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Effect of pre-sowing treatments on seed germination and postgerminative seedling growth of *acacia angustissima* (mill.)

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ABSTRACT

Seeds of *Acacia angustissima* were subjected to a control and five pre-sowing (soaking in cold water for 24 hours, soaking in hot water (100° C) for 5 minutes, immersion in 60 % H_2SO_4 for 30 seconds, immersion in 60 % H_2SO_4 for 5 minutes, and immersion in 60 % H_2SO_4 for 15 minutes) treatments. The number of seeds that sprouted was recorded daily for calculation of germination indices. When there had been no further germination in any of the treatments for four weeks, the height, number of leaves, number of leaflets, and root length of seedlings were determined. One-way analysis of variance was used to test the effects of the pre-sowing treatments on seed germination and seedling growth. Final Germination Percentage, Germination Rate Index, and Seedling Vigour Index were significantly higher in the warm water than other five treatments which displayed similar responses of the traits. Similar, the warm water pre-treatment resulted in the longest seedlings, when shoot height and / or root length was considered. There was no significant effect of treatment on Mean Germination Time, number of leaves, and number of leaflets. The findings suggest that hot water pre-sowing treatment is the most suitable for seed germination and initial seedling height attainment of *Acacia angustissima.*

Contribution/Originality: Assessment of germination related responses of *Acacia angustissima* to pre-sowing treatments is crucial for speedy and mass production of planting stock. This study's findings will be helpful for overcoming physical dormancy, improving germination and seedling vigor to counter low population densities of the species in the western Cameroon Highlands forests.

1. INTRODUCTION

The first developmental step of a plant's life cycle is germination. The process paves the way for postgerminative growth of the new seedling [1]. Rate of water uptake is a key determinant of germination progress. In phase I, water is rapidly imbibed initially by a dry seed to the extent that its tissues become fully hydrated. Phase II is characterized by a limited uptake of water and an increase in metabolic and cellular activity while Phase III which marks the completion of the process is marked by an elevation of water uptake. The timing and rate of germination and the early growth of the emerging seedling are important determinants of competitiveness and establishment success of plants. Moreover, early growth parameters are accurate predictors of long-term performance of plants growing under favourable conditions. For instance, initial height of radiata pine (*Pinus radiata*) and Norway spruce (*Picea abies*) seedlings was found to reflect subsequent growth after 3 years [2] and 40 years [3] respectively, in the field. Similarly, seedlings with large root-collar diameters have greater rates of survival and growth than smaller diameter counterparts due to significant positive correlations between this parameter and root traits that control the uptake of water and nutrients [2, 4-6]. Ensuring an enabling environment for seed germination and early growth of seedlings is made even more relevant because seedlings' pride of place as the most popularly used reforestation stock material [7].

Acacia angustissima (Mill.) Kuntze is a member of the Fabaceae family. The rapidly-growing shrubby or tree-like that is usually 2-3 m tall may attain a height of 7 m or more with a single bole [8]. It is found at altitudes of 0 - 2700 m where its growth is favoured by 5 - 30 °C temperature and 895 - 2870 mm rainfall. Tolerant to free draining acidic, infertile soil and drought conditions, the species thrives in a variety of habitats [9]. *Acacia angustissima* is a multipurpose tree. It is valued for its high production of livestock forage. Its leaves are 10-25 cm long with 10-20 pairs of pinnae and leaflets. On the other hand, the pods that are of length 3-6 cm and width 6-9 mm have been used for food by local peoples. In addition, the tree improves soil nutrient conditions through atmospheric nitrogen fixation [8]. There are also medicinal and agroforestry uses associated with the tree. In the Western Highlands of Cameroon, an abusive use of the plant and unsustainable human behaviour have resulted in reductions in the population and occurrence of the species.

