

Response of *Albizia lebbeck* Seeds to Various Seed Invigoration Techniques under Salt Stress

Sana Rouf¹, Muhammad Farrakh Nawaz^{1,2}, Irfan Afzal³, Sadaf Gul⁴, Amna Sarwar¹, Ayesha Noreen¹, Irfan Ahmad¹, Muhammad Sohail¹, Muhammad Haroon U Rashid^{1*}

1. Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan.
2. Institute of Environmental Studies, University of Karachi, Pakistan
3. Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.
4. Department of Botany, University of Karachi.

*Corresponding author e-mail: haroon.r@uaf.edu.pk

SUMMARY

Salinity is one of the major causes of soil degradation and affects vast areas of the world, particularly Pakistan. Afforestation of degraded lands by suitable tree species is inevitable for soil reclamation, and it is a sustainable option among other available physical and chemical techniques. Understanding the salinity tolerance of different tree species and finding new ways to enhance their potential to germinate and grow in saline soils is desirable. Salinity is the major environmental stress that restricts tree growth because it slows germination and delays the emergence of seedlings. This study analyzed the effect of different pre-sowing seed treatments on *Albizia lebbeck* seeds under 100mM NaCl aqueous solution irrigation. The main objective of the study was to quantify the response of *A. lebbeck* seeds to various seed invigoration techniques under salt stress. Different treatments were applied, including control, soaked in hot water for 3, 5, and 7 min, soaked in cold water for 24, 36, and 48 hours, soaked in sulphuric acid for 5, 10, and 15 seconds, soaked the seeds in Plant Growth Promoting Rhizobacteria (PGPR) for 30 minutes. An evaluation of treatments for germination under salt stress showed significant results. Out of all treatments, sulphuric acid for 15 seconds showed remarkable results on germination parameters with 66.66% final germination, 22.48% speed of accumulated germination, and 22.48% germination index.

Keywords: Salinity, pre-sowing, seed treatments, germination, *Albizia lebbeck*, salinity

INTRODUCTION

Salinity affects approximately 7% of the world's land and approximately 20% of the 230 million ha of irrigated lands (Parihar *et al.*, 2015). This amount is expected to increase due to increased land salinization in the future by artificial saline irrigation, climate variability, and poor practices in agricultural management (Uçarlı, 2020). So, this problem of salinity is becoming very serious. In Pakistan, nearly 75% of its population depends directly or indirectly on the agriculture sector to survive. Pakistan is ranked 8th in the world for salinity-affected land. Approximately 25% of Pakistan's total irrigated area is salinized (Qureshi and Sarwar, 2009). Salt affects the germination process through oxidative, osmotic, and ion-specific effects on seed germination, resulting in decreased germination rates (Munns and Tester, 2002). Salt decreases the amounts of sodium and chloride ions in embryos, impacting their survival (Daszkowska, 2011). Germination of seeds is the most sensitive stage of

plant growth, especially to salt stress (Cuartero *et al.*, 2006). Dormancy and germination are two key traits in the life history of seeds that are considered crucial components in plant growth strategy (Rees, 1997). Dormancy refers to a fertile seed's failure to germinate under favorable conditions. Tree seeds always show some degree of dormancy; more than 50 percent of the 178 species listed in the prescriptions of international certified testing of tree seeds required treatment to break their dormancy (ISTA, 1985). It is important to know the cause of your seed germination problems so that you can implement effective treatments to improve germination (Melo *et al.*, 1998).

Growing multipurpose tree species on salt-affected soils has many benefits. Trees can reduce salt deposition and buildup in the soil's top and surface layers. When trees are grown in salt-affected soils, exchangeable sodium percentage (ESP), electrical conductivity (EC), and soil pH tend to decrease. Trees can help damaged lands improve their physical, chemical, and biological qualities (Shabbir *et al.*, 2018). Almost all members of trees of the Fabaceae family show dormancy due to hard seed coats, which are mostly physical dormancy (Baskin and Baskin, 2005). Physical dormancy is caused by the hard coat of the seed, which is impermeable to water, resulting in either delayed germination or reduced germination rate (Nongrum and Kharlukhi, 2013). Physical dormancy can be overcome in many ways, such as through seed invigoration techniques that enhance germination by up to 80% (Pipinis *et al.*, 2009). Seed Invigoration refers to "post-harvest treatments to improve seedling growth or to facilitate seed delivery to the field" (Taylor *et al.*, 1998).

