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Influence of Hydro, Mechanical, and Chemical Treatments to Seed for Germination and Seedling Growth of *Saraca asoca* (Roxb. De Wilde)

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Abstract: It has been noticed that Saraca asoca (Roxb. de Wilde) populations are drastically declining in the wild. Conserving such populations is crucial because of the numerous ecological, cultural, and economic values. The purpose of our study was to determine if germination and seedling growth could be improved for globally vulnerable Ashoka populations. The study analyzed the effect of various hydro, mechanical, and chemical pre-sowing treatments on the germination and one-year growth of Ashoka seedlings. Our results demonstrated that mechanical (exposing the seed cotyledons) and soaking of seeds in hot water treatments (60 °C) were better than all other water- and chemical-based pre-sowing treatments used in the study of enhancing germination. Nevertheless, chemical treatments were better for the growth and survival of the seedlings. This methodology offers to restore the scattered populations of Ashoka that are facing the risk of extinction in the wild while successfully meeting the commercial demand for this medicinal tree.

Keywords: ex-situ conservation; germination; regeneration; seedling vigor index; sustainability; tree populations

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1. Introduction

Global utilization of traditional medicinal plants, including tree species, has tremendously increased in the last decade because of their extensive harvesting and use in the manufacturing of a vast number of western and traditional medicines and herbal-based products. Ongoing losses of medicinal plant resources are associated with the growing demand for plant-based medicines [1], which results in overexploitation and inadequate knowledge regarding the sustainable management and utilization of wild medicinal plant populations [2]. In addition to these pressures, there is the overarching impact of climate change, which could affect the distribution of these vulnerable populations that are predominantly existing and harvested in the wild [3–5]. Therefore, it is essential to develop and put into practice regeneration and conservation strategies for these overused forest species.

Ashoka (Saraca asoca (Roxb. de Wilde)) is a commercially important medicinal tree species indigenous to Assam, E. Pakistan, Northern Myanmar, Malaysia, Sri Lanka, and

South India [6]. The bark of this valuable medicinal tree, which is used to treat a variety of gynecological disorders, contains a variety of chemicals (amenorrhea, dysmenorrhea, menorrhagia, deficient menstruation, postpartum pain, etc.), bacterial infections, skin conditions, cancer, circulatory and cardiovascular conditions, and numerous other conditions [7–9]. In terms of chemical profiling and secondary metabolite extraction, the various therapeutic, industrial, and pharmaceutical benefits of the species are becoming increasingly important to plant biotechnologists and pharmacologists [10–14].

Ashoka grows as an evergreen understory tree in tropical forest types and shows better growth in moist, well-drained soils [15]. The bark, flowers, and seeds are collected and extracted from the wild population in an unsustainable manner, which has resulted in the rapid decline of the populations [16]. The species is currently listed as globally vulnerable in the IUCN red list due to its scattered wild population with limited distribution and its recalcitrant nature of seeds [17]. Additionally, over extraction of bark due to higher demand by the pharmaceutical industry is now threatening its natural existence [12]. Therefore, Ashoka has been listed as one of the top priority species by the National Medicinal Plants Board (NMPB) and Planning Commission, Government of India, for conservation as well as for the production of quality planting material [14]. In India, the work for conservation, domestication, and popularization of Ashoka among the farmers has been initiated, but very little success has been achieved [11,18].

Saraca asoca is regenerated or propagated by seeds or vegetative means, but large-scale multiplication of the plant is difficult as the seed bearing among the wild population is poor, and these are vastly consumed by wildlife due to the sweet kernels, resulting in seed scarcity [14,19–21]. As a result, it is crucial to standardize the propagation procedures for this species in order to produce top-quality planting materials for extensive planting and conservation. This is possible only through various effective mass multiplication techniques that can ensure the survival of seeds in the early stages of growth [22,23]. In order to conserve and commercialize the species, information on seed quality, seed requirement, pre-sowing treatment, germination behavior, seedling vigor, time, and duration of seed collection is important [24–28]. However, information on naturally available, cost-effective protocols such as growing media, pre-sowing treatment, and their interaction with the growing conditions for multiplication of this medicinal plant is scarce. Farmers from the sub-Himalayan region of West Bengal, India, are currently diversifying their farming through medicinal plant cultivation [29]. However, the region lacks quality planting materials for the species [30]. Hence, this study was carried out to determine the efficacy of hydro-, mechanical, and chemical pre-sowing techniques for improving the seed germination of Ashoka. The second objective was to test the effect of these treatments on seedling vigor. The results of this study are expected to help the growers acquire quality planting material for Ashoka within a short period of time, facilitating less intervention with natural populations in the wild.

2. Materials and Methods

2.1. Experimental Design and Site

The experimental study was conducted in the central forest nursery greenhouse under the Department of Forestry, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India, from May 2018 to May 2020. The study included 15 different treatments and a control; each was replicated three times in a randomized block design (Table 1). The effect of pre-sowing treatments and the one-year growth of seedlings for two consecutive years were analyzed. The study site is in the Terai region of West Bengal, India, and is 43 m above mean sea level with a subtropical climate. According to agro-meteorological data from the Uttar Banga Krishi Viswavidyalaya, the humidity ranged from 80 to 87%, and the total amount of rain received from the south-west monsoon was 200–300 cm, of which 80% was received from June to August. The highest temperature recorded was 34 °C in May, and the lowest was 7.5 °C in January. The summer and winter seasons were both mild. The temperature did not show wide variations from the average climatic measurements for the region during the research

period. In this belt of the Terai region, mature *S. asoca* trees are often found scattered with very little wild population, thereby making it an ideal location to conduct the study.

Table 1. Details of the treatments.