Though a prolific seed producer, poor natural regeneration of *Acacia* species is an obstacle to restoration efforts. They are characterized by a hard testa that presents a physical barrier to the seed uptake of water and exchange of gases for initiation of the process of germination [10]. The potency of various pre-treatment methods in breaking the exogenous dormancy and accelerating germination of *Acacia* seeds have been assessed, including soaking in boiling, hot, cold water, dry heating, acid scarification, clipping, and sandpapering [11-14]. Though effective, the practical applicability of the physical methods is questionable for large seed lots. The effectiveness of pre-treatment in promoting germination will depend the on species and provenance. In a previous study on *Acacia angustissima* with five treatments (control, cold water soaking for 24 hours, hot water soaking for 5 minutes, 20 % H_2SO4 immersion for 20 minutes, 60 % H_2SO_4 immersion for 20 minutes), hot water produced the best outcome while the 60 % H_2SO_4 pre-treatment resulted in the least Final Germination Percentage and Germination Rate Index [15]. However, the concentration of a chemical substance may interact with duration of exposure in influencing germination. In the present investigation of pre-sowing treatments on seed germination and early seedling growth of the species, the duration of immersion in 60 % H_2SO_4 was varied for three intervals under 20 minutes.

2. MATERIALS AND METHODS

2.1. Description of Study Site

The study was executed at a nursery of the Department of Forestry and Wildlife Technology nursery at The University of Bamenda campus is in Bambili (5° 60' 0" - 6° 05' 0" N; 10° 12' 0" - 10° 22' 0" E), Tubah Sub-Division, North West Region of Cameroon [16]. The area experiences a rainy season from March to September and a dry season from September to March. The wettest months are July, August, and September with over 350 mm of monthly rainfall while the driest is January with 6 mm of precipitation. The mean annual rainfall is 2095 mm. The mean annual temperature and relative humidity are 22.51 °C and 75.96 %, respectively. The topography of Bambili is undulating with altitudes varying between about 900 and 2270 m [17].

2.2. Plant Material

Seeds of *Acacia angustissima* were obtained from a forest tree seed dealer in Bambui, a neighboring village to Bambili in Tubah Sub-Division. They were tested for viability by the floatation method using water in a bucket. Upon soaking, seeds that sank to the bottom of the water were taken to be viable while those that floated were considered otherwise and discarded.

2.3. Experimental Design

Treatments were comprised of a control and five pre-sowing treatments. For the pre-sowing treatments, seeds were soaked in cold water for 24 hours, hot water for 5 minutes, 60 % H_2SO_4 for 30 seconds, 60 % H_2SO_4 for 5 minutes, and 60 % H_2SO_4 for 15 minutes. The water for the cold-water pre-treatment was at room temperature while the hot water pre-treatment involved soaking the seeds at 100° C before removing and allowing to cool down to ambient temperature. In the case of the H_2SO_4 dip, the seeds were agitated occasionally so that they would not stick together and then sieved after the corresponding time interval. Treatments were arranged in a randomized complete block design in a non-mist propagator. The substrate was a combination of equal proportions of fine sand and sawdust that had been thoroughly homogenized. There were two replications of each treatment. On 20 April 2023, the seeds were sown at a depth of 0.5 cm and the substrate saturated with water. Each treatment and replication had 40 seeds. As the experiment progressed, irrigation was done based on necessity and weeds were hand-picked. The propagator was stationed in a shade house roofed with aluminum and transparent sheets in an alternating pattern.

2.4. Data Collection

The number of seeds that germinated each day was recorded. On 16 June 2023 when there had been no further germination for four weeks in any treatment, the height, number of leaves and leaflets were determined from a seedling in each treatment and replication. The root system was rinsed free of substrate and its length measured with a ruler. For treatments and / or replications in which only one seed had germinated, the lone seedling was used for the data collection while a random selection was done for that which had more than one seedling. The following germination parameters were calculated:

$$\text{Final Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds planted}} \times 100$$

$$\text{Germination Rate Index} = [(G_1/1) + (G_2/2) + (G_3/3) + \ldots + (G_x/X)]$$

Where, G = Germination on each day after sowing; 1, 2, 3, X = corresponding day of germination [18].

$$\text{Mean Germination Time} = \frac{\Sigma n \times D}{n}$$

Where, n = Number of seeds, which were germinated correspondent to the day D observation and D = number of days counted from the beginning of germination [19].

$$\text{Seedling Vigor Index} = \frac{SL\,(cm) \times GP}{100}$$

Where, SL = Seedling length (shoot + root) and GP = germinated percentage [20].

