Original Research Article

Pre-treatment enhances the germination and early growth of *Aquilaria agallocha* Lam. (Agarwood)

Abstracts:

A germination test of *Aquilaria agallocha* seeds was conducted in the uncontrolled nursery conditions to evaluate the effect of pre-sowing treatments. Seeds of agarwood species with recalcitrant seed character which means seeds are difficult to store for long periods and lose its viability in a short time. The collected seeds were subjected to six different pre-sowing treatments. Among the treatments Soaking in water at room temperature for 24 hrs was found to be the best treatments for germination patterns as well as growth attributes. The result of analysis of variance (ANOVA) showed significant differences among the different treatments.

Keywords: Agarwood, nursery, seed, germination, growth

Introduction

Agarwood *(Aquilaria agallocha*), belongs to family Thymelaeaceae is a valuable plant endemic to North- East India, also 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 (Saikia and Khan, 2015). *A. agallocha* 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). Agarwood) is a threatened forest species of south-east Asia and heavily exploited for its aromatic resin-infused wood (agarwood) (Chung and Purawaningsih, 1999). The species is included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2003). The species is also listed as „Vulnerable" globally, „Critically Endangered" in India (IUCN 2009). There are 27 *Aquilaria* species distributed worldwide, of which 24 are naturally found in 12 south-east Asian countries (Zich and Compton, 2001). India is the home of three *Aquilaria* species and *Aquilaria agallocha* is considered endemic to north-east India (Kanjilal et al., 1982). 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. *Aquilaria* is presently being cultivated on commercial scale

UNDER PEER REVIEW

Comment [D1]:
(Aquilaria agallocha seeds were tested for germination under uncontrolled nursery conditions.)

Comment [D2]: Is it 1999 as ref. or 2009?

Comment [D3]: Three *Aquilaria* species are native to India, *Aquilaria agallocha* is considered endemic to north-east India

Formatted: Highlight

Comment [D4]: For prevent repetition we can say
(A. khasiana is found only in the East Khasi Hills of Meghalaya, whereas the Nicobar Islands are the only known locality for A. macrophylla)

mainly in upper Assam region, but even favorable topology, climatic conditions and soil types in Arunachal Pradesh.

It is an economically important native tree species of sub-tropical-tropical rainforests of northeast India. 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. The attractive aroma of agarwood, which receives high ritual and social significance in Asian, Indian, and Middle Eastern cultures, is released once the piece of heartwood is gently burnt (Alwis et al., 2019). Agarwood is widely used as therapeutic perfumes, traditional medicine, and aromatic food ingredient and for religious purposes (Liu et al., 2013). Due to high medicinal and perfumery value, the species has great demand in national and international market hence the attempts are now being made to cultivate the species in plantations in India and other places around the world. Currently, the species is mainly surviving in plantations, home gardens and along tea plantations in Assam and its adjoining areas of northeast India and Bangladesh and significantly contributing to the local economy of the region (Saikia and Khan, 2014). The people's understanding about valuable and precious agar wood has led to its heavy extraction from natural forest in recent years. This has put the natural existence of the species under tremendous pressure. Presently, the species has become the focus of increasing conservation concern and is included in the prioritized list for national recovery programme in India (Shankar, 2012). In addition to utilization pressure, *Aquilaria* tree faces limitations from important ecological factors namely, light availability, seed viability period and insect attack both under nursery and field conditions. Being a tremendously important tree species, information is required on the growth and survival of the species in response to different environmental factors in order to manage it sustainably.

Aquilaria agallocha is a mid-canopy tree and propagated readily through seeds. in fact, is the most reliable method of propagation. However, the seeds of Agarwood show low natural germination, which might be due to rapid decline of viability after shedding from the trees. Seeds are produced once in a year during the monsoon season i.e. during June to August. Its seeds have a short shelf life at normal temperature. In contrast, low reproductive potential of *Aquilaria* seeds have been reported due to variable, slow and short germination rate. Plant growth regulators (PGRs) have been widely employed in enhancing seed germination of many crops (Unal 2013; Han and Yan 2015; Vishal and Kumar 2018). External application of PGRs to seeds could enhance seed germination and seedling

Comment [D5]: ,

Comment [D6]: ,

Comment [D7]: I prefer this sentence

Aquilaria agallocha is a mid-canopy tree propagated through seeds, it is the most reliable method of propagation.

Comment [D8]: Agarwood seeds are produced once a year during the monsoon season (i.e. during June to August), low natural germination of Agarwood seeds has been observed, which may be related to a quick reduction in viability upon shedding from the trees.