Treatments	
T_1—Soaked in 180 ppm SA for 24 h	T_9—Soaked in NW 24 h
T_2—Soaked in 120 ppm SA for 24 h	T_{10}—Soaked in BW for 5 min
T_3—Soaked in 90 ppm SA for 24 h	T_{11}—Soaked in BW for 3 min
T_4—Soaked in 30 ppm SA for 24 h	T_{12}—Soaked in BW for 1 min
T_5—Rubbed seed coat with sandpaper (mechanical pre-sowing treatment)	T_{13}—Soaked in 100 ppm Tu for 24 h
T_6—Soaked in HW (subsequently cooled) for 12 h	T_{14}—Soaked in 200 ppm Tu for 24 h
T_7—Soaked in HW (subsequently cooled) for 24 h	T_{15}—Soaked in 300 ppm Tu for 24 h
T_8—Soaked in NW for 12 h	T_{16}—No pre-sowing treatment (control)
SA—salicylic acid; ppm—parts per million; hr—hour; NW—normal water; BW—boiling water; m—minute; Tu—thiourea; HW—hot water	

2.2. Collection of Seeds

Trees having more than 10 years of age were selected from different locations in the Terai Zone of West Bengal, India, for seed collection (Table 2). A total of sixty-seven Ashoka trees were marked with their geographical location for seed collection. Mature seeds were collected from pods during May 2018 and 2019. The seeds were then cleaned with normal water, and healthy seeds were selected based on sorting by the water flotation method [31]. The seeds that were submerged in the water were used for the study. According to the International Seed Testing Association, 2,3,5-Triphenyl Tetrazolium Chloride (TTC) was used to test the viability of seeds [32], and 90% of the seeds were found to be viable.

Table 2. Details of the locations of seed collection.

Location	Latitude	Longitude	Altitude	No. of Trees
Rajbari Heritage Park, Cooch Behar, West Bengal	26°19′37.01″ N	89°23′22.98″ E	46 m	20
Nripendra Narayan Park, Cooch Behar, West Bengal	26°19′32.55″ N	89°26′59.91″ E	45 m	15
Rambhola High School, Cooch Behar, West Bengal	26°20′01.01″ N	89°26′37.12″ E	44	05
Manidra Nath High School, Cooch Behar, West Bengal	26°20′49.39″ N	89°26′17.71″ E	46	05
UBKV Campus, Cooch Behar, West Bengal	26°24′13.70″ N	89°23′05.85″ E	43	02
Khagenhat, Dhupguri, West Bengal	26°37′26.45″ N	89°04′53.97″ E	79	10
Bankura, West Bengal	23°12′21.17″ N	87°01′07.08″ E	91	10

2.3. Pre-Sowing Treatments

The study utilized a total of 16 pre-sowing treatments (Table 1). Chemical treatments included soaking in various concentrations of salicylic acid (30, 90, 120, and 180 ppm) and thiourea (100, 200, and 300 ppm) for 24 h. Mechanical pre-sowing treatments included the removal of the seed coat with sandpaper. Hydro-pre-sowing treatment was carried out by soaking the seeds in hot water at 60 °C (subsequently cooled) for 12 and 24 h, soaking in

normal water (water at room temperature) for 12 and 24 h, and soaking in boiling water (100 °C) for 1, 3, and 5 min, respectively. The sixteenth treatment was the control and had no pre-sowing treatment.

2.4. Germination Studies

For each treatment, 120 uniform-sized seeds (40 seeds in each replication) were planted in polybags (5 × 8 cm) filled with nursery soil to analyze the effects of pre-sowing treatments on germination. From the date of sowing, daily observations of seed germination were kept for up to 40 days. Normal water was consumed at room temperature, hot water was consumed at just the human tolerance limit of 60 °C, and boiling water was consumed at 100 °C. According to ISTA guidelines, various germination parameters, including germination initiation, germination%, germination capacity, germination energy, germination index, germination rate, germination value, and peak value, were recorded [33]. Seeds with a minimum of 5-millimeter-long radicals are considered germinated [26].

2.5. Seedling Growth Studies

The seedlings continued to grow in the polybags, and data were recorded every 30 days after sowing (DAS) up to one year and repeated the same the next year on various growth parameters such as shoot length, root length, total seeding length, collar diameter, root diameter, number of branches, number of leaves, fresh root weight, fresh shoot weight, and root-shoot ratio. During the observation period, seedlings were kept in open fields with regular watering in the morning hours. The experiment was carried out from May 2018 to May 2020, over a period of two years. For recording growth observations and mean expression, five seedlings were randomly chosen from each replication in each treatment. The health of the seedlings produced is reflected in the seedling vigor index. It accounts for seedling length and germination percentage [34]. Seedling length recorded at 90 days after sowing (DAS) was used to calculate seedling vigor index (SVI).

$$\text{SVI} = (\text{Germination percent}) * (\text{Seedling length})$$

2.6. Data Analysis

All experiments were conducted twice; the germination and seedling growth were subjected to a one-way ANOVA through a randomized block design, and the pooled mean values were separated by a post-hoc Tukey's honestly significant difference (HSD) test. The software program SPSS Base 10.0 was used for all analyses [35].

3. Results

3.1. Effect of Seed Pre-Sowing Treatments on Germination Attributes

The germination parameters were significantly influenced by the seed pre-sowing treatments ($p < 0.05$) (Table 3). The germination initiation ranged from 16.8 to 26.5 days. In terms of germination initiation, the pre-sowing treatment T_6 (16.8 days) was the fastest, followed by T_5 (17.8 days), T_{13} (18.7 days), and T_{14} (18.8 days), and the slowest was T_{16} (26.5), followed by T_{11} (23.8) and T_{12} (23.0).

Table 3. Effects of pre-sowing treatments on germination attributes for Ashoka seeds.

