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RESEARCH ARTICLE

Effects of Pre-Sowing Treatments on Seed Germination and Seedling Growth Attributes of the Endangered (*Anisoptera scaphula* Roxb.) Species

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Abstract

Anisoptera scaphula (Boilam), one of Bangladesh's tallest native tree species, plays a vital role in the Asian forest ecosystem. It is critically endangered and at high risk of extinction. This research, conducted from April 2022 to February 2023, examined the effects of pre-sowing treatments on seed germination and seedling growth. Seeds collected from a mother tree in Cox's Bazar were subjected to four treatments: untreated (T0), soaked in water for 12h (T1), soaked and air-dried for 12h (T2), and soaked and air-dried for 24h (T3). Results showed T2 had the highest germination rate (84.2%) and fastest germination, while T0 had the lowest (52.3%). Seedlings from T2 also exhibited the greatest height (46.4 cm) and collar diameter (0.76 cm). Around 2000 seedlings are now ready for planting. These findings support large-scale seed production and regeneration efforts to mitigate the extinction risk of *A. scaphula* and restore its ecological role.

Keywords: *Anisoptera scaphula*; Seed germination; Pre-sowing treatments; DMRT; Biodiversity conservation; Endangered

Introduction

Bangladesh is a subtropical monsoon climatic region located between 20°34´ and 26°38´ North latitude and 88°01´ and 92°41´ East longitude (Alam et al., 2023; Miah et al., 2023; Roy et al., 2015). It is bordered by India on its western, northern, and eastern sides, Myanmar to the southeast, and the Bay of Bengal to the south. The climate is humid and warm, influenced by pre-monsoon, monsoon, and post-monsoon circulations, experiencing six seasons annually (Cord et al., 2009; Masum et al., 2022; Mukul et al., 2017; Nazrul et al., 2021; South et al., 2013). January is the coolest month with average temperatures around 26°C (78°F), while April is the warmest, with temperatures ranging from 33°C to 36°C (91°F to 96°F). Most rainfall occurs during the monsoon season (June-September) and is minimal in winter (November-February), making it one of the wettest climates in the world. Most areas receive over 1,525 mm of rain annually, with regions near the hills receiving up to 5,080 mm (Benito et al., 2013; Islam et al., 2021;Tanu et al., 2021; Yudaputra, 2021). The high precipitation and hot, humid temperatures support continuous high forests in Chattogram, the Chattogram Hill Tract, and Cox's Bazar, rich in biodiversity. Bangladesh covers a total area of 147,630 sq. km (57,000 sq. miles), with forest land constituting about 14.47% (BBS, 2022), although only 8-10% has good canopy cover. The forests, primarily located in Chattogram, the Chattogram Hill Tract, Cox's Bazar, and Sylhet, comprise a wide range of timber species.

A total of 224 timber-yielding plant species grows in Bangladesh. Important species include sal (*Shorea robusta*), gamar (*Gmelina arborea*), telsur (*Hopea odorata*), boilam (*Anisoptera scaphula*), jarul (*Lagestroemia speciosa*), teak (*Tectona grandis*), garjan (*Dipterocarpus turbinatus*), and sundari (*Heritiera fomes*) (Bangladesh Bureau of Statistics, 1996). Anisoptera is a genus under the Dipterocarpaceae family (Dutta & Hossain, 2016;Miah et al., 2023; Rahman et al., 2016; Reza & Perry, 2015), consisting of ten species distributed from Chattogram (Bangladesh) to New Guinea. Eight of these species are listed in the IUCN Red List, with four critically endangered and the others classified as endangered. *Anisoptera scaphula*, critically endangered and native to Bangladesh, occurs in small patches in the forested areas of Chattogram, the Chattogram Hill Tract, and Cox's Bazar. This species also thrives in Malaysia, Myanmar, Thailand, and Vietnam.

