Research Article

# Improved Seed Germination Technique for Prosopis cineraria (L.) Druce: A Rare Sacred Plant of Hindus

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**In nature, there are some seeds that can't germinate properly, even if environmental conditions are favourable. This condition is known as seed dormancy. In the case of *Prosopis cineraria* (L.) Druce, the common reason for seed dormancy is the toughness of the seed coat. This type of dormancy is known as physical dormancy (PY). This experiment is conducted to determine the efficiency of different seed priming methods on the seed germination of the Shami plant. The seed priming methods include hydropriming followed by mechanical scarification, lukewarm water, hot water, and sulphuric acid treatments. Five seeds are selected for each treatment to carry out the germination. Then the data for germination percentage is collected one week after planting (WAP). In this study, hydropriming followed by mechanical scarification showed the highest observed germination percentage. However, given the small sample size, further validation is required. It is interesting to reveal that under full exposure to sunlight, the leaflets remain closed in the case of the juvenile Shami plant. This adaptation may be ascribed to resisting water loss by the juvenile plant.**

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## Introduction

The art and science of controlled propagation of plants from existing ones, either by sexual or asexual means, is known as plant propagation. It requires a combination of scientific understanding of plant biology and horticultural competence[1]. It is essential for conserving biodiversity. Various propagation methods, such as seed banking, micropropagation, grafting, etc., help in the conservation of rare, endemic, and threatened plants. Plants that are raised through seeds are known as

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seedlings[2]. However, in vegetative propagation, a part of the plant that is leaves, stem, branch, or root is used to develop new plants, which are called saplings. In some crops, like *Solanum tuberosum* L., *Mangifera indica* L., etc., both the seed and the vegetative part can be used for the multiplication of planting material. The following aspects are needed for plant propagation: knowledge of the chemical, physical, and environmental aspects of propagation, technical skills, working knowledge of seed biology and physiology, and need-based expertise on specific plant species. Flowering plants are mostly propagated through seeds. They remain in a dormant condition until the advent of favourable conditions for seed germination[3]. The aim of propagation is to produce more plants. A seed is a fertilized and matured ovule. It contains three main parts: the embryo, the endosperm, and the seed coat. The endosperm acts as a nutrient reservoir that supplies nutrients to the developing embryo during germination. The embryo is a minute plant that is present in a dormant state. The seed coat is protective in nature. Propagation by seed is a natural process. Plant breeders adopt seeds as a means for the genetic improvement of cultivated plants. Physiologically, seed germination is the metabolic activity of the seed following hydration, which results in the radicle poking through the seed coat[4]. Water, oxygen, and an adequate temperature are the basic needs for seed germination, with additional requirements such as light and hormones. It occurs in three stages: imbibition, plateau stage, and embryo elongation. The second stage, also known as the lag phase, is characterized by an increase in metabolic activity in the embryo, which results in enzyme activity and formation. The third stage is known as embryo elongation, where radicle protrusion occurs. Seed germination is mainly of two types: epigeal and hypogeal germination. Another special type of germination is viviparous germination, in which seeds germinate and connect to the plant[5].