Albazia lebbeck (L.) Benth., a plant of the Fabaceae family, is native to India, Australia, Malaysia, Bangladesh, Nepal, Indonesia, Myanmar, Nepal, Pakistan, and Thailand. This tree species is deciduous and characterized by its rapid growth, ability to improve soil structure, and ability to fix nitrogen (Faisal *et al.*, 2012). It survives in a wide range of climates, with annual rainfall ranging from 600 to 2500 mm. However, it can also survive better when the yearly rainfall is only 400 mm. Although the tree may survive in many different types of soil, from acid to alkaline and saline, it thrives in well-drained, moist soils (Prinsen, 1986). *A. lebbeck* is mostly propagated by seeds. *A. lebbeck* seeds have a stiff seed coat, which causes physical dormancy and poor seed germination (Azad *et al.*, 2012). Numerous studies have demonstrated that Albizia species exhibit poor seed germination when the seed is planted without using any seed invigoration strategies (Nongrum and Kharlukhi, 2013).

Seed invigoration techniques are necessary to enhance seed propagation and shorten germination. There are several methods for overcoming the physical dormancy of seeds, including scarification and bio-priming (Baskin and Baskin, 2004). Seed scarification, i.e., the physical breakage of the hard coat of seeds without lowering the quality of the seed, has been studied for more than a century (Dittus and Muir, 2010).

Hot water soaking is the best seed treatment from the perspective of least damage, efficiency, economy, and application. The treatment involves treating the seed with an amount of hot water sufficient to kill the pathogen yet not enough to damage the seed, making it an effective way to regulate several seed-borne diseases (Muniz, 2001). Using hot water to treat seeds and plant material as plant protection is an eco-friendly and effective method far more effective than using chemicals. Acid

scarification of tree species possessing hard seed coats is known to be highly effective in improving their germination (Youssef, 2008). Seeds are soaked in sulphuric acid for a pre-determined period as per species, followed by washing in running water. In hot water, seeds are dipped to lose the seed coat.

Similarly, seeds are dipped in cold water for a specified time. Priming is a well-established technique that can be used to enhance seed quality. A technique involving seed priming combined with a low dose of beneficial microorganisms is becoming increasingly popular to change the versatility of tree seed performance (Rakshit *et al.*, 2015).

According to several studies, salt stress has been linked to considerable reductions in seed vigor and slowed seedling growth in numerous species (Bybordi, 2010). Thus, effective seed invigoration techniques are needed to promote seed germination in saline soil, and these techniques are especially important for plants growing in saline soils (Li *et al.*, 2014).

MATERIAL AND METHODS

Study area

This investigation was conducted in the postgraduate experimental area (31 ° 25'57" N, 73° 04' 21" E) of the Department of Forestry and Range Management, University of Agriculture, Faisalabad. Climatic conditions during the pot experiment were collected from the Agricultural Metrology Cell, UAF, and described in Table 1.

Table 1: Climatic conditions data during the year 2022

Month	Average Max. Temp. (°C)	Average Min. Temp. (°C)	Rain Fall	Relative Humidity	Pan Evaporation	Sun Shine
March	31.2	18.4	2.1	63	4.2	9.3
April	41	22	1.9	42.5	7.3	10.3
May	42.2	27.3	1.1	43.4	10.9	11
June	53.9	26.5	48.3	47.2	8.1	10.5

Seed Collection

A. lebbek mature pods were collected from the Arboretum of the Department of Forestry and Range Management, University of Agriculture, Faisalabad. Seeds were extracted from pods, washed, and air-dried at room temperature overnight. Seeds with any seed infestation were discarded, and disease-free seeds were retained. Moreover, the seeds were immersed in tap water for a fundamental viability test based on their specific gravity. Then, the seeds were separated into groups: i-e, "immediately sunken," and "floating." The seeds in the former category were selected for further experimentation, whereas those in the latter category were discarded.

Tetrazolium Test

To rapidly estimate germination potential, a tetrazolium viability test was conducted on 100 seeds from the same seed lot. Tetrazolium salt produces a red stain in every living cell in the seed embryo. It is a simpler method of determining seed viability than a germinating test. Tetrazolium staining detects respiration on a cellular level, providing positive proof of survival. Non-viable tissues do not respond and do not stain as a result.