Dipterocarpaceae, a family of 16 genera and about 695 known species (Dutta & Hossain, 2016; Głowacka & Flis-Olszewska, 2022; Rahman et al., 2010; Miah et al., 2023; Rahman et al., 2016; Reza & Perry, 2015; Uddin et al., 2020), includes *A. scaphula*, one of the tallest tree species in Bangladesh, reaching 30-45 m in height with a diameter of 1.0-1.5 m. *A. scaphula* is a resinous tree with grey bark, thick simple leaves, and terminal racemes. Its flowers are sub-globose, and the fruiting calyx tube is constricted at the mouth, with two large calyx lobes. It is an evergreen species, partially flowering from December to January, with fruits ripening during April-May. Native to Bangladesh, Myanmar, and Thailand, it is found in low altitudes and deep valleys in seasonal forests (Chowdhury et al., 2019; de Souza Valente et al., 2020; Dutta & Hossain, 2016; Głowacka & Flis-Olszewska, 2022; Rahman et al., 2010; Majumdar et al., 2014; Miah et al., 2023; Mohd-Taib et al., 2020; Rahman et al., 2016, 2017; Reza & Perry, 2015; Scherer et al., 2021; Uddin et al., 2020), tending to be gregarious in more seasonal forests.

A. scaphula is a flagship species in Bangladesh. It plays a dominant role in Asian forest ecology, providing shelter for avian fauna and orchids. Its timber is hard, rigid, durable, and suitable for various construction purposes. The wood is easy to work with and takes polish well, extensively used for general light construction, furniture, boats, and plywood. A small amount of resin is obtained from the tree, used locally for torches and caulking boats (Hossain et al., 2006; Kalanzi & Mwanja, 2023; Ojha et al., 2009; Ray et al., 2018). However, biodiversity in Bangladesh is depleting due to ineffective implementation of biodiversity safeguards in political and market systems. *A. scaphula*, despite being a flagship species, is on the verge of extinction and serves as an ambassador for conservation efforts. Scattered plantations are raised in the Chittagong Forest Division and Chittagong University campus, with 38 Boilam trees identified. Natural regeneration is hindered by the lack of mother trees and seeds, ground vegetation competition, and bad seed production. The seeds are recalcitrant (Chowdhury et al., 2023), complicating collection and storage. Techniques for seed germination need development for successful artificial regeneration. Pre-sowing treatments and proper germination techniques are crucial for effective plantation programs.

The study aimed to evaluate the germination potential of *A. scaphula* for large-scale plantations, determining the effects of pre-sowing treatments (Devi & Sharma, 2014; Hossain et al., 2006; Kalanzi & Mwanja, 2023; Ojha et al., 2009; Ray et al., 2018; Sahoo et al., 2020), and investigating the initial growth performance of seedlings under control and different treatments. These objectives aim to optimize seedling development and enhance plantation efforts.

Materials and methods

Description of the study area

The study was conducted at the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Bangladesh, over one year from April 2022 to February 2023. The nursery is situated in an area with about 60% hilly terrain, with elevations ranging from 14 to 87 meters above mean sea level (Mia et al., 2015). The soil in this region is characterized as moderately coarse to fine-textured sandy loam to sandy clay loam, with moderate to strong acidity and poor fertility. Specifically, the soil has a pH of 5.5, organic matter content of 2.0%, base saturation percentage (BSP) of less than 40%, and cation exchange capacity (CEC) of less than 10 me/100g (Mehta et al., 2021).The nursery benefits from a tropical monsoon climate, marked by hot, humid summers and mild, dry winters. The region receives an average annual rainfall of 2,500 to 3,000 mm, with the majority occurring between June and September. The mean monthly minimum temperature is 21.24°C. May, the warmest month, has an average daily high temperature of 33°C, with maximum temperatures reaching up to 29.75°C. The period from May 2 to May 24, 2015, was notably warm. July experiences the highest percentage of days with recorded precipitation, around 84%. In contrast, May typically records the highest temperatures, averaging 32.60°C, with a minimum temperature of 14.10°C (El-Keltawi & Abdel-Rahman, 2010; Sahoo et al., 2020). This climate and soil profile provides a specific environmental context for the nursery, which plays a crucial role in understanding and managing the conditions under which seed germination and seedling growth are studied.

Seed collection

Seeds of *Anisoptera scaphula* were collected from a mother tree in Saplapur, Teknaf Upazila, Cox's Bazar, with GPS coordinates used to precisely locate the source (El-Kassaby et al., 2008; Lúcio & Sari, 2017; Ranal et al., 2009; Sultana et al., 2021) (Figure 1).