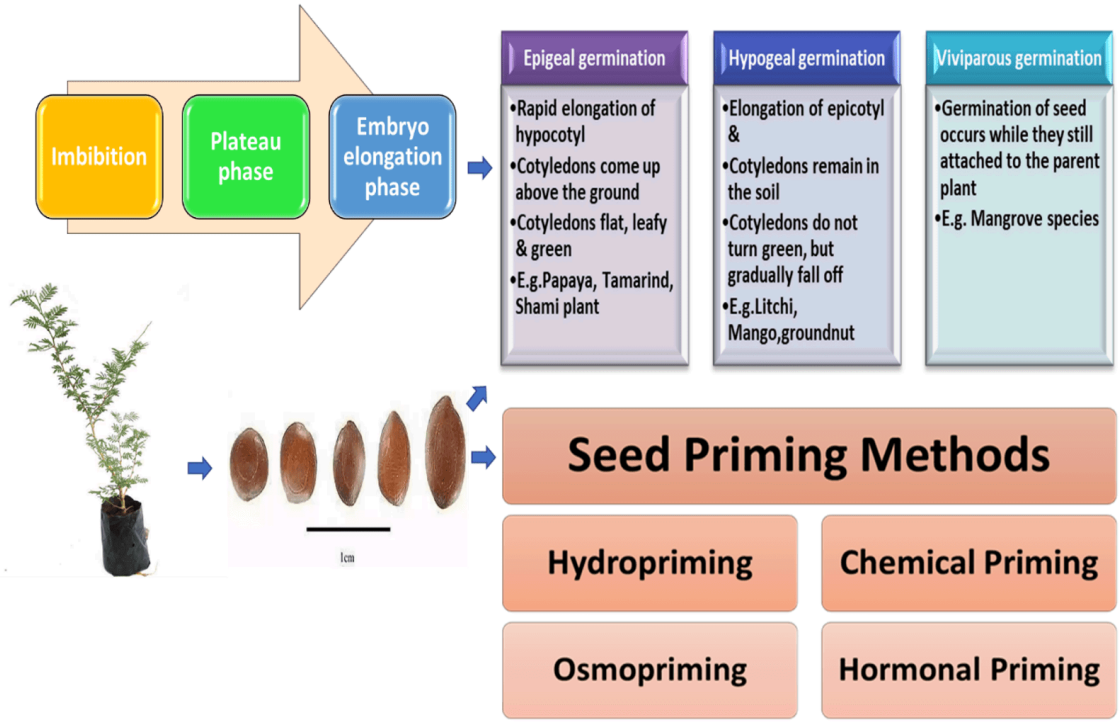


**Figure 1.** Stages of different seed germination methods of *Prosopis Cineraria*.

Various internal and external factors are present that affect the germination potential of the seeds. When seeds are dormant, they are unable to germinate under favourable conditions. Seed dormancy is an adaptive character that enhances the survival chance of seeds during adverse conditions such as heat, cold, and salinity, etc. The ratio of ABA:GA is the vital predictor of seed dormancy and germination[6]. The amount of the two phytohormones is regulated by their rates of biosynthesis versus deactivation and is regulated at the molecular level. An increase in gibberellin biosynthesis indicates that the DELLA proteins are degraded. Expression of DELLA proteins indicates the biosynthesis of ABA (Abscisic acid), which represses seed germination[7]. The balance between the activities of ABA and GA in seeds is under both developmental and environmental control. During the early stages of seed development, ABA sensitivity is high, while GA sensitivity is low, which favours seed dormancy. But in the later phase of seed development, ABA sensitivity declines while GA sensitivity increases progressively, which favours germination. At the same time, seeds become progressively sensitive to environmental factors such as temperatures and light, which can stimulate or inhibit germination. Seed priming is a pre-sowing technique that causes seeds to germinate more efficiently[8]. Various seed priming methods are used to enhance the germination potential of seeds.