2.5. Statistical Analysis

Following testing of the data distribution, the effects of pre-sowing treatments on seed germination and seedling growth were tested with one-way ANOVA. The Least Significant Difference (LSD) test was used for means comparison. The statistical analysis package was Data Desk 6.01 in which the probability level was set at 0.05.

3. RESULTS

Three of the four germination indices investigated were significantly influenced by pre-sowing treatment. Among the growth parameters, on the other hand, only height and root length were affected (Table 1).

Table 1. ANOVA p-values for the effect of pre-sowing treatments on seed germination and seedling morphology of Acacia angustissima.

Source	FGP	GRI	MGT	H	NL	NLl	RL	SVI
Pre-treatment	0.0005	0.0209	0.2972	0.0228	0.4441	0.4715	0.0095	0.0026

Note: FGP = Final germination percentage; GRI = Germination rate index; MGT = Mean germination time; H = Height; NL = Number of leaves; NLl = Number of leaflets; RL = Root length; SVI = Seedling vigor index.

The pattern of response was same for the germination indices. Final Germination Percentage, Germination Rate Index, and Seedling Vigor Index were significantly greater in hot water than the other pre-sowing treatments which showed similar responses of the traits (Figure 1, 2).

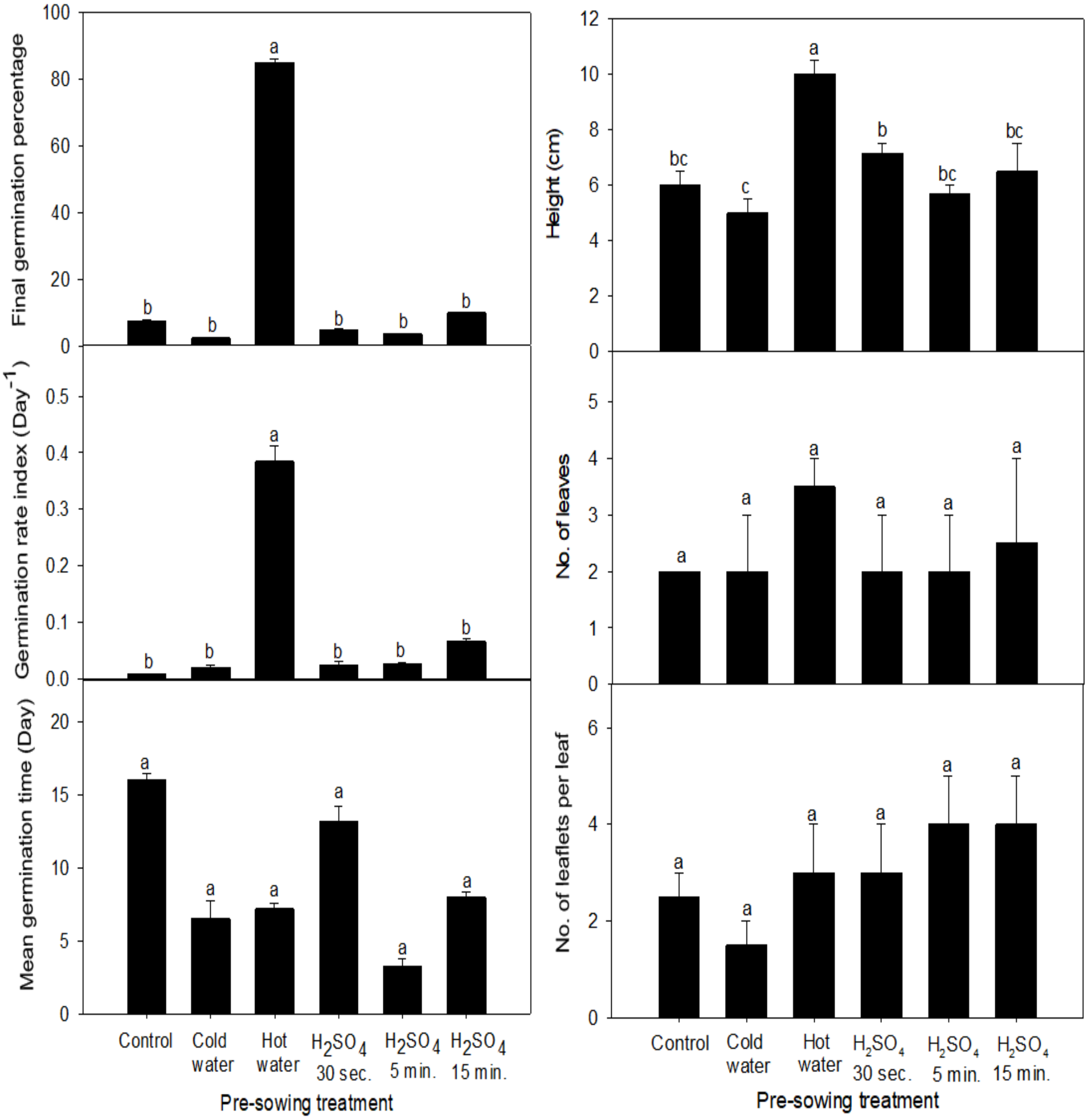

Figure 1. Effect of pre-sowing treatments on seed germination and seedling morphology of *Acacia angustissima*. Means without a common letter differ significantly.

Note: Means underneath the same letter are not significantly different from each other.

Values of height were highest in hot water and lowest in cold water. The other four pre-sowing treatments resulted in comparable responses of this trait. Furthermore, the differences between the cold-water pre-treatment and either the control, immersion in H_2SO_4 for 5 or 15 minutes were not statistically significant (Figure 1). On its part, root length declined from warm water and 30 seconds immersion in H_2SO_4 to the other four pre-sowing treatments which displayed similar responses of the parameter. Similarly, the warm water and 30 seconds H_2SO_4 immersion pre-treatments did not differ for root length (Figure 2).