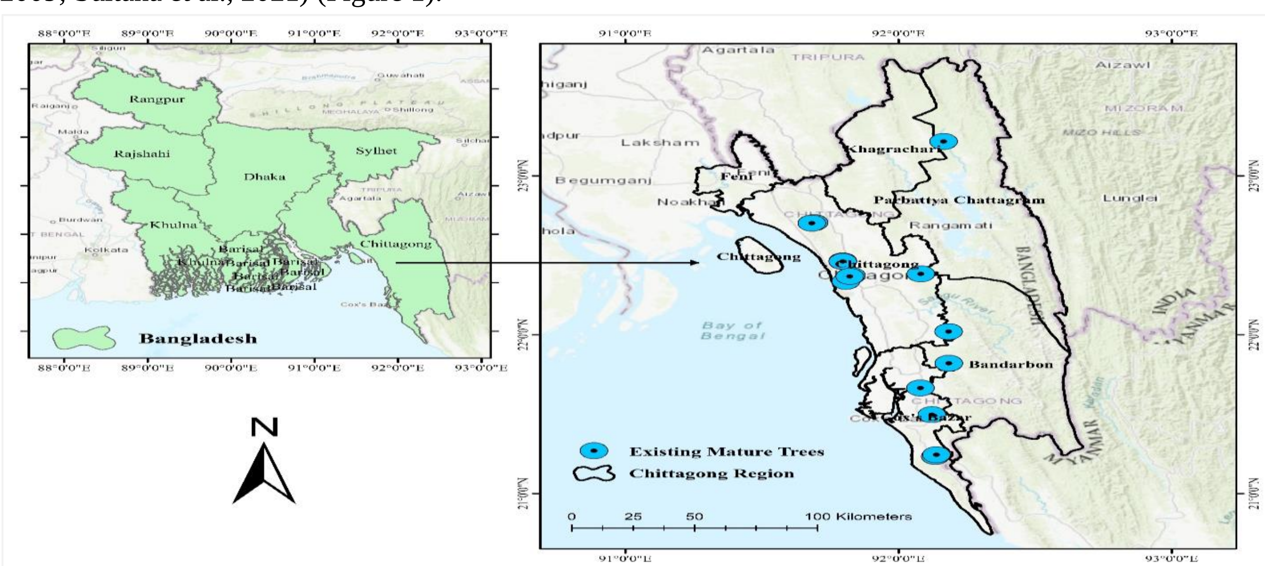

Figure 1. The map shows the geographic location of the recorded only few existing mature tree species of *A. scaphula* all over Bangladesh.

Seed purity was assessed by measuring the weight of pure seeds in the sample and calculating the percentage of pure seed. To ensure consistency in germination trials, freshly collected seeds of uniform size were selected, as seed size is positively correlated with germination success and seedling vigor (Chowdhury et al., 2024). The trials involved dewinged seeds subjected to four pre-sowing treatments: no treatment (T0), soaking in water for 12 hours (T1), soaking in water for 12 hours followed by 12 hours of air drying (T2), and soaking in water for 12 hours followed by 24 hours of air drying (T3). Each treatment was applied to 300 seeds per replicate, with three replicates per treatment, totaling 3,600 seeds. The seeds were sown inverted in polybags filled with a mixture of forest soil and decomposed cow dung at the IFESCU nursery (Sultana et al., 2021), following the methodology described by Hossain et al. (2014). This approach aimed to evaluate the effects of different pre-sowing treatments on seedling growth parameters such as germination rate, height, and collar diameter. The findings are expected to provide insights into optimizing seedling development and enhance reforestation and conservation efforts.

Statistical analysis

Germination percentage was estimated using the following formula,

Germination percentage (%) = germinated seeds /total seeds sown × 100

Mean germination time is a measure of the rate and spread of germination over time (Ranal et al., 2009). To analyze the significant differences among control and treatments, mean germination time, biomass of seedlings were tested with Duncan's multiple range test at 95% confidence intervals.