Hydropriming is defined as a process that partially hydrates seeds and simultaneously inhibits the sprouting process. Chemical priming is the process of treating seeds with various chemicals (for example: sulphuric acid, ascorbic acid, urea). Using an osmotic solution for treating seeds is known as osmopriming[9]. *Prosopis cineraria* (L.) Druce is a valuable, versatile plant species that flourishes well in arid climates such as the Thar Desert, Arabian Peninsula, etc. It is a dicotyledonous, armed, moderate-sized tree. It is a gift from nature to mankind due to its numerous values. It is the only tree that thrives well despite all environmental conditions of a desert. It is a phreatophyte, nitrogen-fixing legume[10]. It is endemic to the hot deserts of India. It is also known as 'Kalpatru' or 'King of the Desert.' This plant has religious significance. It represents one of the Navagrahas, Lord Shani. In Hindu tradition, *Prosopis cineraria* is associated with Lord Shani and is considered one of the Navagrahas. According to traditional beliefs, it is often planted in the west direction. In some Hindu rituals, its leaves are offered to deities such as Lord Shiva and Sri Ganesh, with the belief that they bring strength and success. *Prosopis cineraria* (L.) Druce is a versatile tree possessing great nutritional and medicinal importance. It is often defined as the "Lifeline" of the desert as it has been crucial for rural livelihood and economy[11]. This species embodies all five F's: Forest, Fiber, Fuel, Fodder, and Food. Because of dormancy in seeds of this plant, propagation through sexual methods is very poor. Vegetative propagation of this species is also difficult. As seed dormancy prevents germination, it is crucial to overcome this problem for successful germination[12]. Seed vigor is an important parameter for determining seed quality. Inconsistent seed germination and slow development of seedlings reduce crop output. One low-cost method of overcoming dormancy and inconsistent seed germination is seed priming. The main goals of seed priming are to speed up germination and shield the seed from a wide range of adverse environmental circumstances[13]. As described by Kubala *et al.*, priming is a two-step procedure that involves soaking seeds under control conditions, followed by drying them back to their natural moisture content. Pill and Necker (2001) reported seed priming methods that involve soaking the seeds in water before sowing. This method allows the imbibition process while inhibiting the other two phases. Impermeability of the seed coat to water is one of the factors that delay germination[14]. There are various methods available for breaking the dormancy of a seed, such as stratification, scarification, hormonal treatment (hormonal priming), osmopriming, etc. Scarification refers to a process that mechanically ruptures the seed coat. One of the key factors in the beginning of germination is the presence of fractures in the seed coat[15]. Scarification can be of the following types: mechanical scarification, acid scarification, hot water scarification, and warm moist

scarification, etc. Mechanical scarification is one of the common priming techniques used to overcome hard seed coat-imposed dormancy. It physically makes nicks on the seed surface to increase water uptake by the seeds. Acid scarification is a chemical method to soften the tough seed coat[16]. The acid concentration (concentrated or dilute) during scarification techniques can be varied according to the species. The most common acids used in acid scarification techniques are sulphuric acid ($H_2SO_4$) and hydrochloric acid (HCl)[17]. $H_2SO_4$ is the most popular and effective chemical to reduce hard seed dormancy in legumes. In this technique, dry seeds are placed in concentrated acid for a certain period. The mixture should be stirred at intervals for uniform results. Hot water scarification involves dropping seeds into hot water. The water can be classified as boiling water and lukewarm water (20℃ to 40 ℃) according to temperature variations[18]. The seeds should be sown immediately after the treatment. This procedure aids in softening the seed coat. The primary sources of fulvic and humic acids, which function as plant bio-stimulants, are the biodegradation of plant organic matter that contains lignin (Malan, 2015). To speed up germination, it could be preferable to extract fulvic acid from composts[19]. Fulvic acid dissolves readily in diluted alkali. Gill *et al.* (2015) used fulvic acid as a seed priming agent in *Prosopis cineraria* and *Acacia tortalis*. Seeds were softened by immersing them in concentrated $H_2SO_4$ for fifteen minutes with distilled water. The seeds were treated with 0.5% of fulvic acid fraction in water. It was found that fulvic acid enhanced seed germination (at 1%), with a 27% increase in *Prosopis cineraria* over the control[20]. He used 0.5% and 1% concentrations of fulvic acid for seed priming methods based on the findings of Khang (2011), who reported that concentrations higher than 1% decreased plant height. Fatima *et al.* (2023) primed seeds of the Shami plant (*Prosopis cineraria*) using different priming techniques such as osmo-, hydro-, acid-, and hormone priming. In the hydropriming treatment, Shami seeds were immersed in hot water for 1-5 minutes, followed by immediate washing with cold water[21]. In osmopriming, the seeds were treated with various osmotica such as $KNO_3$ (0.5% and 2%), KCl (1% and 2%), and PEG 6000 (10% and 20%), etc. In acid priming, seeds were treated with 25% $H_2SO_4$ (for 10, 20, or 30 minutes) and 50% $H_2SO_4$ (for 2, 5, or 10 minutes), followed by washing with distilled water. Hormone priming of seeds was done using gibberellic acid and BA[22]. In acid-hormone priming, seeds were treated with 50% sulphuric acid for 2 minutes, then washed with distilled water, followed by immersing them in BA. Before being sown, the seeds were cleaned with distilled water and allowed to dry at room temperature overnight in the dark. It was found that the highest germination rate was observed in hydro-primed Shami seeds. The germination percentage was negatively affected in osmopriming treatments[23]. The acid priming of Shami seeds