Treatments	Initiation of Germination (Days)	Germination (%)
T_1	21.5 ^{bcd}	64.9 ^{d}
T_2	19.3 ^{def}	66.5 ^{cd}
T_3	19.0 ^{def}	61.7 ^{de}
T_4	18.5 ^{efg}	65.8 ^{cd}
T_5	17.8 ^{ef}	86.1 ^{a}

Table 3. *Cont.*

Treatments	Initiation of Germination (Days)	Germination (%)
T_6	16.8 ^{f}	84.7 ^{a}
T_7	19.7 ^{cdef}	64.3 ^{d}
T_8	19.3 ^{def}	76.8 ^{b}
T_9	20.5 ^{bc}	67.8 ^{cd}
T_{10}	22.3 ^{ab}	19.7 ^{f}
T_{11}	23.8 ^{b}	17.6 ^{f}
T_{12}	23.0 ^{ab}	21.5 ^{f}
T_{13}	18.7 ^{def}	66.1 ^{cd}
T_{14}	18.8 ^{def}	73.3 ^{bc}
T_{15}	19.8 ^{cdef}	81.5 ^{ab}
T_{16}	26.5 ^{a}	55.9 ^{e}
SE_M	**1.6**	**4.0**
SE_D	**1.1**	**2.8**
HSD	**3.6**	**8.2**

T—treatments (Table 1); SE_M—standard error of the mean; SE_D—standard error of difference; HSD—Tukey's honestly significant difference. Different letters (a, b, c, d, e, f and g) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different.

Pre-sowing treatments significantly influenced (both increased and decreased) seed germination compared to control. Mechanical pre-sowing (T_5) was recorded with the highest germination (86.1%), which was closely followed by hot water pre-sowing (T_6, 84.7%) and chemical pre-sowing (T_{15}, 81.5%). For the control (T_{16}), 55.9% germination was obtained when seeds were not subjected to any pre-sowing treatments. These three treatments, however, were statistically on par with each other. On average, treatments T_5, T_6, and T_{15} enhanced germination by 28.7–30.1% compared to control. Soaking the seeds in boiling water for one (T_{12}), three (T_{11}), and five (T_{10}) minutes reduced germination for both years as compared to control by 35.0–43.2% and 30.6–37.5%, respectively. Remaining pre-sowing treatments with normal water ($T_{8–9}$), 24 h soaking in hot water (T_7), dipping in thiourea (100 and 200 ppm; $T_{13–14}$), and dipping in salicylic acid (180, 120, 90, and 30 ppm; $T_{1–4}$) also significantly enhanced germination of *S. asoca* by 5.8–20.8%. The poorest germination recorded was 17.6–21.5% when seeds were soaked in boiling water for 1–5 min ($T_{10–12}$).

Initiation of germination in *S. asoca* was significantly influenced by the pre-sowing treatments (Table 3). With the exception of subjecting the seeds to boiling water for 1–5 min ($T_{10–12}$), all other seed pre-sowing treatments, including control, had a statistically similar influence on seed germination capacity. On average, days taken for germination (IG) were significantly different in treatments $T_{3,5,15}$ (9.3–13.0 days) and T_{11} (18.8 days) than in the control (T_{16}—15.8 days), while the rest of the treatments (14.7–18.1 days) had no statistical difference from the control. Treatment T_{15} took the minimum time to complete germination (9.3 days), while seeds subjected to boiling water for three minutes (T_{11}) took the maximum time to complete germination (18.7 days). Pre-sowing treatments found to have higher germination were also recorded with higher germination energy, germination capacity, germination value, peak value, and other associated parameters (Appendix A, Table A1).

Increasing the soaking time of seeds in hot (T7) and normal (T9) water by 12 h (up to 24 h) reduced germination by 20.4 and 9.0%, respectively, compared to 12 h soaking in hot (T6) and normal (T8) water. Moreover, the associated germination parameters also generally decreased non-significantly ($p = 0.061$) with an increasing soaking time of 12 h. It was also observed that subjecting the seeds to increasing temperatures of water from normal room temperature (25–30 °C) to hot water (70 °C) insignificantly increased the

germination and its associated parameters, while concurrently increasing the soaking time also insignificantly decreased the germination. Further increasing the temperature of water to the boiling point significantly decreased germination. Similarly, in the present study, increasing the concentration of salicylic acid from 30 ppm to 180 ppm ($T_{1–4}$) and thiourea from 100 ppm to 300 ppm ($T_{13–15}$) with the same exposure time of 24 h of soaking progressively increased the germination over control (T_{16}) by 5.8–10.6% and 10.2–25.6%, respectively. These pre-sowing treatments were statistically similar to each other except treatments T_{13} and T_{15}, which differed significantly ($p = 0.04$). The increase in germination with these chemical pre-sowing treatments was also significant over control (T_{16}), except for treatment T_3, which was statistically similar to control. On average, thiourea-based pre-sowing treatments gave 8.9% higher germination than with salicylic acid. Thus, among the chemical-based pre-sowing treatments, thiourea was a more efficient germination enhancer for *S. asoca*.

3.2. Effect of Pre-Sowing Treatments on Seedling Growth

3.2.1. Shoot, Root, and Total Seedling Length

Shoot and root length of *S. asoca* seedlings also showed varying results when compared to control. The overall trend in growth over the months can be visualized in Table 4. Shoot and root length after 90 and 365 days of sowing (DAS) were significantly influenced by different seed pre-sowing treatments (Table 4). All the pre-sowing treatments were recorded with longer shoot and root length than the control, but not all treatments were significant ($p = 0.07$). The longest mean shoot length recorded at 365 DAS was 68.1 cm with thiourea 200 ppm (T_{14}), followed by 59.6 cm with salicylic acid 120 ppm (T_2), 54.5 cm with salicylic acid 90 ppm (T_3), 53.8 cm with hot water for 12 h (T_6), 52.4 cm with normal water 24 h (T_9), and 50.4 cm with normal water 12 h (T_8). Despite being statistically similar to one another, these treatments had significantly longer shoots than the control (T_{16}—33.73 cm) ($p = 0.001$). A similar trend was also observed for root length at 90 and 365 DAS when subjected to the seed treatments.

Table 4. Effect of pre-sowing treatments on seedling shoot and root length (cm).

