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Comprehensive evaluation of seed germination and physical traits in *Aquilaria agallocha* (Agarwood)

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Abstract
Agarwood (*Aquilaria agallocha*) is a highly prized tropical tree valued for its aromatic resin, widely used in perfumery, traditional medicine, and cultural practices. However, its natural regeneration is constrained by factors such as low seed viability, excessive harvesting and vulnerability to fungal infections. Agarwood seeds exhibit recalcitrant characteristics, losing viability rapidly upon desiccation, which limits their storage potential. Sustainable cultivation of agarwood is hampered by a limited understanding of its seed biology, including germination behaviour and physical traits. This study aimed to evaluate the effects of various pre-sowing treatments on seed germination, as well as to analyse the physical traits of agarwood seeds. Various pre-sowing treatments have been investigated to enhance agarwood seed germination rates. Germination trials were conducted under controlled conditions with seven pre-sowing treatments at ICAR Research Complex for NEH region, Manipur Centre. Results obtained showed that 24 hour soaking in water at room temperature yielded the highest percentage of germination at 63.33%. The longer soaking time significantly reduced germination and treated seeds with concn. H_2SO_4 had extreme damage on the seed upon germination. Physical analysis revealed that the seed of agarwood is small size and smooth coated. By employing improved germination techniques and sustainable propagation strategies, the conservation and commercial cultivation of agarwood can be significantly enhanced, ensuring its long-term economic and ecological viability.

Keywords: Agarwood, seed germination, seed traits, conservation, propagation

Introduction
Agarwood, extracted from the tree *Aquilaria agallocha* belonging to Thymelaeaceae family, is one of the highly priced natural products that emit fragrant resin, which is part of cultural, medical, and economic activities for thousands of years (Thompson *et al.* (2022) [20], Abdulah *et al.* (2022)) [1]. The resin, as an outcome of fungal infection or physical injury, is widely used in traditional medicine, perfumes, and religious rituals (Shivanand *et al.* (2022) [18]; Baldovini (2022) [5]. Due to its increasing demand globally, agarwood has been over-harvested and habitat destroyed, placing several species of *Aquilaria*, including *A. agallocha* at risk of endangerment (Hazarika *et al.* (2023) [7], Nath *et al.* (2023) [14]. Further, natural regeneration of agarwood trees is limited by low seed viability, and susceptibility to fungal infections. Agarwood seeds have recalcitrant behavior: they lose viability rapidly upon desiccation (Dutta and Bhuyan (2023) [6], Jin *et al.* (2023) [11], Piñon *et al.* (2023) [16]. Fresh seeds exhibit high germination potential, but viability drops drastically within weeks if not properly managed. Several environmental factors, including humidity, light exposure, and substrate conditions, affect the successful germination process. Conservation and cultivation of agarwood are now imperative for the continuation of its ecological and economic importance. The ability to successfully propagate and cultivate agarwood is related to characteristics of its seeds and the pre-sowing treatments used to enhance germination. The physical seed traits identified include size, weight, length etc., provides a set of information useful for understanding the reproductive ecology of *A. agallocha*. Seed propagation is still the most viable method for large-scale plantation efforts. However, very little information on the seed biology of *A. agallocha* exists in terms of germination behaviour and dormancy mechanisms that might hinder successful cultivation. *A. agallocha* seeds are

recalcitrant with a high moisture content and short viability period that needs to be sown immediately after harvest (Hoque *et al.* (2019) [9], Abdullah *et al.* (2024) [2]. Besides, sensitivity to environmental factors like temperature, light, and composition of substrate during germination makes the germination protocols rather more critical. This study aims to fill these gaps by evaluating the seed traits and germination behavior of *Aquilaria agallocha*. This research explores the effects of pre-sowing treatments on germination success, providing a basis for better propagation practices. The findings will contribute to the sustainable cultivation and conservation of this ecologically and economically important species.