Results and discussions

Seed germination performance

Germination percentage of Boilam (*A. scaphula*) seeds was significantly affected by pre-sowing treatments. The germination percentage of dewinged Boilam seeds sown invertedly in polybags (Hossain et al., 2014) varied from 52.3% to 84.2% among the treatments. The highest germination percentage (84.2%) was obtained in T2 (Dewinged seeds soaked in water for 12 hours followed by air drying for another 12 hours), followed by 74.7% in T1 (Dewinged seeds soaked in water for 12 hours). The lowest seed germination percentage (52.3%) was observed in T0 (control; dewinged seeds without any treatment) (Figure 2). In a similar study on *Scaphula glabra*, Laurie (1940) reported 66% germination when seeds were sown superficially at 0.64 cm depth invertedly, i.e., with the apex downward and the stalk end upward. Hossain et al. (2014) reported a maximum germination percentage (65%) when dewinged *A. scaphula* seeds were sown invertedly (apex, wing side down) without soaking in water. Therefore, it has been shown clearly that the germination percentage of Boilam seeds significantly enhanced with soaking in water followed by air drying for 12 hours (Hossain et al., 2020, 2020). Expanding on these findings, several factors can be identified as influential in the successful germination of Boilam seeds. Pre-sowing treatments such as soaking in water and air drying appear to activate certain physiological and biochemical processes within the seeds (Dwivedi & Chopra, 2014). Soaking seeds in water likely facilitates the softening of the seed coat, allowing for better water absorption and subsequent activation of metabolic pathways essential for germination. This process, followed by air drying, could be contributing to the stabilization of the seed's internal moisture content, which is crucial for maintaining the viability of the seeds during the initial stages of germination (Hossain et al., 2018). Additionally, the practice of dewinging seeds, as noted in the study, removes the seed's natural dispersal apparatus, which might otherwise act as a physical barrier to water uptake (Golev et al., 2020; Wallertz et al., 2018; Ward et al., 2000). By removing the wings, the seeds are more uniformly exposed to the soaking treatment, leading to more consistent germination results (Figure 3). The inversion of seeds during sowing, with the apex downward and the stalk end upward, is another critical factor. This orientation may promote more efficient penetration of water into the seed and facilitate the emergence of the radicle, the first part of the seedling to emerge during germination. Comparing these findings with Laurie's study on *Scaphula glabra*, it is evident that seed orientation and sowing depth also play a significant role in germination success (Golev et al., 2020; Johansson et al., 2013; Lashley et al., 2017; Mickaël et al., 2007a; Wallertz et al., 2018; Ward et al., 2000). Sowing seeds at a shallow depth of 0.64 cm ensures that the seeds remain close to the soil surface, where they can receive adequate moisture and oxygen, both of which are essential for germination. The inverted orientation aligns with the natural tendency of the radicle to grow downward, potentially reducing the energy expenditure required for the seedling to establish itself.