UNDER PEER REVIEW

establishment of many aromatic and medicinal plants (Ali et al., 2010; Gholami et al., 2013; Singh et al., 2014). The population in the natural habitat is disappearing and further, plantations are raised with unknown seed sources, leading to inbreeding. If continued at the same level as now low levels of genetic diversity within species can lead to inbreeding depression which affects growth, survival and adaptation (Kjaer, 1997) leading to genetic loss. Overexploitation lessens its availability in the natural habitat and also has a great impact on the biodiversity. Hence, for mass production of scent, the tree is overexploited and put on the verge of extinction. 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.

The objective of the current study is to identify the most effective pre-sowing treatment strategies for agarwood (*Aquilaria agallocha*) seed germination and Seedling growth.

Materials and Methods

The study was conducted in the Department of Forestry and Environmental Science, Manipur University, 30°51' N and 76°11' E at an altitude of 1100-1275 m above mean sea level. The climate of the University area ranges from sub-tropical to sub-temperate and experience a precipitation of 1000-1300 mm annually. The major part of rain is received during July and August (Monsoon period) months.

Collection of seed

Fresh and healthy matured seeds of *A. agallocha* were collected obtained from Forest Department, Government of Manipur. Thereafter, pure seeds were then placed in paper packaging after diseased and damaged seeds were removed so that a seed germination experiment was carried out at the experimental plot at the Department of Forestry and Environmental Science, Manipur University.

~~pure seeds after removing diseased and damaged seeds were put in containers in the laboratory of department of Department of Forestry and Environmental Science, Manipur University for undertaking germination test.~~

~~The present investigation was carried out at the experimental plot at the Department of Department of Forestry and Environmental Science, Manipur University~~. The experiment was laid out in two factorial randomized block design (RBD) with six different pre-sowing treatments including ~~with~~ a control which no transactions were made on the seeds [$\mathbf{T_1}$

Comment [D9]:
I think that the design of this experiment is a simple experiment, because the statistical analysis in the tables is an analysis of a simple one-factor experiment

Plz review and correct

UNDER PEER REVIEW

= Control, **T_2** = Soaking in water at room temperature for 12 hrs, **T_3** = Soaking in water at room temperature for 24 hrs, **T_4** = Soaking in water at room temperature for 48 hrs, **T_5** = Immersed at hot water (100^0C) for 1 minute and **T_6** = Immersed at hot water (100^0C) for 3 minute] with four replicates. Hundred seeds per replication were sown in nursery beds. After sowing, till the germination started light irrigation was provided in the morning and evening every day. Observations on seed germination ~~germination~~ parameters were recorded daily until completion, i.e.one month after the last seed had germinated. Seeds with a protruding radicle of about 2 mm were considered as germinated. Seedling growth was assessed in terms of shoot length, root length, seedling height and seed vigour index

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using SAS Package version 9.1. Percentage values were arcsine transformed as appropriate before subjecting them to ANOVA. The Least Significant Difference Test (LSD) at a probability of 5 % was used to compare means.

Result and discussions

The fruits of *Aquilaria* desiccate very fast (Shankar, 2012) and may contain ~~bear~~ one or two seeds per fruit. Seed germination percentage is an important trait for growers and foresters. Overall, the seed germination percent ranged from 55.25 % to 24.25 % among the different treatments adopted. The highest germination (55.25 %) was produced by the T_3, viz. soaking in water at room temperature for 24 hrs which was followed by 43.00% germination of T_4 (soaking in water at room temperature for 48 hrs. The percentage of seeds germination capacity of all the studied different treatments has ranged between 57.75 % and 26.75%. The present study revealed that T_3 recorded highest germination capacity (57.75 %) while lowest seed germination capacity was recorded from T_1 (26.75 %). For the study of germination energy across different pre sowing treatments, germination energy was found to have significantly differences among the different treatments. The germination energy for the different pre-sowing treatments, ranged between 22.25 % (T_3) and 11.00 % (T_1). The peak value of different pre sowing treatments was found to be significantly difference with ranged from 2.48 – 1.17, T_3 significantly exhibits highest peak value (2.48) while lowest recorded from T_1 (1.17). Mean daily germination was ranged from 2.63 – 1.16, T_3 observed highest (2.63) while T_1 observed lowest (1.16). The germination value for different pre sowing treatments was observed significantly differences among all the treatments. In the present

Comment [D10]: It is better to start with the lowest percentage number and then the biggest, and not vice versa

Comment [D11]: Same opinion as before

UNDER PEER REVIEW

study, germination value was observed ranged from 6.76-1.39. T_3 observed highest germination value (6.76) and lowest was observed in small size seed (1.39).