Seed Treatments

Eleven treatments were applied to the seeds: control, soaked in hot water for 3, 5, and 7 min; soaked in cold water for 24, 36, and 48 hours. Soaked in sulphuric acid for 5, 10, and 15 seconds, Soaked the seeds in Plant Growth Promoting Rhizobacteria (PGPR) for 30 minutes. In hot water treatment, water was warmed to about 100°C. Hot water was poured into a 250ml beaker. Sixty seeds were placed in a loose cotton bag and submerged in water for three, five, and seven minutes. The water was kept at a uniform temperature throughout the treatment. The water was continuously stirred. The bag was not allowed to touch the bottom after the completion of treatment. The seeds were taken out and cooled for 24 hours at room temperature. In cold water treatment, cold water was poured into a 250ml beaker for cold water treatment. Sixty seeds were placed in a loose cotton bag and immersed in cold water for 24 hours, 36 hours, and 48 hours. The seeds were planted on the same day after removing the water. In sulphuric acid treatment, concentrated sulphuric acid (98%) was added to a 250ml beaker. Sixty seeds were placed in a loose cotton bag and soaked in concentrated sulphuric acid for 5, 10, and 15 seconds. The acid was continuously stirred. The seeds were properly washed for 30 minutes with running water before being dipped in distilled water. In plant growth-promoting rhizobacteria (PGPR) treatment, the seed was surface sterilized and then soaked in a Plant Germination-Promoting Rhizobacteria solution. The seeds were pre-soaked in water for 12 hours, thoroughly mixed with the PGPR, and left to stay for 30 minutes.

Seedbed preparation, irrigation, and weeding, harvesting time

Two seed beds with dimensions of 10 ft long and 6 ft wide were prepared. The debris and stones were removed. Polythene bags of sizes 10cm long and 4 cm wide are perforated by making 9 holes and later filled with nutrient-rich soil. Each prepared seedbed had a capacity of accommodating 330 polythene tubes. One seed pre-polythene tube was sown at a depth of 1.5cm. The seed bed was immediately irrigated with water. A sample size of 60 seeds per treatment with 3 replications each was used. Moisture is a very important factor in the emergence and performance of seeds. Later, the seedbed was irrigated periodically (the NaCl aqueous solution used for irrigation was 100mM) for one week, along with removing potentially competing weeds.

One day before harvesting, the seed bed was flooded with water to minimize root breakage while uprooting the seedlings. The seedlings were harvested on 1st June 2022. The seedlings were kept in paper bags for further parameter recording.

Growth parameters

After harvesting, all the growth parameters were recorded, such as shoot height, shoot diameter, root length, seedlings fresh and dry biomass separately for shoot and root, and number of leaves by appropriate methods.

Germination indices

The following germination indices were calculated as follows;

a. Time is taken to 50% germination (day)

50 days are needed for 50% of the total no. of seeds to germinate (Josep and Maria, 2002)

b. Mean germination time (days)

The formula was used to determine the mean germination time (MGT).

$M\text{-days} = [(N1 \times T1) + \dots + (Nn \times Tn)] / [N1 + N2 + N3 + \dots + Nn]$

Tn is the percentage of seeds germinating in each treatment, and N is the number of seeds utilized in each replication (Czabator, 1962).

c. Final germination (%)

The following equation was used to compute the germination percentage (GP): (Ranal *et al.*, 2009).

$GP = \text{number of seeds that germinated} / \text{total seeds} \times 100$

e. Speed of germination (S)

$$S = \sum_{i=1}^k \frac{N_i}{T_i}$$

Where Ti is the amount of time that has passed since the experiment began up until the ith interval, Ni is the number of seeds that germinated during that interval (not the overall number). K is the total no of periods (Bradbeer, 1988).

f. Speed of Accumulated germination (AS)

$AS = [N1/1 + N2/2 + N3/3 \dots + Nn/n]$

N1, N2, N3, Nn: Cumulative number of seeds which germinate at time 1, 2, 3, ... N. (Haugland and Brandsaeter, 1996)

g. Coefficient of the rate of germination (CRG)

$CRG = 100 \times [(N1 + N2 + N3 + \dots + Nn) / [(N1 \times T1) + \dots + (Nn \times Tn)]]$

N1: the quantity of seeds germination at time T1. N2: number of seeds now in germination Number of seeds that have sprouted at time Tn (T2 Nn) (Bewley and Black, 1985)

h. Germination index

The Association of Official Seed Analysis (1983) provided the following formula for calculating the germination index (GI):

$GI = \text{number of germinated seeds} / \text{First Count Days} + \dots + \text{Number of germinated seeds} / \text{Last Count Days}$

Data Collection and Analysis

All the collected data were statistically analyzed with the help of statistical software named "Statistix 8.1". Complete Randomized Design (CRD) (Steel and Torrie, 1980) with LSD study of variance technique was adopted to determine the significance of collected data at a 5% significance level.