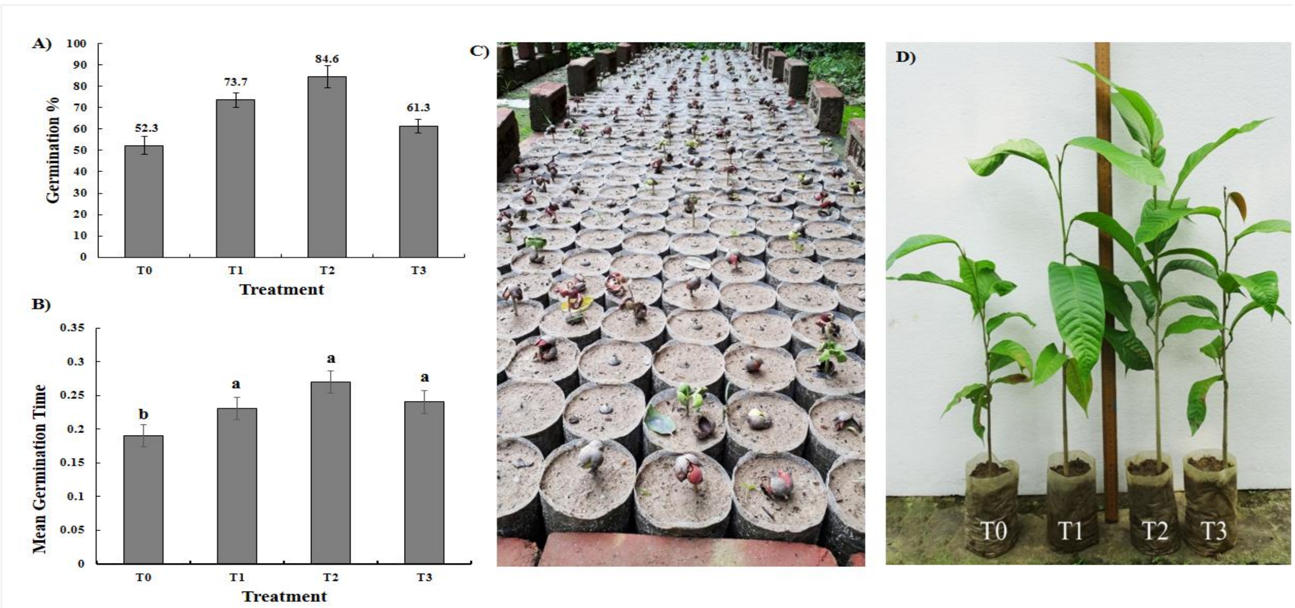

Figure 2. Effects of various pre-sowing treatments on germination potentials of the seeds of *A. scaphula* are shown in Figure 2A and 2B, the error bar indicates the standard error of means, and different alphabets (a, b) in the bar chart (Figure 2B) indicate the significant differences among different methods of pre-sowing treatment of *A. scaphula*. Figure (2D) shows the different physiological conditions of *A. scaphula* at the sapling stage, where, T0 is controlled, T1 is the pre-sowing treatment where soaking of seeds in water for approximately12 hours, T2 is the soaking of seeds in water for 12 hours followed by air drying for 12h, and T3 is the soaking of seeds in water for 12 hours followed by air drying for 24 hours.

Moreover, the data suggests that the absence of pre-sowing treatments results in significantly lower germination percentages. The control group in the study, which did not receive any pre-sowing treatment, exhibited the lowest germination rate of 52.3%. This stark contrast highlights the importance of pre-sowing treatments in enhancing germination efficiency (Cuesta et al., 2010; Häggström et al., 2021; McKee et al., 1984).

In practical applications, these findings can be utilized to improve the propagation techniques for Boilam seeds, especially in reforestation and conservation projects where high germination rates are critical for the successful establishment of plant populations. Implementing pre-sowing treatments such as soaking and air drying can significantly increase the germination success of Boilam seeds, thereby ensuring a more robust and resilient population of this critically endangered species (Garnett et al., 2004; Parker et al., 2004, 2006).

Figure 3. Physiological condition and growth performance of *A. scaphula* seedlings from various pre-sowing treatment (T0, T1, T2, T3), where, seedling from T0 is control, seedling from T1 is soaking of seeds in water for 12 hours, seedling in T2 is soaking of seeds in water for 12 hours followed by air drying for 12 hours, and seedling from T3 is soaking of seeds in water for 12 hours followed by air drying for 24 hours.

Overall, the study underscores the significance of carefully considered pre-sowing treatments and seed-handling techniques in maximizing the germination potential of Boilam seeds. By adopting these methods, forestry practitioners and conservationists can enhance the success rates of their planting efforts, contributing to the preservation and restoration of Boilam populations in their natural habitats (Häggström et al., 2021). This research adds valuable knowledge to the field of seed science and offers practical solutions for improving seed germination outcomes in forestry and conservation contexts.

Seed germination period

Dewinged seeds sown invertedly were found to start germination one week after sowing them in the polybag and continued for up to 20 days (Parker et al., 2006). The fastest germination (least imbibition period; 6 days) was observed in T2 (seeds soaked in water for 12h followed by air drying for 12 h) and T1(seeds soaked in water for 12h) and delayed (highest imbibition period, 20 days) was observed in T0 (control). Germination started with the development of a radicle from the winged side of the seed. Highest mean germination time (0.27) was found in T2, and lowest Mean Germination Time was in T0. There is no significant difference at $p<0.005$, Duncan's Multiple Range Test (DMRT) (Figure 2).

Seedling Growth Performance

Seedling growth parameters such as height and collar diameter were significantly influenced by the seed treatments. The maximum average height (46.4 cm) of the seedlings was observed in T2, followed by 37.9 cm in T1, with the lowest height (28.8 cm) recorded in T0 (control). Similarly, the collar diameter of the seedlings was also greatest in T2 (0.76 cm), followed by T1 (0.64 cm), and the smallest diameter was found in T0 (0.54 cm) (Figure 4). These results suggest that the pre-sowing treatment involving soaking seeds in water for 12 hours followed by air drying for another 12 hours (T2) is highly effective in promoting seedling growth. This treatment appears to provide an optimal balance of moisture absorption and subsequent drying (Dwivedi & Chopra, 2014; Kuniyal et al., 2013), which may activate and stabilize the physiological processes necessary for robust seedling development. The increased height and collar diameter of seedlings in T2 indicate a healthier and potentially more resilient plant, which is crucial for successful establishment in the field (Mickaël et al., 2007b). The substantial difference in growth parameters between treated and untreated seeds (T0) highlights the importance of appropriate pre-sowing treatments in forestry practices. The untreated seeds (T0) produced seedlings with significantly lower growth metrics, which may affect their survival and competitiveness in natural settings (Figure 3) (Dey et al., 2022; King & He, 2005; Matilla, 2019; Van Assen et al., 2015). This underscores the value of implementing evidence-based pre-sowing treatments to enhance seedling performance and ensure the success of reforestation and conservation efforts.