T	SL		RL	
	90 DAS	**365 DAS**	**90 DAS**	**365 DAS**
T_1	18.7 ^{abc}	43.4 ^{bc}	14.9 ^{cdefg}	41.1 ^{abc}
T_2	19.6 ^{abc}	59.7 ^{ab}	21.4 ^{a}	45.7 ^{ab}
T_3	19.3 ^{abc}	54.6 ^{bcd}	13.7 ^{defg}	31.8 ^{cd}
T_4	19.5 ^{abc}	42.7 ^{cde}	13.7 ^{defg}	33.4 ^{bcd}
T_5	21.7 ^{ab}	47.5 ^{bcd}	21.8 ^{a}	33.0 ^{cd}
T_6	22.4 ^{a}	53.8 ^{abc}	18.7 ^{abc}	28.6 ^{d}
T_7	19.2 ^{abc}	48.3 ^{bcd}	13.6 ^{defg}	26.9 ^{d}
T_8	18.2 ^{bc}	50.5 ^{bcd}	17.6 ^{abcde}	31.7 ^{cd}
T_9	18.8 ^{abc}	52.4 ^{bcd}	15.9 ^{bcdef}	25.7 ^{d}
T_{10}	18.9 ^{abc}	39.8 ^{de}	13.3 ^{efg}	24.0 ^{d}
T_{11}	18.3 ^{bc}	42.0 ^{cde}	16.6 ^{abcdef}	25.8 ^{d}
T_{12}	18.8 ^{abc}	43.2 ^{cde}	17.6 ^{abcde}	30.5 ^{cd}
T_{13}	18.2 ^{bc}	42.7 ^{cde}	12.2 ^{fg}	32.4 ^{cd}
T_{14}	21.0 ^{abc}	68.1 ^{a}	21.8 ^{a}	46.7 ^{a}
T_{15}	18.9 ^{abc}	45.9 ^{bcde}	14.0 ^{defg}	30.4 ^{cd}
T_{16}	16.4 ^{c}	33.7 ^{e}	8.6 ^{g}	21.4 ^{d}
SE_M	**1.2**	**7.1**	**2.33**	**5.99**
SE_D	**0.9**	**5.0**	**1.64**	**4.24**
HSD	**3.9**	**14.3**	**4.76**	**12.08**

SL—shoot length (in cm); RL—root length (in cm); DAS—days after sowing; SE_M—standard error of the mean; SE_D—standard error of difference; HSD—Tukey's honestly significant difference. Different letters (a, b, c, d, e, f and g) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different.

Seed treatments also increased root length as compared to control, but not always significantly ($p = 0.067$). The longest mean root length was 46.7 cm with T_{14}, followed by 45.7 cm with T_2, and 41.1 cm with T_1. These treatments were statistically similar ($p = 0.08$) to each other but significantly increased the mean root length over control (T_{16}—21.4 cm). Similarly, the longest seedlings (shoot and root) were produced when the seeds were subjected to thiourea 200 ppm for 24 h (T_{14}), followed by 99.8 cm with salicylic acid 120 ppm for 24 h (T_2), 89.8 cm with hot water for 12 h (T_6), and 89.1 cm with normal water for 12 h (T_8). From the table, it is seen that these treatments significantly increased the total seedling length compared to the control (T_{16}—63.8 cm), though they were statistically similar to each other ($p = 0.073$).

3.2.2. Seedling Vigor Index

The seedling vigor index was significantly influenced by the pre-sowing treatments (Table 5). This parameter indicates the quality (health, vigor, and uniformity) of seedlings, i.e., a higher index value implies a better quality of seedlings. On average, pre-sowing treatments improved the index significantly (2105–3748) as compared to control (T_{16}—1541), except for boiling water treatments ($T_{10–12}$), which significantly reduced the index (616–789). Treatments with higher germination ($T_{5,6,14}$) were also recorded to have a higher seedling vigor index because seeds subjected to faster and higher germination, i.e., rubbing by sandpaper (T_5), soaking in hot water for 12 h (T_6), and soaking in thiourea at 200 ppm for 24 h (T_{14}), produced more healthy, vigorous, and uniform seedlings than when subjected to other treatments. The overall trend in the effect of pre-sowing treatment on seedling index is given in Table 5.

Table 5. Effects of pre-sowing treatments on seedling vigor index.

Treatment	1st Year	2nd Year	MEAN
T_1	2060 ^{abcd}	2241 ^{cd}	2171 ^{cd}
T_2	1941 ^{abcd}	2967 ^{abcd}	2443 ^{abcd}
T_3	1752 ^{bcd}	2813 ^{abcd}	2254 ^{cd}
T_4	1494 ^{de}	2731 ^{abcd}	2105 ^{d}
T_5	3966 ^{a}	3528 ^{a}	3748 ^{a}
T_6	3093 ^{b}	3421 ^{a}	3279 ^{b}
T_7	1564 ^{bcd}	2684 ^{abcd}	2116 ^{cd}
T_8	2657 ^{abcd}	2898 ^{abcd}	2777 ^{abc}
T_9	1972 ^{cd}	2579 ^{bcd}	2300 ^{bcd}
T_{10}	534 ^{ef}	759 ^{e}	648 ^{e}
T_{11}	374 ^{ef}	892 ^{e}	616 ^{e}
T_{12}	520 ^{ef}	1133 ^{e}	798 ^{e}
T_{13}	1769 ^{bcd}	2593 ^{bcd}	2198 ^{cd}
T_{14}	3168 ^{b}	2744 ^{abcd}	2827 ^{ab}
T_{15}	2198 ^{abcd}	2879 ^{abcd}	2571 ^{abcd}
T_{16}	977 ^{ef}	2123 ^{d}	1541 ^{d}
SE_M	**236.05**	**346.22**	**187.08**
SE_D	**333.82**	**489.63**	**264.57**
HSD	**679.98**	**997.34**	**538.91**

SE_M—standard error of the mean; SE_D—standard error of difference; HSD—Tukey's honestly significant difference. Different letters (a, b, c, d, e and f) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different.

