asian Journal of Biological Sciences. 6(2): 96-105.
21. SAIKIA P & KHAN M L. 2011. "Agar (*Aquilaria malaccensis* Lam.): a promising crop in the home gardens of Upper Assam, Northeastern India," Journal of Tropical Agriculture. 5: 8–14.
22. SAIKIA P & KHAN ML. 2013. Population structure and regeneration status of Aquilaria malaccensis Lam. in home gardens of Upper Assam, northeast India. Tropical Ecology. 54(1): 1-13.
23. SALAM N, AWAL A, & ABDULLAH S. 2019. Embryogenic Callus Induction of *Aquilaira Malaccensis* Lam. and *Aquilaria Subintegra* Ding Hou. International Journal of Engineering and Advanced Technology. 9(1): 5746-5751.
24. SARMA DR, SARMAH J, GUPTA A, & MISHRA RK. 2015. *Aquilaria malaccensis*, an ayurvedic medicinal herb found in assam – its therapeutical and pharmacological aspect. Indian Journal of Tropical Biodiversity. 23(2): 218-222.
25. SEN S, DEHINGIA M, TALUKDAR NC, & KHAN M. 2017. Chemometric analysis reveals links in the formation of fragrant bio-molecules during agarwood (*Aquilaria malaccensis*) and fungal interactions. Scientific Report. 1-14. https://doi.org/10.1038/srep44406.

26. SHANKAR U. 2012. Effect of seed abortion and seed storage on germination and seedling growth in *Aquilaria malaccensis* Lamk. (*Thymelaeaceae*). 102(4): 596-604.

27. SIAH CH, PARAMESWARI, & MOHAMED R. 2014. Transcriptome reveals senescing callus tissue of *Aquilaria malaccensis*, an endangered tropical tree, triggers similar response as wounding with respect to terpenoid biosynthesis Journal of Forestry Research. 25(3): 535−540. https://doi.org/ 10.1007/s11676-014-0492-8.

28. TABIN T, & SRIVASTAVA K. 2014. Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (Thymelaeaceae). Asian Journal of Plant Science and Research. 4(6):41-46.

29. YAACOB, S. (1999). Agarwood: Trade and CITES Implementation in Malaysia. Unpublished report prepared for TRAFFIC Southeast Asia, Malaysia.

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Conflict of interest

No conflict of interest was reported by the authors.