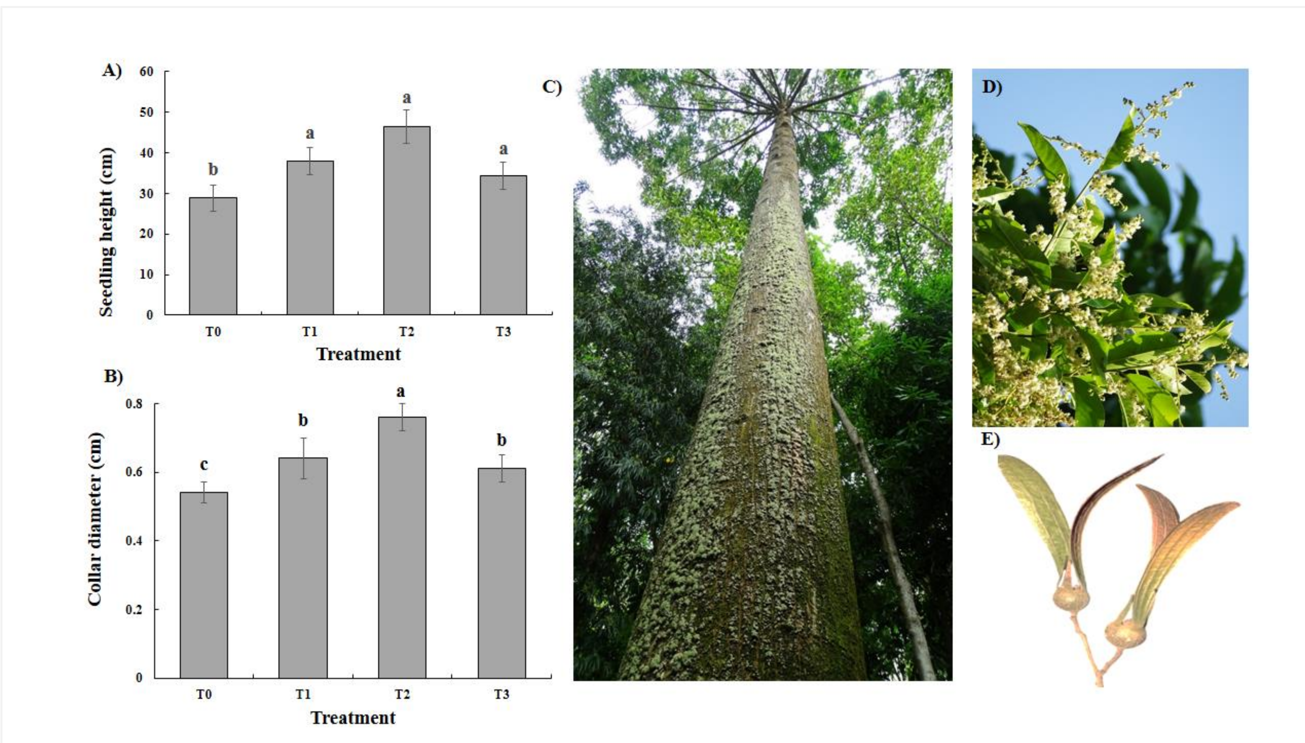

Figure 4. Height and diameter growth of Boilam seedlings develop through various pre-sowing treatment six months after sowing the seeds. T0: Control, T1: Soaking of seeds in water for 12h, T2: Soaking of seeds in water for 12h followed by air drying for 12h and T3: Soaking of seeds in water for 12h followed by air drying for 24h. Note: the same letter(s) indicates no significant difference at p≤0.05, according to DMRT. I (error bar) indicates the standard error of means. C) Mother tree, D) Flowers, E) Seeds of Boilam.