with different concentrations for different durations clearly improved the germination rate. The maximum seed germination (100%) was observed in three treatments: acid alone, BA alone, and the combined treatment of acid + BA 20 DAS. The treatment with 200 ppm BA alone or in combination with 50% sulphuric acid can be recommended as the optimal treatment for improving the germination rate in Shami seeds[24]. Primed Shami seeds could be stored at room temperature or 4°C for more than 10 days with no adverse effects on seed germination. During the present study, the standard methodology of Ffolliott & Thames (1983) was adopted. Vilela *et al.* (2001) applied these scarification methods to germinate the seeds of the Shami plant (*Prosopis cineraria*). This method involves the following processes: by mechanical methods, seeds were nicked with a new razor blade; by chemical procedures, seeds were treated with sulphuric acid 1N for 15 minutes, then washed in running tap water for 2 minutes. Seeds were dipped in boiling water until the water reached room temperature, which was also utilized as a thermal process[25]. Hassan *et al.* (2023) treated seeds with sulfuric acid (50%) and cytokinin (888 micromolar), increasing germination percentages from 11% to 75%. The combination of sulfuric acid and cytokinin was the most effective treatment, initiating germination 3 days after sowing. Sporadic research has been undertaken to explore multiple methods of breaking seed dormancy in seeds of the Shami plant[26]. However, there is still an opportunity for development, which is why this research work was carried out. After careful scrutiny, it was revealed that the method design and available seed germination techniques for *Prosopis cineraria* (L.) Druce are partially effective[27]. As this taxon, a rare sacred plant of Hindus, is in high demand, an improved method for effective seed germination has been envisioned[28]. To approach the problem, the following objectives have been adopted: to study seed priming techniques and to determine the germination percentage of seeds after completion of various priming techniques.

## Materials and Methods

Dry pods of Shami were collected from the Shami plant found at the Gopinath Panigrahi Botanic Garden of Fakir Mohan University, Balasore, Odisha. Seeds were removed from the dried pods of Shami (Sangri), and then they were surface sterilized with 70% ethyl alcohol for 30 seconds, followed by washing twice with distilled water. The entire experiment was adopted after Fflolliott & Thames (1983). A set of five seeds was taken for each of the treatments. The seeds were subjected to four groups of priming methods such as mechanical scarification combined with hydropriming, lukewarm water, hot water, and acid[29]. A set of five seeds was placed in hot water (100°C) for approximately 3

minutes, followed by immediate washing with tap water to avoid damage to the embryo from the heat. Then, the seeds were nicked with a new razor blade. Some seeds were dipped in lukewarm water for 24 hours. After 24 hours, seeds were sown. Seeds were immersed in hot water (100°C) for 2 minutes. After the treatment, seeds were immediately placed in cold tap water for 5 minutes to stop the heating reaction[30]. Then, they were washed with distilled water three times and dried on tissue paper at room temperature overnight in darkness before sowing. Seeds were soaked in 50% $H_2SO_4$ (sulphuric acid) for 2 minutes. The mixture was stirred cautiously at intervals during the treatment to get uniform results. Then, the primed seeds were dried on blotting paper overnight in darkness before sowing[31]. The pots were filled with 750 g of garden soil. A set of five primed seeds from each treatment was taken and sown in the garden soil. During germination, the seeds were supplied with 60-120 ml of water daily at regular intervals. Data for the germination rate was collected one week after planting (WAP). The germination percentage was calculated using the following formula.

$$\text{Germination Percentage} = \left( \frac{\text{Number of seeds germinated}}{\text{total number of seeds sown for germination}} \right) \times 100$$

For soil sample collection and growth of seedlings, the pots were filled with 750 g of soil in each. Then the seedling was planted in soil samples and soil-vermicompost mix (1:1) in a pot and exposed to two different climatic conditions (open field condition and partially-shaded condition)[32]. All the seedlings were watered at regular intervals (60-120 ml of water daily, twice). Then, the seedling growth was observed for a month (30 days). After 30 days, the seedlings were uprooted, and growth parameters such as shoot length, root length, seedling length, and number of leaves emerged were measured.