RESULTS

Germination

The analysis of variance (Table 2) indicated that the model was significant at $p \leq 0.01$. In *A. lebbeck* seed treated with sulphuric acid for 15 seconds (T_9) showed the highest final germination FGP value of 66.66% with a minimum value recorded in soaked in hot water for 7 min (T_3) 36.66% (Table 2). The highest MGT was recorded in seeds subjected to cold water for 48 hours (T_6) at 15.06%, with the lowest mean value recorded in hot water for 3 min (T_1) at 8.65% (Table 2). Similarly, the highest speed of accumulated germination S value was observed in sulphuric acid for 15 seconds (T_9) at 1.95%, and the least mean value was recorded in hot water for 7 min (T_3) at 0.85% (Table 2). Maximum germination index GI value was recorded in seed treated with sulphuric acid for 15 seconds (T_9) at 22.48%, and least mean value was recorded in seed treated with hot water for 7 mins (T_3) at 10.99% (Table 2).

Table 2. Effect of seed treatments on the germination indices of *A. lebbeck*

Treatment	FGP (%)	MGT (d)	T ₅₀ (d)	S	AS	CRG
T ₀	53.33 abcd	10.95 ab	9.83 a	1.23 bc	16.26 bc	9.51 abc
T ₁	41.66 bcd	8.65 b	7.11 bcd	1.06 bc	12.77 bc	11.71 a
T ₂	50.00 abcd	9.59 b	6.62 cd	1.31 bc	15.93 bc	11.28 ab
T ₃	36.66 d	11.18 ab	9.00 abc	0.85 c	10.99 c	9.01 abc

Figures not sharing the same letters differ significantly at $p = 0.05$. T₀ = Control; T₁, T₂, and T₃ = soaked in hot water for 3, 5, and 7 min, respectively; T₄, T₅, and T₆ = Soaked in cold water for 24, 36, and 48 hours respectively; T₇, T₈ and T₉ = Soaked in sulphuric acid for 5, 10 and 15 seconds respectively; T₁₀ = Soaked the seeds in Plant Growth Promoting Rhizobacteria (PGPR) for 30 minutes. FGP, Final germination percentage; MGT, mean germination time; T₅₀, Time taken to 50% germination; S, Speed of germination; AS, Speed of Accumulated germination; CGR, Coefficient of the rate of germination; GI, germination index.

Growth

The seed treatment on *A. lebbeck* seeds showed different impacts on their growth parameters. The best result was shown in T₀ (control) with 0.176% shoot diameter, and the minimum shoot diameter was shown by T₄ (cold water 24hrs) with 0.065% shoot diameter (Fig. 1b). T₃ (hot water 7 min) with 34.150% showed the highest no. of leaves, and the minimum no. of leaves showed in T₇ (sulphuric acid 5 sec) with 20.469% (Fig. 1d). T₅ (cold water 36hrs) with 34% showed the highest shoot length, and the minimum results of shoot length showed in T₁₀ (PGPR 30 min) with 22.192% (Fig. 1a). T₈ (sulphuric acid 10sec) with 14.833% gave the highest root length and the minimum results of root length showed in T₀ (control) with 11.313% (Fig. 1c). T₃ (hot water 7 min) with 4.263% showed peak shoot fresh weight and the minimum results of shoot fresh weight showed in T₁₀ (PGPR 30min) with 2.088% (Fig. 2a).