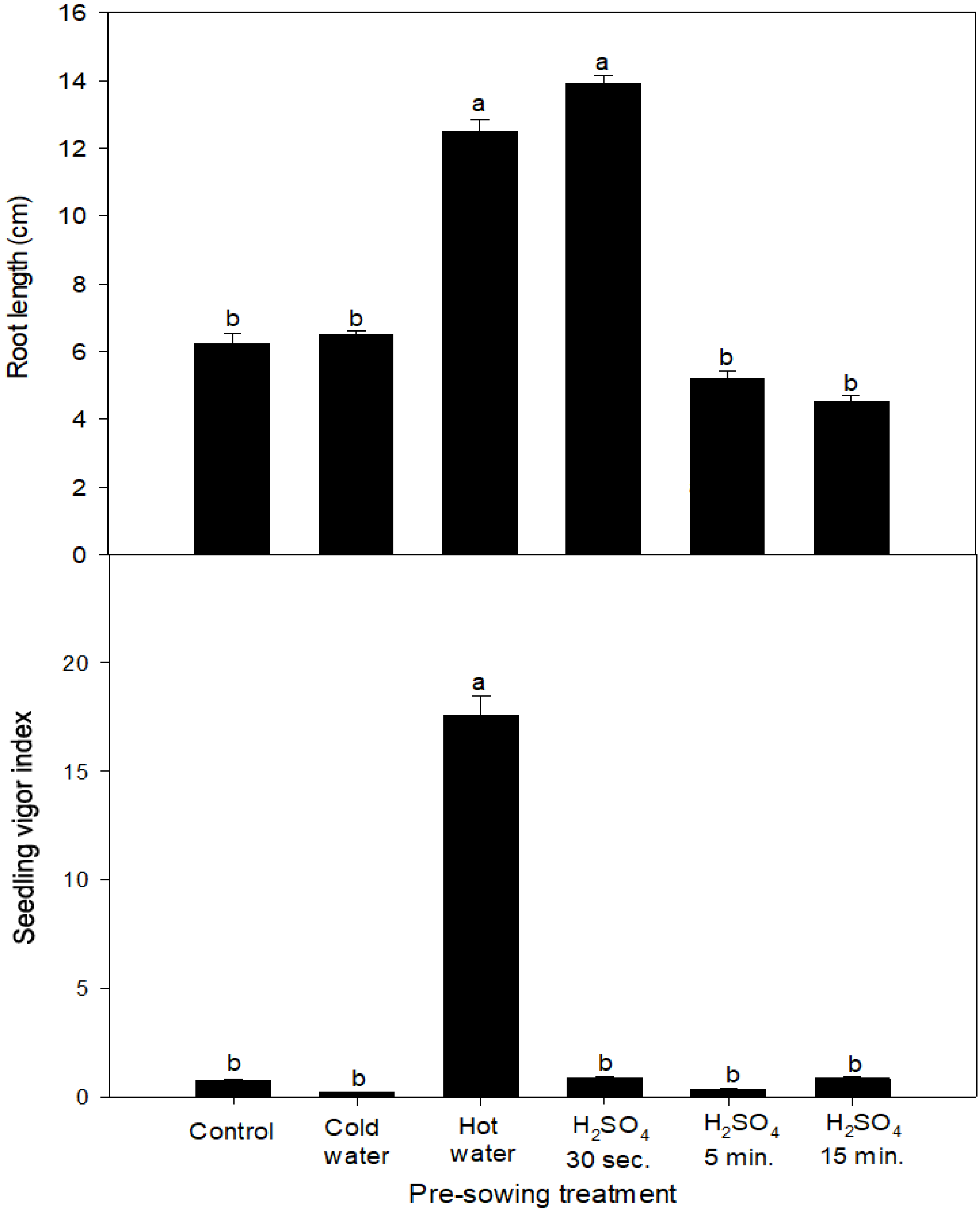

Figure 2. Effect of pre-sowing treatments on root length and seedling vigor index of *Acacia angustissima*. Means without a common letter differ significantly.

Note: Means underneath the same letter are not significantly different from each other.

4. DISCUSSION

Germination is affected by many factors, including maturity, viability, and storage conditions of the seed as well as environmental conditions at sowing and treatments applied to break dormancy. The effectiveness of a dormancy-breaking method depends on the type of dormancy and the species. In the present study, warm water treatment proved to be the suitable agent for breaking seed coat dormancy and improving germination in *A. angustissima* by augmenting the germination percentage, speed and showing a tendency towards reduction of the mean germination time. In contrast, neither culturing seeds in cold water for 24 hours nor immersing them in H_2SO_4 for various durations was beneficial to germination. The importance of hot water treatment has been confirmed in other species including *Acacia saligna* [14] *Acacia senegal* [21] *Mimosa bimucronata* [22] *Iliamna remota* [23] *Cassia fistula* [24] *Albizia zygia* [25] and *Aegilops tauschii* [26].

The action of the hot water is related to softening of the seed coat and eliminating waxes therefrom so that entry of water into the seed is possible. Upon disruption of the macrosclerid layer, lifting and cracking of the strophiole water gets into the seed to the advantage of biochemical processes essential for germination [27, 28]. Aside from aiding water uptake, the heat in the water may exert a shock on the embryo that modifies its metabolic processes with the outcome that germination is either favored or hindered depending on the species [29].

Several morphological attributes constitute important predictors of subsequent field performance [2, 30, 31]. In Norway spruce, for instance, initial heights of seedlings reflected subsequent growth even after four decades [3, 32]. The greater height, root length, and vigor of seedlings from the hot water treatment is suggestive of a higher potential for growth and survival. Significant correlations have been found between root length and other root parameters like root surface area and root volume [33] that relate strongly with a capacity for exploitation of soil volume for growth resources.

5. CONCLUSION

Various methods have been employed in breaking physical seed-coat dormancy of forest plants. It has been confirmed here that hot water dip is the suitable pre-treatment for seed germination and initial seedling growth of *A. angustissima*. In the face of substantial declines in density and representation of the species across the landscape of the western Highlands of Cameroon, the findings of this study are of utmost relevance for the supply of quality planting material given that the value of natural regeneration from seed is reduced by germination related difficulties.

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