Materials and Methods

The experiment was conducted in the polyhouse at research farm of ICAR Research for NEH region, Manipur Centre, Lamphelpat during July, 2023. The study area is located in Imphal West District of Manipur, India and situated at approximately 24.8251° N latitude and 93.9089° E longitude. The area experiences a humid subtropical climate, characterized by cool, dry winters and a warm monsoon season. Average temperatures range from around 14°C in January to approximately 25°C during the monsoon season in July. The region receives about 1,381 mm of annual rainfall, with June and July being the wettest months.

Seed Collection and Preparation: Mature seeds of *A. agallocha* were collected from natural forests in Jorhat, Assam. After harvesting, seeds were cleaned and damaged or immature seeds were discarded. Only fresh seeds were used to minimize viability loss.

Physical Traits Analysis: Seed dimensions (fruit length, fruit width, fruit thickness and seed length, seed width, seed thickness) were measured using digital calipers. Weight was recorded using a precision balance.

Germination test

The effect of different pre sowing treatment conditions on seed germination of *A. agallocha* was tested under polyhouse conditions. Seven treatments with four replication were applied i.e., T_1: Control; T_2 Soaking in water at room temperature for 12 hours; T_3 Soaking in water at room temperature for 24 hours; T_4 Soaking in water at room temperature for 48 hours; T_5 Boiling water treatment and soaking for 12 hours; T_6 Boiling water treatment and soaking for 24 hours; T_7 Concn. H_2SO_4 2 min. dip and then washing. Each treatment consisted of 60 seeds, totalling 240 seeds per treatment. Seeds were sown in polybags containing the medium soil and sand (1:1) in a Completely Randomized Design and the testing period was 28 days. Germination percent (%), Germination Energy (%), Germination Speed, Mean Daily Germination, Peak Value and Germination Value were calculated.

Data analysis

The data were analysed using SPSS version 26 and the results were subjected to the analysis of Duncan's multiple range test (DMRT) at 5% level.

Germination per cent (GP): The per cent germination was calculated by a number of seeds in a given sample that actually germinated.

$$GP\ (\%) = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Germination energy (GE): Germination energy was calculated on the basis of the percentage of the total number of seed that had germinated when the germination reached its peak.

$$GE = \frac{\text{Number of seeds germinated upto the time of peak germination}}{\text{Total number of seeds sown}} \times 100$$

Germination value (GV): Germination value is the index combining speed and completeness of seed germination.

$$GV = PV \times MDG$$

PV= Peak Value
MDG = Mean daily germination

Results and Discussion

Physical Traits of Seeds: The data describes various physical traits of *A. agallocha* seeds, indicating that the seed is relatively small and compact (Table 1). The fruit has a weight of 1.89 grams, a length of 27.34 mm, a width of 18.04 mm, and a thickness of 13.21 mm, all of which suggest it is a miniature fruit. The seed characteristics further support this observation, with a seed weight of 0.21 grams, a length of 13.89 mm, and a width of 5.13 mm. These traits could also reflect environmental or genetic factors influencing fruit and seed development. Overall, the data provides valuable insight into the morphological characteristics of the agarwood seeds. The physical seed traits of *A. agallocha* provide insight into their ecological adaptability and the challenges they face in their natural environment. Each trait is uniquely adapted to facilitate reproduction and dispersal. Seed and fruit size as well as moisture retention capacity are also pivotal traits influencing germination success (Klupczyńska and Pawłowski, (2021) [12]. While the small size of *A. agallocha* seeds facilitates easier dispersal, it also makes them prone to rapid desiccation. The findings are in line with Alzate and Onstein (2022) [3] and Hernández-Brito *et al.* (2021) [8], who suggested that small seeds are more easily dispersed over long distances. This sensitivity highlights the importance of immediate sowing or the application of pre-sowing treatments to enhance germination rates. Furthermore, understanding the variations in seed morphology across different *A. agallocha* can provide insights into their adaptive strategies, enabling the selection of traits best suited for plantation environments.