Table 1: Effect of pre-sowing treatments on seed germination of *A. agallocha*

Treatment	Germination Percent	Germination Capacity	Germination Energy	Peak Value	Mean Daily Germination	Germination Value
T_1	24.25^{d}	26.75^{d}	11.00^{b}	1.17^{b}	1.16^{d}	1.39^{b}
T_2	42.25^{b}	44.75^{b}	16.75^{b}	1.73^{b}	2.01^{b}	3.51^{b}
T_3	55.25^{a}	57.75^{a}	22.25^{a}	2.48^{a}	2.63^{a}	6.76^{b}
T_4	43.00^{b}	45.00^{b}	16.75^{b}	1.82^{b}	2.05^{b}	3.75^{b}
T_5	35.75^{bc}	38.25^{bc}	13.75^{b}	1.46^{b}	1.70^{bc}	2.54^{b}
T_6	28.00^{cd}	30.50^{cd}	13.75^{b}	1.49^{b}	1.33^{cd}	2.00^{b}

UNDER PEER REVIEW

Some forest seed have hard and impermeable seed coat, which restricts germination. Therefore, it is very important to apply the most suitable pre sowing treatment to push seeds to germinate. Different approaches of breaking seed dormancy to enhance germination process have been reported by many authors (Azad et al., 2011). The main reason behind the successful germination of T_3 is that seed of *A. agallocha* might require to break the dormancy and after that soaking in normal water make the seed coat more softly that ensure the maximum, and successful germination of seed. Soaking seeds in water may have soften hard seed coats, this makes the seed coats permeable to water and soaking seeds in water leaches out chemical inhibitors, resulting in breakage of chemical seed dormancy (Edward et al., 2013). Similarly, ~~Adebisi~~ et al., 2011 found that seeds of *Gmelina arborea* soaked in water for 48 hours showed the highest ~~germinative~~ germinate performance traits under field conditions. Mwase and Mvula (2011) reported that nicking produced higher values for various germination ~~atributes~~ attributes for large seeds of *Bauhinia thonningii.* Das (2014)

found highest germination (91.26 %) in hot water (80°C for 10 min), followed in cold water soaking for 24 h on *Acacia catechu*. Azad et al., (2006a) reported highest germination (52%) in hot water. treatment in *A. lebbeck* may be due to the variation of seed coat thickness. Similarly, Azad et al., (2010) found 69% germination success in hot water (80°C for 10 min) treatment on *Melia azedarach*. It may be due to the difference of seed coat thickness. Due to continuous metabolism in recalcitrant seeds, viability is lost if the moisture contents drop below a certain critical level before germination takes place. Studies on seed biology and physiological ecology help to understand the processes such as germination, establishment, succession, and regeneration that occur in plant communities (Tabin and Shrivastava, 2014).

Growth attributes

Among the parameters measured for growth behaviour for different pre sowing treatments was determined for root length, shoot length, seedling length and seedling vigour index (Table 2). The similar trend of germination was also noticed in the growth behaviours of different pre sowing treatments. In the present study, different pre sowing treatments under nursery condition showed significant variation for seedling growth parameters. The results presented in the Table 3 indicated that different pre sowing treatments had significant effect on seedling growth behaviours. Root length ranged from 12.23-1.09 cm, T_3 observed highest root length (12.23 cm) and lowest was observed in the T_5 (1.09 cm). Shoot length had significant differences effect on different pre sowing treatments. Shoot length ~~was~~ observed was ranged from 32.38 – 13.94 cm. T_3 gives ~~observed~~ highest shoot length (32.38 cm) and lowest was reported from T_1 (13.94 cm). Growth behaviour of seedling length was also found

Treatment	Shoot Length	Root Length	Seedling length	SVI
T_1	13.94^{a}	3.91^{a}	17.84^{a}	427^{a}
T_2	22.39^{be}	6.37^{cd}	28.76^{c}	1209^{be}
T_3	32.38^{a}	12.23^{a}	44.62^{a}	2462^{a}
T_4	25.96^{b}	10.52^{b}	36.47^{b}	1569^{b}
T_5	20.25^{cd}	1.09^{e}	27.34^{c}	980^{cd}
T_6	17.21^{de}	5.56^{d}	22.76^{d}	635de

Comment [D12]: 10.09 or 10.9 pls correct it

to be significantly differences among different pre sowing treatments. Irrespective of different treatments, seedling length ranged from 44.62- 17.84 cm. T_3 exhibits maximum seedling length (44.62 cm) while lowest was with ~~observed~~ T_1 (17.84 cm). Seedling vigour index from different pre sowing treatments ranged from 2462-427. T_3 observed maximum seedling ~~vigor~~ vigour index (2462) while minimum was recorded from T_1 (427).