Consequently, approximately 2,000 Boilam seedlings are ready for planting in the next planting season (June-July 2023) as part of the germplasm establishment and restoration program. These seedlings, having undergone effective pre-sowing treatments, are expected to exhibit better growth and higher survival rates once planted. This initiative is a crucial step towards the conservation of the critically endangered Boilam species, contributing to the restoration of its natural habitats and the maintenance of biodiversity. In practical terms, the preparation of 2,000 seedlings demonstrates a significant scaling up of efforts to establish a sustainable population of Boilam trees (Hasnat et al., 2019; Moïse et al., 2005; Shinde & Chavan, 2017). The insights gained from this study on pre-sowing treatments can be applied to other species with similar ecological requirements, thereby broadening the impact of these findings on forest restoration practices. By ensuring that seedlings are well-prepared before planting, forestry managers can optimize resource use and improve the outcomes of their restoration projects. Overall, the data strongly support the use of specific pre-sowing treatments to enhance seedling growth parameters, such as height and collar diameter (Mariam & Alamgir, 2022). These treatments are not only beneficial for the immediate growth of the seedlings but also for their long-term survival and establishment in reforestation and conservation programs. The successful propagation and planting of Boilam seedlings will contribute to the genetic diversity and resilience of forest ecosystems, aiding in the overall goal of environmental sustainability and biodiversity conservation.

Biomass of seedlings

The study revealed that leaf fresh weight was heterogeneous, with Treatment 2 (T2) (0.83>p=0.05) significantly differing from Treatments T0, T1, and T3 (Alpha<p=0.05). Homogeneity was observed in Treatment T0, which showed no significant difference with T2 (0.83>p=0.05).