## Results

Before sowing, seeds were treated with various priming agents. After the completion of the seed priming treatment, the seeds were sown the next day. Seeds began to germinate on $7^{th}$ March 2024. The total time taken by the seeds to germinate was approximately 5 days. From this experiment, it was revealed that all the seed priming methods have a significant impact on seed germination. Primed seeds germinated faster than the non-primed seeds. The seed dormancy of this plant is due to the toughness of the seed coat. These seed priming methods generally soften the tough seed coat, allowing water uptake.


**Figure 2.** Development of seedlings after germination.

| Seed priming method | No of seeds sown | No of seeds germinated | Germination percentage (%) |
|---|---|---|---|
| Hydropriming + mechanical scarification | 5 | 5 | 100% |
| Hydropriming (40°C) | 5 | 4 | 80% |
| Hydropriming (100°C) | 5 | 3 | 60% |
| Acid priming (50% $H_2SO_4$ for 2mins) | 5 | 4 | 80% |
| Acid priming (Conc. $H_2SO_4$) | 5 | 3 | 60% |

**Table 1.** Seed germination percentage in different priming methods.

The highest germination percentage was observed in seeds treated with hydropriming, followed by mechanical scarification. The second highest germination was found in seeds treated with lukewarm

water and acid priming (50% $H_2SO_4$ for 2 minutes). Seeds that were primed with 50% $H_2SO_4$ for 2 minutes showed an 80% higher germination percentage than other priming methods except hydropriming, followed by mechanical scarification methods. When seeds were primed with concentrated sulfuric acid for 1 minute, the germination percentage was 60%, but after some days, it was found that out of five germinated seedlings, two had shown abnormal growth and died. Seeds that were primed with hot water (100 ℃) followed by mechanical scarification methods showed a higher germination percentage than the other priming methods. The increase in germination percentage may be due to the softening of the tough seed coat and the nick facilitating water uptake. Hydropriming of seeds with lukewarm water also enhances the germination percentage up to 80%. The temperature of the lukewarm water generally ranges between 36.5 to 40 ℃. The overnight soaking of the seeds generally enhances the uptake of water, which hastens the imbibition rate. There is a decrease in germination percentage when seeds are hydro-primed with hot water rather than lukewarm water. It indicates that the boiling temperature of the water may have negatively affected the embryo. When seeds were primed with concentrated sulphuric acid, there is a decrease in germination percentage compared to diluted sulphuric acid. It indicates that concentrated sulphuric acid may negatively affect the embryo which is present inside the seed. This approach of seed priming agrees with the findings of other authors. The acid priming of seeds may soften the tough seed coat, which leads to an optimal level of water uptake and oxygen adsorption, which are prerequisites for seed germination. From the soil testing analysis, it was found that the pH of the soil is 4.70, which is acidic, and the organic carbon content was also low. Acidic pH and low organic carbon content of soil affect plant growth.

| Characterisation methods | Control | Experimental |
|---|---|---|
| pH | 4.70 | 7.50 |
| TSS (ds/m) | 0.07 | 2.84 |
| Organic carbon (Kg/ha) | 101.85 | 363.75 |
| Phosphorus (Kg/ha) | 27.30 | 8.10 |
| Potassium (kg/ha) | 90.00 | 100.00 |