Germination Performance: The table 2 shows germination characteristics under different pre sowing treatments. Germination percent (%), germination energy (%), germination speed, mean daily germination, peak value and germination value were highest in T_3, with values of 63.33%,41.75%, 48.08, 2.36, 3.87 and 8.79, respectively, indicating optimal conditions for germination in this treatment. Treatment T_2 follows with moderate germination performance, while T_1 and T_4 show intermediate values. Treatments T_5, T_6, and T_7 demonstrate poor germination performance, with T_7 having the lowest values across all parameters, including a germination percentage of just 3.70%. These results suggest that T_3 provides the most favorable conditions for seed germination, while T_7 is the least effective. The pre-sowing treatments applied to *A. agallocha* seeds showed a significant difference in germination rates. Among the treatments, soaking in water at room temperature for 24 hours (T_3) significantly improved the germination rate because it probably hydrates the seed, breaks dormancy by activating metabolic processes. This is in agreement with Leishangthem

and Rana (2017) [13] and Jaganathan and Harrison (2024) [10], who were pointed out that moisture plays a critical role in improving seed viability for tropical species. However, seeds that received concn. H_2SO_4 2 min. dip and then washing (T_7) had a low germination rate. This is due to the possible damage caused by the acid to the seed coat and embryonic tissues (Amri (2010) [4]; Rana *et al.* (2017) [17]. More prolonged acid exposure can also cause osmotic stress, thus preventing germination. The germination rate increased with soaking duration until it reached 48 hours after which it began to decline. The boiling water treatments, which are meant to simulate the natural scarification processes, yielded poor rates of germination. Overexposure to boiling water or extremely high temperatures can denature proteins and break the embryo, lowering the seed's germination rate. Therefore, optimizing the soaking time and water temperature during treatment is crucial to prevent seed viability destruction and ensure successful germination results (Paparella *et al.* (2015) [15]; Tanwar *et al.* (2023) [19]. The results show the significance of the optimization of soaking durations in the balance between hydration and seed health.

Table 1: Seed traits of *A. agallocha*

Traits	
Fruit weight (g)	1.89
Fruit length (mm)	27.34
Fruit width (mm)	18.04
Fruit thickness (mm)	13.21
Seed weight (g)	0.21
Seed length (mm)	13.89
Seed width (mm)	5.13

Table 2: Effect of pre-sowing treatments on the seed germination of *A. agallocha*

Treatments	Germination per cent (%)	Germination energy (%)	Germination speed	Mean daily germination	Peak value	Germination value
T_1	33.03^{d}	17.2^{d}	15.69^{d}	1.12^{c}	1.43^{d}	1.45^{d}
T_2	56.05^{b}	36.65^{b}	37.27^{b}	2.02^{a}	3.15^{b}	5.84^{b}
T_3	63.33^{a}	41.75^{a}	48.08^{a}	2.36^{a}	3.87^{a}	8.79^{a}
T_4	43.90^{c}	31.38^{c}	31.38^{c}	1.49^{b}	2.45^{c}	3.73^{c}
T_5	20.40^{e}	12.25^{e}	13.74^{d}	0.73^{d}	1.14^{de}	0.86^{e}
T_6	16.58^{f}	9.98^{e}	11.79^{d}	0.58^{d}	0.91^{e}	0.56^{ef}
T_7	3.70^{g}	2.18^{f}	2.78^{e}	0.15^{e}	0.24^{f}	0.03^{f}
C.D.	2.36	4.37	4.41	0.37	0.34	0.53
S.E(m)	0.79	1.46	1.47	0.12	0.11	0.18
SE(d)	1.12	2.06	2.08	0.17	0.16	0.25

Conclusion

The findings emphasize the need for careful selection and optimization of pre-sowing treatments to maximize germination outcomes. By providing insights into the germination behavior and physical traits of *A. agallocha* seeds, this study contributes to the development of effective propagation practices, ensuring the sustainable cultivation and conservation of this economically important species.

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