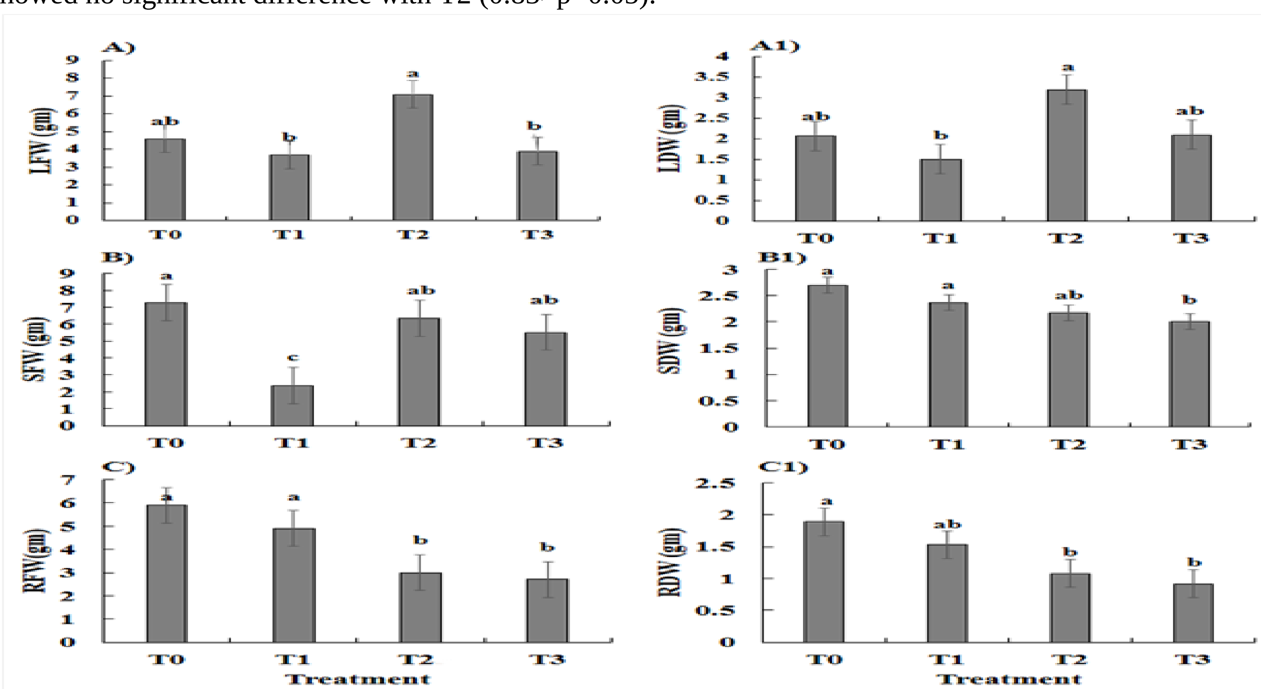

Figure 5. Effects of different pre-sowing treatments on Leaf, Shoot and Root fresh and Dry weight of *Anisoptera scaphula* seedling in Nursery, LFW(g)= Leaf Fresh Weight, LDW(g)= Leaf Dry Weight, SFW(g)= Shoot Fresh Weight, SDW(g)=Shoot Dry Weight, RFW(g) =Root Fresh Weight, RDW(g) =Root Dry Weight. Note: the same letter(s) indicates no significant difference at p≤0.05, according to DMRT. I (error bar) indicates the standard error of means.

The highest leaf dry weight was recorded in T2 (3.2g), significantly differing ($p<0.005$) from T1, T0, and T3. For shoot fresh weight, the highest was recorded in T0 (7.28g), which significantly differed from T1, T2, and T3. Similarly, the highest shoot dry weight was recorded in T0 (2.70g) with no significant difference from T2, T1, and T3. Root fresh weight was highest in T0 (5.6g), differing significantly ($p<0.005$) from Treatments T1, T2, and T3, and the highest root dry weight was also in T0 (1.89g), significantly differing ($p<0.005$) from T1, T2, and T3 (Figure 5). Based on these findings, it is recommended to adopt T2 for leaf production due to its superior leaf dry weight, while T0 is suggested for maximizing root and shoot biomass, balancing the overall plant growth for optimal reforestation and conservation efforts (Lúcio & Sari, 2017; Ranal et al., 2009; Sultana et al., 2021).

Conclusion and Recommendations

The study investigated the germination performance and initial growth of seedlings, focusing on the effects of various pre-sowing treatments. The findings underscore that pre-sowing treatments significantly enhance seed germination and seedling growth, offering a cost-effective and time-efficient method for producing quality seedlings of the native tree species *Anisoptera scaphula*. The results showed that pre-sowing treatments notably influenced seed germination potential. Specifically, dewinged seeds sown invertedly began germination one week after sowing and continued for up to 20 days. The fastest germination, with an imbibition period of six days, was observed in T2 (seeds soaked in water for 12 hours followed by air drying for 12 hours) and T1 (seeds soaked in water for 12 hours). In contrast, the control group (T0) exhibited the slowest germination, with an imbibition period of 20 days. The germination percentage was highest in T2 at 84.2%, followed by T1 at 74.7%, with the lowest germination percentage of 52.3% in T0. Furthermore, the mean maximum height of seedlings was recorded as 46.4 cm in T2, 37.9 cm in T1, and 28.8 cm in T0. Similarly, the collar diameter of seedlings was greatest in T2 at 0.76 cm, followed by 0.64 cm in T1, and 0.54 cm in T0. T2 also exhibited the highest leaf fresh weight, while T0 had the highest root and shoot fresh and dry weight. The outcomes of this research have significant implications for mass-scale seed production and conservation efforts. By adopting the T2 treatment, the species stands a better chance of being removed from the endangered list and can potentially reestablish its dominance in the ecosystem, providing habitat for various other flora and fauna. Based on the study’s findings, several recommendations can be made: Adoption of T2 Treatment for Large-Scale Plantations: The T2 treatment (seeds soaked in water for 12 hours followed by air drying for 12 hours) should be utilized for large-scale plantation efforts. This method has demonstrated the highest germination rates and optimal seedling growth, making it the most effective for conservation and reforestation projects.

Declaration

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Ethics approval/declaration: Required ethics were followed.

Consent to participate: All the authors gave consent for participation.

Consent for publication: All the authors gave their consent for the paper to be published

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request, included within the article and its supplementary materials, etc. This statement ensures transparency and reproducibility of the research findings.

Authors' Contribution: Author 1: Atia Hoque Subah, Data collection, data curation, formal analysis, investigation, methodology, writing original draft. Author 2: Md. Aktar Hossain, conceptualization and supervision, validation. Author 3: Mohd Imran Hossain Chowdhury, research design, project administration, validation, manuscript writing. Author 4: Sumya Asma Cynthia, formal analysis, data visualization, data collection, manuscript editing and writing. Author 5: Mehedi Hasan Rakib, manuscript writing and editing." All authors have read and agreed to the published version of the manuscript.

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