T₆ (cold water 48hrs) with 1.56% showed maximum root fresh weight and the minimum results of root fresh weight showed in T₀ (control) with 0.835% (Fig. 2b). T₃ (hot water 7min) with 3.55% showed highest shoot dry weight and the minimum results of shoot dry weight showed in T₁₀ (PGPR 30min) with 1.55% (Fig. 2c). T₆ (cold water 48hrs) with 1.01% give highest root dry weight and the minimum results of root dry weight showed in T₀ (control) with 0.61% (Fig. 2d). T₃ (hot water 7min) with 4.40% showed highest seedling biomass and the minimum results of seedling biomass showed in T₁₀ (PGPR 30min) with 2.23% (Fig. 3).

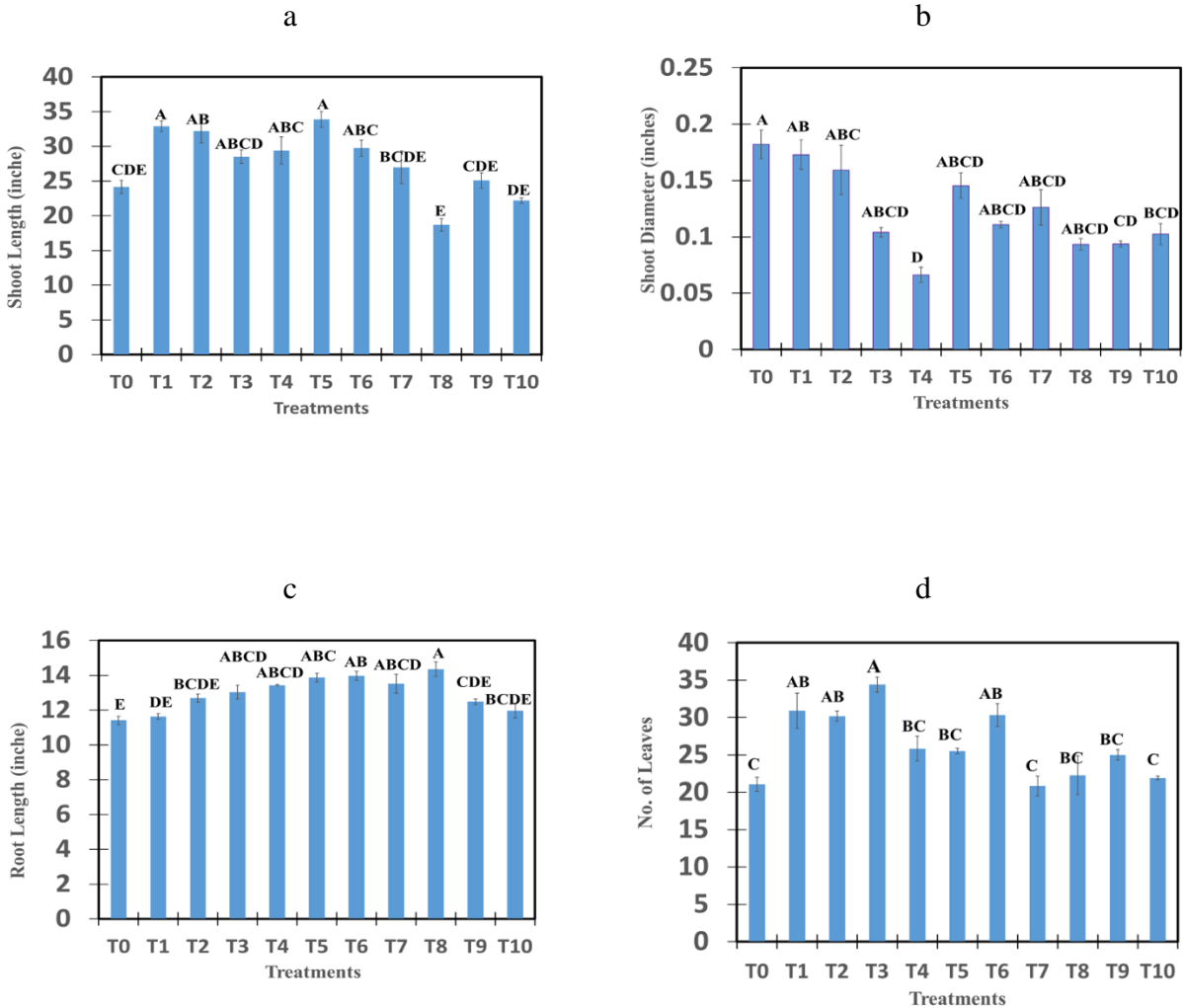


Figure 1: Effect of pre-sowing seed treatments on growth parameters of *A. lebbek* seedlings. a) shoot length; b) shoot diameter; c) root length; d) No. of leaves per plant.