**Table 2.** Soil profiles of control and experimental samples.


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It is interesting to reveal that under full exposure to sunlight, the leaflets remain closed. The adoption may be ascribed to resisting water loss by the juvenile plant. For both root and shoot, the maximum length was observed in seedlings of T2 (soil: vermicompost pot containing seedlings exposed to partially shaded conditions) than in the control. As the soil pH is acidic and low in organic carbon content, the seedling growth also decreased. The shoot and root lengths and number of leaves were also reduced. In the roots of seedlings of T2, three root nodules were also observed. The root nodules were primarily responsible for nitrogen fixation in the case of leguminous plants. From the above experiment, it is concluded that the juvenile plant is sensitive to high temperatures. The growth and development of the juvenile plant were more favorable in partially shaded conditions than in full exposure to sunlight.

| **Soil type** | **Seedling length (In cm)** | **Root length (In cm)** | **Shoot length (In cm)** | **No of leaves** |
|---|---|---|---|---|
| Soil (Control) | 22.5 | 11 | 11.5 | 8 |
| Soil & vermicompost (T1) | 23 | 13.5 | 9.5 | 15 |
| Soil & vermicompost (T2) | 33 | 8.5 | 24.5 | 32 |

**Table 3.** Comparative growth of the shami plant in different conditions. (T1: Open field condition; T2: Partially-shaded condition)


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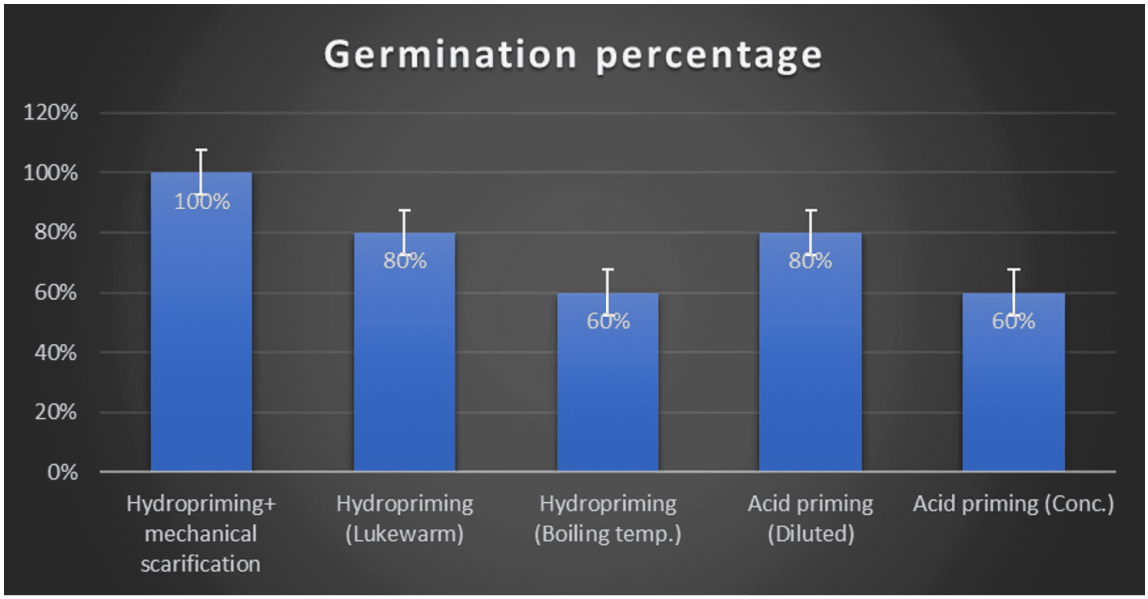