3.2.3. Collar and Root Diameter of Seedlings

The collar and root diameter of the seedlings increased with pre-sowing treatment at 90 and 365 DAS, but not always significantly (Table 6). Soaking the seeds with thiourea at 200 ppm for 24 h (T_{14}) produced seedlings with both the highest collar (4.4 and 11.0 mm) and roots (3.06 and 8.44 mm) diameter at 90 and 365 DAS, respectively. Both the collar and root diameters recorded with treatment T_{14} were significantly higher than the control (2.4 and 6.3 mm and 1.3 and 5.4 mm at 90 and 365 DAS, respectively). The overall trend in the effect of pre-sowing treatments on seedling collar and root mean diameter is given in Table 6.

Table 6. Effects of seed treatments on seedling collar and root diameter.

T	CD		RD	
	90 DAS	**365 DAS**	**90 DAS**	**365 DAS**
T_1	3.1 ^{bc}	8.0 ^{bc}	2.4 ^{abc}	7.4 ^{ab}
T_2	3.5 ^{ab}	10.9 ^{ab}	2.7 ^{ab}	7.5 ^{ab}
T_3	2.9 ^{bc}	7.9 ^{bc}	2.6 ^{abc}	7.5 ^{ab}
T_4	2.6 ^{c}	7.7 ^{c}	2.5 ^{abc}	6.8 ^{abc}
T_5	3.2 ^{bc}	7.1 ^{c}	2.0 ^{bcde}	6.5 ^{abc}
T_6	3.1 ^{bc}	7.2 ^{c}	2.0 ^{bcde}	6.8 ^{abc}
T_7	3.0 ^{bc}	7.2 ^{c}	1.6 ^{de}	6.4 ^{abc}
T_8	2.9 ^{bc}	7.8 ^{bc}	2.4 ^{abcd}	6.6 ^{abc}
T_9	2.8 ^{bc}	7.1 ^{c}	1.4 ^{e}	6.5 ^{abc}
T_{10}	2.7 ^{bc}	6.6 ^{c}	1.6 ^{cde}	6.0 ^{bc}
T_{11}	2.8 ^{bc}	6.9 ^{c}	2.0 ^{bcde}	6.5 ^{abc}
T_{12}	3.1 ^{bc}	7.2 ^{c}	2.6 ^{ab}	7.0 ^{abc}
T_{13}	2.9 ^{bc}	7.2 ^{c}	2.2 ^{abcde}	5.5 ^{c}
T_{14}	4.4 ^{a}	11.0 ^{a}	3.0 ^{a}	8.4 ^{a}
T_{15}	3.0 ^{bc}	7.1 ^{c}	2.1 ^{bcde}	7.6 ^{ab}
T_{16}	2.4 ^{c}	6.3 ^{c}	1.3 ^{e}	5.4 ^{c}
SE_M	**0.38**	**1.03**	**0.42**	**0.82**
SE_D	**0.27**	**0.73**	**0.30**	**0.58**
HSD	**0.78**	**2.07**	**0.86**	**1.66**

CD—collar diameter (in mm); RD—root diameter (in mm); T—treatments; SE_M—standard error of the mean; SE_D—standard error of difference; HSD—Tukey's honestly significant difference. Different letters (a, b, c, d and e) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different.

3.2.4. Number of Branches and Leaves per Seedling

The mean numbers of branches and leaves per seedling were also significantly influenced by seed pre-sowing treatments (Table 7).

Table 7. Effects of pre-sowing treatments on the number of branches and leaves.

T	NOB		NOL	
	180 DAS	**365 DAS**	**90 DAS**	**365 DAS**
T_1	1.0 ^{b}	2.1 ^{c}	10.8 ^{abcde}	50.6 ^{cd}
T_2	1.1 ^{ab}	4.0 ^{ab}	13.6 ^{ab}	118.0 ^{ab}
T_3	1.1 ^{ab}	2.3 ^{bc}	8.3 ^{de}	38.5 ^{cd}
T_4	1.0 ^{b}	2.1 ^{c}	7.6 ^{e}	34.6 ^{cd}
T_5	1.0 ^{b}	2.1 ^{c}	12.8 ^{abc}	51.6 ^{cd}
T_6	1.5 ^{a}	3.3 ^{abc}	13.5 ^{ab}	95.8 ^{bc}
T_7	1.1 ^{ab}	2.1 ^{c}	11.1 ^{abcde}	67.1 ^{bcd}
T_8	1.1 ^{ab}	2.5 ^{bc}	12.0 ^{abcd}	52.9 ^{cd}
T_9	1.0 ^{b}	2.1 ^{c}	9.1 ^{de}	53.6 ^{cd}
T_{10}	1.0 ^{b}	1.6 ^{c}	9.6 ^{bcde}	46.6 ^{cd}
T_{11}	1.0 ^{b}	1.8 ^{c}	13.0 ^{abc}	58.3 ^{cd}
T_{12}	1.1 ^{ab}	2.3 ^{c}	12.1 ^{abc}	51.6 ^{cd}
T_{13}	1.0 ^{b}	3.1 ^{abc}	9.0 ^{cde}	47.6 ^{e}
T_{14}	1.5 ^{a}	4.6 ^{a}	16.3 ^{a}	178.0 ^{a}
T_{15}	1.1 ^{ab}	2.8 ^{bc}	12.0 ^{abcd}	52.3 ^{bcd}
T_{16}	1.0 ^{b}	1.50 ^{c}	7.17 ^{e}	26.17 ^{d}
SE_M	**0.21**	**0.88**	**2.15**	**1.77**
SE_D	**0.15**	**0.62**	**1.52**	**2.46**
HSD	**0.43**	**1.78**	**4.34**	**4.02**

T—treatment; NOB—number of branches; NOL—number of leaves; DAS—days after sowing; SE_M—standard error of the mean; SE_D—standard error of difference; HSD—Tukey's honestly significant difference. Different letters (a, b, c, d and e) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different.