Figures not sharing the same letters differ significantly at $P = 0.05$. T_0 = Control; T_1 , T_2 , and T_3 = soaked in hot water for 3, 5, and 7 min, respectively; T_4 , T_5 , and T_6 = Soaked in cold water for 24, 36, and 48 hours respectively; T_7 , T_8 and T_9 = Soaked in sulphuric acid for 5, 10 and 15 seconds respectively; T_{10} = Soaked the seeds in Plant Growth Promoting Rhizobacteria (PGPR) for 30 minutes.

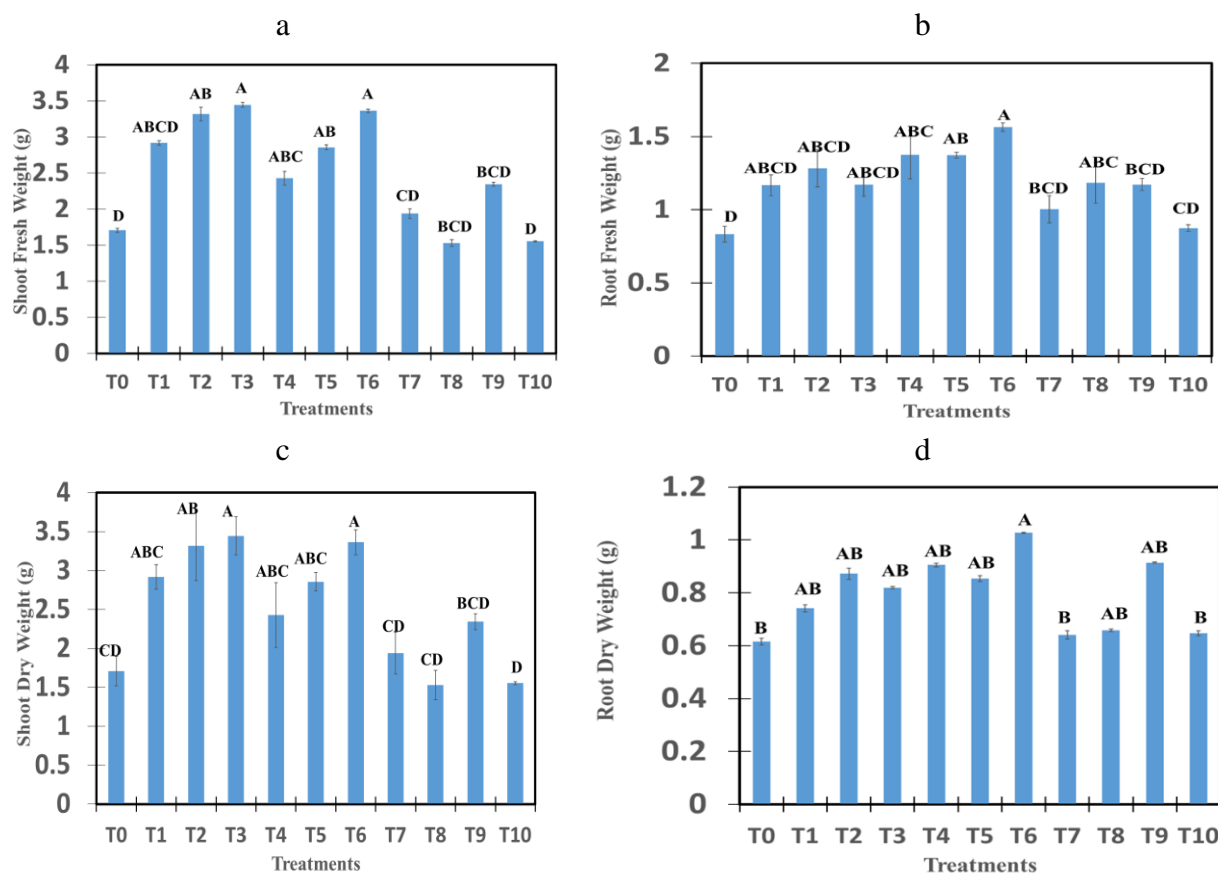


Figure 2: Effect of pre-sowing seed treatments on growth parameters of *A. lebbeck* seedlings. a) shoot fresh weight; b) Root fresh weight; c) Shoot dry weight; d) Root dry weight.

