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

Abstract

The imposition of pre-sowing treatment has been playing a crucial role for enhancing the germination percentage of the forest species and its succeeding growth stages. On this contention, a preliminary study was conducted to understand the impact of different pre-sowing treatments on *Aquilaria agallocha* Lam. (Agarwood) and its early growth behaviour. In this present study, the seeds were subjected to six different pre-sowing treatments. Among the pre-sowing treatments, soaking in water at room temperature for 24 hrs (T_3) has resulted the maximum germination percentage (55.25%) as compared to other counterparts. On an average, there was an increase of 49 %, 47 %, 38 %, 40% and 49% of germination percentage, germination capacity, germination energy, peak value and mean daily germination respectively in T_3 over control (T_6). Similarly, the early seedling growth of the agarwood was also influenced by imposition of different pre-sowing treatments. And all the seedling growth parameters viz., shoot length (cm), root length (cm), seedling length (cm) and seedling vigour index were recorded maximum in T_3 as compared to rest of the treatments.

Keywords: Pre-sowing, Germination, Water Soaking, Seedling Growth

Introduction

Agarwood *(Aquilaria agallocha*), belonging to the 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 is 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 a commercial scale mainly in the 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 in therapeutic perfumes, as traditional medicine, an aromatic food ingredient and for religious purposes (Liu et al. 2013). Due to its high medicinal and perfumery value, the species has great demand in the national and international markets hence 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 forests 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 concerns and is included in the prioritized list for the 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 seed is being considered as the the most reliable method of propagation. However, the seeds of Agarwood show low natural germination, which might be due to its rapid decline of viability after shedding from the trees. Seeds are produced once in a year during the monsoon season

i.e. from June to August. Its seeds have a short shelf life at normal temperature. In contrast, the low reproductive potential of *Aquilaria* seeds has been reported due to variable, slow and short germination rate. Plant growth regulators (PGRs) have been widely employed in enhancing the 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 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 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 them from extinction, and hence to improve the economy.

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 experiences a precipitation of 1000-1300 mm annually. The major part of the rain is concentrated during July and August (Monsoon period) months.

Collection of seed

Fresh and healthy matured seeds of *A. agallocha* were collected from the Forest Department, Government of Manipur. Thereafter, pure seeds after removing diseased and damaged seeds were put in containers in the laboratory of department of Forestry and Environmental Science, Manipur University for undertaking germination tests.

The present investigation was carried out at the experimental plot at the 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 a control [$\mathbf{T_1}$ = Control, $\mathbf{T_2}$ = Soaking in water at room temperature for 12 hrs, $\mathbf{T_3}$ = Soaking in water at room temperature for 24 hrs, $\mathbf{T_4}$ = Soaking in water at room temperature for 48 hrs, $\mathbf{T_5}$ = Immersed at hot water (100^0C) for 1 minute and $\mathbf{T_6}$ = Immersed at hot water

(100^0C) for 3 minutes] with four replicates. A 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 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 One way Analysis of Variance (ANOVA), Duncan Multiple Test (DMRT) at $p<0.05$ was carried out using SPSS 17.0 window version to understand the effect of different of pre-sowing treatments of germination and early growth parameters of agarwood.

Result and discussions

The fruits of *Aquilaria* desiccate very fast (Shankar, 2012) and may bear one or two seeds per fruit (Table 1). Seed germination percentage is an important trait for growers and foresters. Overall, the seed germination percentage 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. (Table 1) 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 the highest germination capacity (57.75 %) while the 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 significant differences among all the treatments. In the present study, the germination value ranged from 6.76-1.39 where T_3 observed the highest germination value (6.76) and the lowest was recorded in small size seed (1.39). Some forest seeds have hard and impermeable seed coats which restricts germination.

Therefore, it is very important to apply the most suitable pre sowing treatment. Different approaches to breaking seed dormancy to enhance the germination process have been reported by many authors (Azad et al., 2011). The main reason behind the successful germination of T_3 is the seed of *A. agallocha* might require to break the dormancy and after that soaking in normal water make the seed coat more softly that ensuring the maximum and successful germination of the seed. Soaking seeds in water may soften hard seed coats, this makes the seed coats permeable to water and soaking seeds in water leaches out chemical inhibitors, resulting in the 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 performance traits under field conditions. Mwase and Mvula (2011) reported that nicking produced higher values for various germination attributes for large seeds of *Bauhinia thonningii*. Das (2014) found that the 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 the 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 in 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).

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}

Values followed by different alphabets in parenthesis is significantly different at $p<0.05$ based on DMRT.

Growth attributes

The imposition of different pre-sowing treatments had significant influenced on the early growth parameters *i.e.*, root length, shoot length, seedling length and seedling vigour index of agarwood. (Table 2). In the present study the root length ranged from 12.23-1.09 cm where T_3 observed the highest root length (12.23 cm) and the lowest was recorded in the T_5 (1.09 cm). Similarly, the shoot length of the agarwood was also significantly influenced by the imposition of different pre-sowing treatments. Shoot length was observed ranged from 32.38

– 13.94 cm where T_3 recorded the highest shoot length (32.38 cm) and T_1 (13.94 cm) attained the minimum shoot length. The growth behaviour of the seedling length was also found 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 the maximum seedling length (44.62 cm) while the lowest was observed in T_1 (17.84 cm). While the seedling vigour index in different pre sowing treatments ranged from 2462-427 and T_3 observed the maximum seedling vigor index (2462) while the minimum value was recorded in T_1 (427).

Table 2: Effect of different 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}

Values followed by different alphabets in parenthesis is significantly different at $p<0.05$ based on DMRT.

The values obtained for different growth parameters in untreated seeds were lower than the different treatments. Agarwood seeds have low germination percentage 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 enhancing 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, 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 improve survival and growth for large scale plantation of the species. Hence, this can be recommended to enhance the germination in mass scale establishments.

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