3.2.5. Fresh Shoot Weight, Root Weight, and Root Shoot Ratio

Most of the treatments differed significantly in terms of the mean number of branches per seedling over two years (Table 7) as compared to the control, except for treatments T_6 and T_{14} (1.5 each) at 180 DAS and T_{14} (4.6) and T_2 (4.0) at 365 DAS (Table 8). However, the treatments increased the mean number of leaves per seedling by two years at 90 and 365 DAS, but there was no significant difference (p = 0.08). Treatment T_{14} produced the highest number of leaves at 90 (16.3) and 365 (178) DAS, followed by T_2 with 13.7 and 118.0 leaves at 90 and 360 DAS, respectively (p = 0.08). Treatments T_2 and T_{14} produced a statistically similar number of leaves per seedling but significantly produced a greater number of leaves than the control (7.17 and 26.17 number of leaves at 90 and 365 DAS, respectively).

Table 8. Effect of seed treatments of *S. asoca* on mean fresh root and shoot weight and root-shoot ratio.

T	FSW		FRW		FRSR	
	90 DAS	**365 DAS**	**90 DAS**	**365 DAS**	**90 DAS**	**365 DAS**
T_1	4.3 ^{bcd}	43.3 ^{bc}	2.7 ^{bcd}	27.5 ^{ab}	0.6 ^{a}	0.6 ^{bcde}
T_2	5.1 ^{abc}	92.2 ^{ab}	3.3 ^{abc}	29.3 ^{ab}	0.6 ^{acd}	0.3 ^{cde}
T_3	5.1 ^{abc}	40.0 ^{bc}	2.6 ^{cd}	21.9 ^{bc}	0.5 ^{bcd}	0.5 ^{bcde}
T_4	3.7 ^{cd}	27.9 ^{c}	2.5 ^{cd}	17.3 ^{bc}	0.6 ^{abcd}	0.6 ^{bcde}
T_5	6.2 ^{a}	30.9 ^{c}	2.7 ^{bcd}	18.6 ^{bc}	0.4 ^{bcd}	0.6 ^{cde}
T_6	5.4 ^{ab}	32.7 ^{c}	3.7 ^{ab}	27.6 ^{ab}	0.6 ^{abcd}	0.8 ^{de}
T_7	4.1 ^{bcd}	30.7 ^{c}	2.9 ^{bcd}	15.9 ^{c}	0.7 ^{abcd}	0.5 ^{bcde}
T_8	4.4 ^{bcd}	36.9 ^{c}	3.1 ^{abcd}	22.1 ^{bc}	0.7 ^{abcd}	0.6 ^{cde}
T_9	4.1 ^{bcd}	29.2 ^{c}	2.6 ^{cd}	18.5 ^{bc}	0.6 ^{abcd}	0.6 ^{bcd}
T_{10}	3.8 ^{cd}	21.6 ^{c}	2.3 ^{cd}	14.5 ^{c}	0.5 ^{cd}	0.6 ^{bcd}
T_{11}	4.2 ^{bcd}	29.5 ^{c}	2.8 ^{bcd}	15.9 ^{c}	0.6 ^{abcd}	0.5 ^{bcde}
T_{12}	5.1 ^{abc}	31.6 ^{c}	3.0 ^{abcd}	21.7 ^{bc}	0.6 ^{ab}	0.6 ^{bc}
T_{13}	3.7 ^{cd}	37.8 ^{bc}	2.5 ^{cd}	21.3 ^{bc}	0.6 ^{abcd}	0.5 ^{bcde}
T_{14}	6.1 ^{a}	113.3 ^{a}	4.0 ^{a}	34.3 ^{a}	0.6 ^{abcd}	0.3 ^{cde}
T_{15}	4.9 ^{abc}	38.9 ^{bc}	2.8 ^{bcd}	27.8 ^{ab}	0.5 ^{cd}	0.7 ^{ab}
T_{16}	3.4 ^{d}	17.2 ^{c}	2.1 ^{d}	13.4 ^{c}	0.6 ^{abc}	0.7 ^{a}
SE_M	**0.75**	**14.58**	**0.50**	**5.25**	**0.07**	**0.15**
SE_D	**0.53**	**10.31**	**0.23**	**3.71**	**0.25**	**0.10**
HSD	**1.52**	**29.38**	**0.66**	**10.58**	**1.37**	**0.30**

FSW—fresh shoot weight (in grams); FRW—fresh root weight (in grams); FRSR—fresh root shoot ratio; DAS—days after sowing; T—treatments; SE_M—standard error of the mean; SE_D—standard error of difference; HSD—Tukey's honestly significant difference. Different letters (a, b, c, d and e) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different.

All the pre-sowing treatments improved the biomass of the seedlings as compared to the control, but not all treatments differed significantly from the control, and no particular trend was observed for root shoot ratio. Hormonal treatment using 120 ppm salicylic acid for 24 h and 200 ppm thiourea for 24 h significantly increased the growth biomass of seedlings as compared to the control and all other treatments. T_{14} (treatment with thiourea 200 ppm) produced maximum biomass (10.2 g and 147.6 g at 90 and 365 DAS), followed by T_2 (120 ppm salicylic acid) (8.56 g and 121.51 g at 90 and 365 DAS). Even though the remaining treatments produced higher biomass than the control, they were far inferior compared to T_2 and T_{14}. Moreover, variations in the concentration of these hormones had a drastic effect on seedling growth. This indicates that these hormones, beyond a particular concentration, can act as growth inhibitors instead of promoters or regulators. It can be said that for optimum and vigorous growth of *S. asoca* seedlings in the nursery, a treatment with thiourea at 200 ppm or salicylic acid at 120 ppm is optimal, as it also facilitates uniform and faster germination. Thiourea or salicylic acid applied to the seeds proved to be the better pre-sowing treatment for the growth of *S. asoca* seedlings because all growth parameters are better than other pre-sowing treatments at concentrations of 100–300 ppm, whereas the best germination boosters are mechanical (T_5) and hot water treatments (T_6).