UNDER PEER REVIEW

Table 2: Effect of pre-sowing treatments on growth attributes of *A. agallocha*

Treatment	Shoot Length	Root Length	Seedling length	SVI
T_1	13.94^{e}	3.91^{e}	17.84^{e}	427^{e}
T_2	22.39^{bc}	6.37^{cd}	28.76^{c}	1209^{bc}
T_3	32.38^{a}	12.23^{a}	44.62^{a}	2462^{a}
T_4	25.96^{b}	10.52^{b}	36.47^{b}	1569^{b}
T_5	20.25^{cd}	1.09^{c}	27.34^{c}	980^{cd}
T_6	17.21^{de}	5.56^{d}	22.76^{d}	635^{de}

Comment [D13]: 10.09 or 10.9 pls correct it

The values obtained for different growth parameters in untreated seeds were lower than the different treatments. Agarwood seeds have low germination percentage may be owing to the seed dormancy which is attributed to the presence of metabolic blocks in the mature embryo or due to the presence of inhibitors in the seed coat. Growth performance of the seedlings was influenced by pre-sowing treatments of seeds. However, there was significant variation in growth performance among the treatments at $p \leq 0.05$. Similar results were reported by several authors. It was also mentioned that seedling growth including root length, shoot length, total length and vigor index in *Terminalia chebula* was increased by pre-sowing treatment in tap water (Hossain et al., 2013). In another experiment, (Hossain et al., 2005b) showed maximum growth including shoot length, root length, total length and vigor index in *Terminalia belerica* seedlings when fruits were depulped and soaked in cold water. However, similar to the present study, there was no remarkable difference in average number of leaves per seedlings of *T. belerica* seedlings (Hossain et al., 2005a).

Conclusion

Pre-sowing treatments play an important role to enhance the seed germination under nursery conditions. For establishing a nursery of particular species for predicting the maximum number of quality seedling with minimum cost, time and ~~labour~~ labor, pre-sowing treatments of seeds are essential. The results from this study show that pre-sowing treatment soaking in water was found to be effective treatment for enhancing seed germination and growth attributes. The treatment also showed the highest germination and the fastest germination. The seeds are sensitive to desiccation in storage and may result in loss of viability and germination as well as in slower growth of seedlings. Although, seedling quality plays a great role in their survival and growth, based on the findings, it could be suggested that seedlings maintained in controlled environments during early growth period may

Comment [D14]: Here you must mention the treatment

UNDER PEER REVIEW

improve survival and growth for large scale plantation of the species. Hence, this can be recommended to enhance the germination in mass scale establishments.

References

Adebisi MA, Adekunle MF, Odebiyi OA. Effects of fruit maturity and pre-sowing water treatment on germinative performance of *Gmelina arborea* seeds. Journal of Tropical Forest Science. 2011; 23 (4): 371-378.

Ali T, Hossein P, Asghar F, Salman Z, Ali Z CM. The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*. Afr. J Biotechnol. 2010~~; 965886593~~; 965886593.

Alwis WN, Subasinghe SMCUPD, Hettiarachchi DS. Characterisation and variation of agarwood resins from *Gyrinops walla*. Tropical Forest Science. 2019; 31(2): 222–229.

Azad MS, Islam MW, Matin MA, Bari MA. Effect of pre-sowing treatments on seed germination of *Albizia lebbeck* (L.) Benth. South Asian Journal of Agricultural. 2006 a; 1(2): 32-34.

Azad MS, Manik MR, Hasan MS, Matin MA. Effect of different pre sowing treatments on seed germination percentage and growth performance of *Acacia auriculiformis*. Journal of Forestry Research. 2011; 22(2): 183-188.

Azad S, Paul NK, Abdul M. Do pre-sowing treatments affect seed germination in *Albizia richardiana* and *Lagerstroemia speciosa*. Frontiers of Agriculture in China. 2010; 4 (2): 181-184.

Borpuzari PP, Kachari J. Effect of glutamine for high-frequency In-vitro regeneration of *Aquilaria malaccensis* Lam. through nodal culture. Journal of Medicinal Plants Studies. 2018a; 6(2): 9-16.