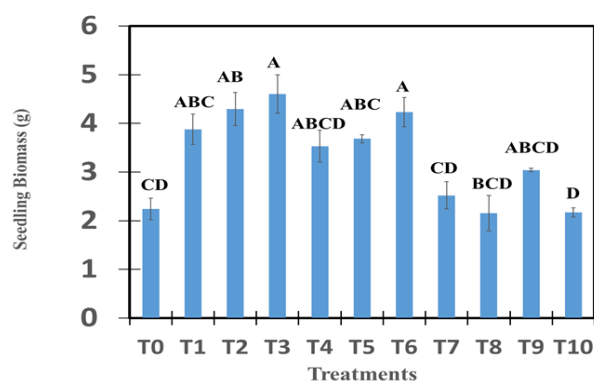


Figure 3: Effect of pre-sowing seed treatments on seedling biomass of *A. lebbeck* seedlings.

DISCUSSION

The main focus of this investigation was to check the effect of different seed invigoration techniques on the growth of *A. lebbeck* in saline soils. Salinity also impacts cell shape and stomatal conductivity (Hussain *et al.*, 2019). Because saline soils contain a higher concentration of salts, their osmotic potential is low, and plants growing in these soils must keep their internal osmotic potential low to avoid exosmosis. As a result, oxidative stress and dehydration develop. Salinity stress also causes nutritional imbalance, with nutrients such as N, Ca, K, P, Fe, and Zn being substituted by harmful ions, decreasing their uptake by the shoot. (Verma *et al.*, 2019).

Six types of multifunctional tree seeds were examined for salt tolerance Using fresh and saline (NaCl) solutions. *Al. lebbeck* has demonstrated the best ability to germinate under various salinity conditions (Rashid *et al.*, 2004).

The present study showed that seeds soaked in Sulphuric acid for 15 seconds gave the best 66.66% final germination, 22.48% speed of accumulated germination, and 22.48% germination index under saline soil (Table 2). Acid scarification also greatly increased species germination with hard seed coats (Youssef, 2008). Chemical treatments soften seed coats and make them permeable to water. Seed dormancy in *A. lebbeck* claimed that sulfuric acid treatment effectively broke dormancy; *albizia* seeds are light-insensitive, and the temperatures applied to the seeds did not affect germination (Dutra and Medeiros, 2009).

Sulphuric acid has been proven to be highly effective in seed germination of *A. lebbeck* seeds as it showed significant results when its seeds were treated for 15 seconds. It showed 95% seed germination (Tomar *et al.*, 2011). It may be due to the disintegration effects of the acid on the seed coat of *A. lebbeck* seeds and its ability to stimulate the biochemical and physiological activities necessary for germination (Akinyele *et al.*, 2020).

Another research that examined five species of medicinally significant woody plants, including *A. lebbeck* (L) benth also indicated that the highest germination rates of 50% were recorded with H₂SO₄ for 5 minutes. The optimum pre-sowing treatment may meet the demand for medicinally significant raw materials and maintain ecological balance and environmental stability (Khanduri *et al.*, 2010).

Six types of multifunctional tree seeds were examined for salt tolerance using fresh and saline (NaCl) solutions of 7.5, 15, and 22.5 mmhos cmG to examine the effects of salt on seedling growth, germination period and the decrease in germination with rising salt levels. As salinity increased, the germination period and germinative energy decreased, and germination patterns changed. Species' salt tolerance has been assessed through observation and *Al. lebbeck* has demonstrated the best ability to germinate under various salinity conditions (Rashid *et al.*, 2004).

Another study conducted two experiments to evaluate the seed dormancy and its effect on germination of *Albizia lebbeck* and the effect of temperature & light on germination. In the first experiment, to break the seed dormancy of *A. lebbeck*, seeds were treated with hot water, H₂SO₄, humid heat, and scarification. In the 2nd experiment, seeds were treated first with H₂SO₄ for 10 minutes and then subjected to germination under different combinations of the controlled environment of light and

temperature. Results clearly showed that *A. lebbeck* seeds possess dormancy and H₂SO₄ treatments were ideal for breaking the seed dormancy in the case of *A. lebbeck*. Light and temperature do not significantly affect seed germination (Dutra and Filho, 2009).

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