4. Discussions

The study represented the effects of seed pre-sowing treatment on the improvement of germination, seedling vigor index, seedling length, and weight. In our study, the mechanical treatment (T_5, i.e., exposing the cotyledons), hot water treatment (T_6, i.e., soaking the seeds in hot water), and chemical treatment ($T_{13–15}$, i.e., seed dipping in thiourea) were found to be better than all other water- and chemical-based pre-sowing treatments for enhancing germination. These treatments removed and softened the seed coats, allowing moisture and air into the embryo for germination without any disturbance. These results are in accord with similar studies with other plant species, which also reported that scarification with sandpaper and hot water pre-sowing treatment of seeds for 12 h boosted seed germination. These procedures stripped away and softened the seed coats, allowing moisture and air to enter the embryo without impeding germination [36–39]. These treatments promote pre-germinative metabolic events by supporting and increasing embryo growth, thereby improving germination [40–44]. A variety of pre-sowing treatments have been found to significantly improve *S. asoca* seed germination. Mechanical treatments such as exposing the plumule and soaking in hot water are examples, as are chemical treatments such as salicylic acid and thiourea [45]. The rate of moisture loss in the seeds has also been found to have an effect on germination, with the highest occurring four weeks after harvest [13]. The optimal germination temperature has been determined to be 30–50 °C, with no dormancy period observed [46]. These findings support our research.

Soaking the seeds in boiling water for 1–5 min ($T_{10–12}$) destroyed the embryo, causing germination to fail, and increasing soaking time with water might have destroyed the embryo due to oxygen deficiency [26]. Increasing the soaking time at a higher temperature decreases the solubility of oxygen in the seed and destroys the carbohydrates, thus reducing germination [45,47]. Increasing temperature elevates the consumption of oxygen required for respiration, causing hypoxic effects on respiration due to soaking injury [48]. Further, increasing the water temperature to a boiling point significantly decreased germination due to the mortality of the developing embryo [26,49,50]. We observed the lowest mean germination time recorded for mechanical treatment (T_5), i.e., the fastest germination among the seed pre-sowing treatments studied, and vice versa for the poor-performing pre-sowing treatments [51–53]. The higher germination rate indicates faster germination due to suitable conditions developed for plumules to emerge out of the seed coat due to exposure to these external stimuli [35,54,55]. Pre-sowing treatments with higher germination were also recorded with higher germination energy, germination capacity, germination value, and peak value (Table A1), indicating higher, faster, and smoother germination [26,56,57]. Increasing the soaking time ($T_{7,9}$) might have saturated the seed with water, thus disturbing the oxygen and water balance in the seed and causing a deficiency of oxygen for the developing embryo, hindering its metabolic process and restricting germination [58]. Thus, a decrease in germination can be attributed to insufficient aeration [59]. Thiourea and salicylic acid were also commonly used to enhance germination in tree species with various concentrations, especially the higher concentrations reported to give better results [60–62]. On average, thiourea-based pre-sowing treatments ($T_{1–4}$) gave 8.87% higher germination than salicylic acid ($T_{13–15}$). Among the chemical-based pre-sowing treatments, thiourea was a more efficient germination enhancer for Saraca asoca.

Even though all the treatments showed an increase in growth aspects compared to the control, the longest shoot length recorded was with thiourea at 200 ppm (T_{14}), followed by salicylic acid at 120 ppm (T_2). According to studies, salicylic acid actually encouraged seed germination under stress rather than being crucial for germination under normal growth conditions [63–69]. In contrast, thiourea is a known stimulator of germination [65,68,70]. Salicylic acid was found to promote seed germination under stress rather than being crucial for seed germination under normal growth conditions, according to studies [65,71,72]. In addition, seed pre-sowing treatment, either with water or chemicals, activates DNA replication, increases RNA impairment and protein synthesis, promotes embryo growth, repairs deteriorated seed, and reduces metabolite leakage, which improves germination [73–76].

Low seed set, heavy predation of *S. asoca* seeds, and commercial exploitation have led to its existence in a fragmented population, making it endangered [8,76]. Through public participation, the conservation of *S. asoca* can be possible by developing its plantations in the agricultural landscape. However, the success of such plantations will depend on achieving uniform and optimum germination of *S. asoca* seeds for mass multiplication [58] through seed pre-sowing treatment, as it improves germination, germination speed, and seed vigor [77–81] through softening, puncturing, wearing away, or splitting the seed coat, permitting gaseous and moisture exchange [82].

Application of thiourea ($T_{13–15}$) or salicylic acid ($T_{1–4}$) in low concentration to the seeds proved to be the best pre-sowing treatment for the seedling growth of *S. asoca*, as all the growth parameters are better than others because at 100–300 ppm, thiourea and salicylic acid act as growth hormones, which enhance growth by increasing biomass [83,84]. Application of 200 ppm thiourea or 120 ppm salicylic acid treatments enhanced seedling growth as these biomolecules might have induced faster division and elongation of meristematic cells along with increased transport of metabolites, growing tips, and activity of hydrolyzing enzymes, which aided growth and development of the seedlings [15,83,84]. The development of roots and shoot growth may be enhanced through unrestricted nutrients obtained from soil and photosynthates in the growing meristems, ensuring the survivability of the seedlings [85,86]. This was demonstrated by the substantial impact that these treatments had on the germination and growth characteristics of *S. asoca* seedlings. The pre-sowing treatments had enhanced seed germination, germination speed, and seed vigor; thus, they helped in the establishment of *S. asoca* seedlings through uniform and vigorous growth [77–81].