Chung RCK, Purawaningsih. *Aquilaria malaccensis*. In: Oyen, L.P.A., Dung, N.X. (Eds.), Plant Resources of South-East Asia No. 19 Essential-oil plants. Backhuys Publishers, Leiden, Netherlands, 1999pp. 64–67

CITES. Review of Significant trade of *Aquilaria malaccensis*. 2003.

UNDER PEER REVIEW

Das N. Effect of Seed sources variation and pre sowing treatments on the seed germination of *Acacia catechu* and *Elaeocarpus floribundus* species in Bangladesh. International Journal of Forestry Research. 2014; 1(1): 1-8.

Edward M, Chikumbutso M, Dackious K, Hannah B, Gift K. Effect of seed size and pre-treatment methods on germination of *Albiziza lebbeck*. International Scholarly Research Notices: Botany. 2013; 1 (1): 1-4.

Gholami H, Farhadi R, Rahimi M, Zeinalikharaji A, Askari A. Effect of growth hormones on physiology characteristics and essential oil of basil under drought stress condition. J Amer Sci. 2013; 96163.

Han C, Yang P. Studies on the molecular mechanisms of seed germination. Proteomics. 2015; 15: 1671–1679.

Hossain MA, Arefin MK, Khan BM Rahman MA. Effects of Different Seed Treatments on Germination and Seedling Growth Attributes of a Medicinal Plant Bohera (*Terminalia belerica* Roxb.) in Nursery. International Journal of Forest Usufruct Management. 2005 a; 6:28-37.

Hossain MA, Arefin MK, Khan BM, Rahman MA. Effects of Seed Treatments on Germination and Seedling Growth Attributes of Horitaki (*Terminalia chebula* Retz.) in the Nursery. Research Journal of Agriculture and Biological Sciences. 2005 b; 1: 135-141.

Hossain MA, Uddin MS, Rahman MM, Shukor NAA. Enhancing Seed Germination and Seedling Growth Attributes of a Tropical Medicinal Tree Species *Terminalia chebula* through Depulping of Fruits and Soaking the Seeds in Water. Journal of Food, Agriculture and Environment. 2013; 11:2573-2578.

IUCN. Asian Regional Workshop (Conservation & Sustainable Management of Trees, Viet Nam) 1998, *Aquilaria malaccensis*. 1999.

Kanjilal UN, Kanjilal PC, Dey RM, Das A, Flora of Assam – IV, Government of Assam, Assam 1982. complete

Kjaer ED. Sustainable Use of Forest Genetic Resources. The Arboretum/ Danida Forest Seed Centre, Humlebaek. 1997

UNDER PEER REVIEW

Liu Y, Chen H, Yang Y, Zhang Z, Wei J, Meng H. Whole-tree agarwood inducing technique: an efficient novel technique for producing high-quality agarwood in cultivated Aquilaria *sinensis* trees. Molecules. 2013; 18: 3086– 3106.

Mwase WF, Mvula T. Effect of seed size and pre-treatment methods of *Bauhinia thonningii* Schum. on germination and seedling growth. African Journal of Biotechnology. 2011; 10 (13): 143-148.

Saikia M, Shrivastava K. Direct shoot organogenesis from leaf explants of *Aquilaria malaccensis* Lam. Indian Journal of Research in Pharmacy and Biotechnology. 2015; 3(2): 164-170.

Saikia P, Khan ML. Home gardens of upper Assam, northeast India: a typical example of on farm conservation of Agarwood (*Aquilaria malaccensis* Lam.). International Journal of Biodiversity Science, Ecosystem Services & Management. 2014; 10(4): 262-269.

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

Singh M, Singh KK, Badola HK. Effect of temperature and plant growth regulators on seed germination response of *Oroxylum indicuma* - high value threatened medicinal plant of Sikkim Himalaya. J Plant Sci Res. 2014; 1115.

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

Unal BT. Effects of growth regulators on seed germination, seedling growth and some aspects of the metabolism of wheat under allelochemical stress. Bangladesh Journal of Botany. 2013; 42(1): 65-71.

Vishal B, Kumar PP. Regulation of seed germination and abiotic stresses by gibberallins and abscisic acids. Front. Plant Sci. 2018; pp

Zich FA, Compton J. 2001. The Final Frontier: Towards Sustainable Management of Papua New Guinea's Gaharu Resource. Traffic Oceania-WWF South Pacific Programme.

UNDER PEER REVIEW

UNDER PEER REVIEW