5. Conclusions

Pre-sowing treatments of *S. asoca* included in the study significantly influenced the germination parameters estimated as compared to the control (no pre-sowing treatment). As rubbing the seed with sandpaper for mass multiplication will take more time unless we develop any suitable method or equipment to remove the seed coat from the seed carefully without harming the embryo, soaking the seeds in hot water for 12 h is advised for the Terai region of West Bengal. However, to achieve uniform, healthy, and vigorous seedlings of *S. asoca*, treatment of seeds with either 200 ppm thiourea or 120 ppm salicylic acid is recommended, as with these treatments, better growth parameters of seedlings were achieved. However, the seedling vigor index estimated with these mechanical and water pre-sowing treatments is higher than either of the two chemical treatments. Enhancing germination with these low-cost methods will help in mass production strategies for population enrichment or ex-situ conservation of this vulnerable tree species.

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Appendix A

Table A1. Effects of pre-sowing treatments on various germination attributes for Ashoka seeds.

T	IG	G%	GC	GE	GI	GR	GV	MDG	PV	MGT
T_1	21.5 ^{bcd}	64.9 ^{d}	79.8 ^{de}	22.9 ^{cd}	24.5 ^{f}	50.1 ^{c}	9.9 ^{ab}	2.1 ^{bc}	4.0 ^{a}	15.7 ^{bc}
T_2	19.3 ^{def}	66.5 ^{cd}	80.9 ^{cde}	34.7 ^{a}	29.6 ^{cdef}	52.5 ^{c}	8.4 ^{abc}	2.3 ^{ab}	5.0 ^{a}	14.7 ^{cd}
T_3	19.0 ^{def}	61.7 ^{de}	80.9 ^{cde}	22.0 ^{cd}	26.8 ^{def}	46.4 ^{c}	7.1 ^{abc}	1.8 ^{ef}	3.5 ^{a}	13.0 ^{de}
T_4	18.5 ^{efg}	65.8 ^{cd}	78.5 ^{e}	19.3 ^{de}	26.9 ^{cdef}	56.4 ^{bc}	9.3 ^{ab}	2.6 ^{a}	3.3 ^{ab}	14.3 ^{cd}
T_5	17.8 ^{ef}	86.1 ^{a}	93.3 ^{ab}	26.8 ^{bc}	24.7 ^{ef}	67.8 ^{a}	7.1 ^{abc}	2.1 ^{bcd}	4.1 ^{a}	11.8 ^{e}
T_6	16.8 ^{f}	84.7 ^{a}	95.0 ^{a}	27.2 ^{bc}	26.8 ^{cdef}	63.3 ^{ab}	5.7 ^{c}	1.6 ^{fg}	4.1 ^{a}	17.2 ^{ab}
T_7	19.7 ^{cdef}	64.3 ^{d}	85.7 ^{bcde}	19.3 ^{de}	28.7 ^{abcd}	50.0 ^{c}	8.4 ^{abc}	2.30 ^{b}	3.6 ^{a}	17.7 ^{ab}
T_8	19.3 ^{def}	76.8 ^{b}	88.6 ^{abc}	25.8 ^{bc}	29.7 ^{abc}	55.9 ^{bc}	6.6 ^{bc}	1.9 ^{cde}	4.1 ^{a}	16.1 ^{bc}
T_9	20.5 ^{bc}	67.8 ^{cd}	86.4 ^{bcd}	27.2 ^{bc}	27.9 ^{abcd}	52.0 ^{c}	5.2 ^{c}	1.1 ^{h}	3.8 ^{a}	17.8 ^{ab}
T_{10}	22.3 ^{ab}	19.7 ^{f}	38.5 ^{g}	9.7 ^{f}	29.0 ^{abcd}	16.7 ^{e}	5.1 ^{bc}	1.6 ^{fg}	1.6 ^{c}	18.1 ^{ab}
T_{11}	23.8 ^{b}	17.6 ^{f}	32.6 ^{g}	14.5 ^{ef}	30.5 ^{ab}	14.0 ^{e}	5.1 ^{bc}	1.4 ^{gh}	2.0 ^{bc}	18.7 ^{a}
T_{12}	23.0 ^{ab}	21.5 ^{f}	47.5 ^{f}	11.8 ^{f}	28.2 ^{abcd}	29.0 ^{d}	8.6 ^{abc}	2.2 ^{bc}	2.0 ^{bc}	17.7 ^{ab}
T_{13}	18.7 ^{def}	66.1 ^{cd}	89.9 ^{ab}	19.1 ^{de}	28.9 ^{abcd}	50.8 ^{c}	7.5 ^{abc}	2.0 ^{cde}	3.8 ^{a}	17.3 ^{ab}
T_{14}	18.8 ^{def}	73.3 ^{bc}	89.6 ^{ab}	19.1 ^{de}	32.1 ^{a}	55.5 ^{bc}	10.3 ^{a}	2.0 ^{bcde}	4.0 ^{a}	17.5 ^{ab}
T_{15}	19.8 ^{cdef}	81.5 ^{ab}	89.9 ^{ab}	30.4 ^{ab}	27.4 ^{cde}	50.9 ^{c}	9.6 ^{ab}	2.6 ^{a}	3.8 ^{a}	9.38 ^{f}
T_{16}	26.5 ^{a}	55.9 ^{e}	80.6 ^{de}	26.2 ^{bc}	25.8 ^{def}	46.4 ^{c}	7.6 ^{abc}	1.8 ^{def}	3.3 ^{a}	15.7 ^{bc}
SE_M	**1.6**	**4.0**	**3.8**	**2.78**	**1.42**	**5.01**	**1.21**	**0.14**	**0.69**	**1.20**
SE_D	**1.1**	**2.8**	**2.7**	**1.97**	**1.01**	**3.54**	**1.71**	**0.10**	**0.49**	**0.85**
HSD	**3.6**	**8.2**	**7.8**	**5.6**	**2.91**	**10.21**	**3.48**	**0.29**	**1.42**	**2.44**

Different letters (a, b, c, d, e, f, g and h) within each treatment value indicate a significant difference at $p < 0.05$. Means with the same letter are not significantly different. T—treatment; IG—initiation of germination; G%—germination percentage; GC—germination capacity; GE—germination energy; GI—germination index; GR—germination rate; GV—germination value; MDG—mean daily germination; PV—peak value of germination; MGT—mean germination time.

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