**Figure 3.** Graph showing seed germination percentage in different seed priming.

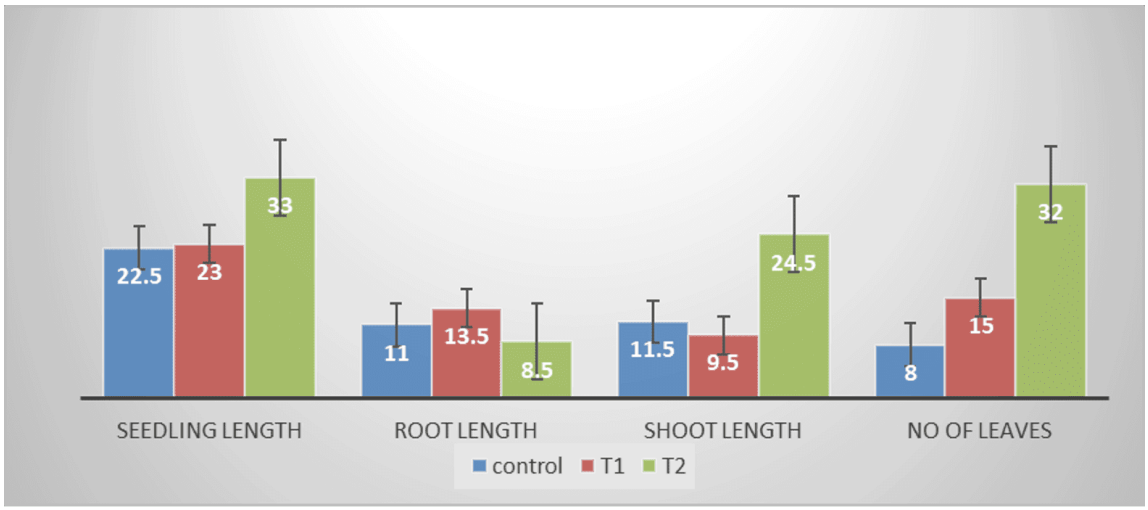


**Figure 4.** Graph showing the comparative growth of the shami plant in different conditions.

## Discussion

*Prosopis cineraria* seeds possess a hard, impermeable seed coat that inhibits water absorption and delays germination. Scarification is an effective method to overcome this physical dormancy. It holds immense cultural, economic, and ecological significance, particularly in Hindu tradition, where it is

considered sacred[33]. Its role in maintaining the ecological balance in arid and semi-arid regions is also crucial. However, seed germination of *P. cineraria* can be a challenge due to its hard seed coat and dormancy. To improve seed germination rates and facilitate its propagation, researchers have developed several methods. Below are some of the improved techniques for germinating *Prosopis cineraria* seeds effectively[34]. The seed coat is gently nicked or scratched using sandpaper or a sharp blade to expose the embryo to water and air. Seeds can be soaked in concentrated sulfuric acid for 30 minutes to 1 hour. This helps in softening the seed coat and promoting water uptake. Soaking seeds in hot water (80-90°C) for 5-10 minutes helps in breaking dormancy. After soaking, the seeds are cooled down to room temperature and then sown[35]. This method softens the seed coat and accelerates the germination process. Placing the seeds in a moist environment at temperatures of 4°C for 10-15 days can simulate the natural cold winter conditions and trigger germination. Soaking seeds in a dilute solution of nitric acid (5-10%) for about 10-15 minutes can improve germination by weakening the seed coat[36]. The ideal temperature for germination is between 25-30°C, and maintaining consistent moisture is critical for successful germination. Using a germination tray with a humidity dome can help maintain moisture levels and prevent the drying of seeds, which could result in poor germination rates[37]. Seeds should be sown in well-drained, sandy, or loamy soil. Heavy soils may retain too much water, leading to fungal infections or seed rot. Lightly covering the seeds with a thin layer of soil ensures proper aeration and moisture retention. Once seeds have germinated, the young seedlings should be transplanted carefully into larger pots or into the field[38]. They should be kept in a partially shaded area initially to reduce stress. Gradually increasing sunlight exposure as the seedlings grow helps in acclimatization and strengthens the plants. *Prosopis cineraria* is a nitrogen-fixing tree. Inoculating seeds with rhizobial bacteria before sowing can promote better growth and development of seedlings, improving their overall survival and vigor.

## Conclusion

The improved seed germination techniques for *Prosopis cineraria* aim to overcome the natural dormancy mechanisms of the seed, ensuring faster and more efficient propagation. By applying scarification, soaking in hot water, using growth regulators, and optimizing environmental conditions, the germination rate can be significantly enhanced[39]. These methods are essential for promoting the cultivation of this culturally significant and ecologically vital tree species in arid regions[40]. These improvements not only enhance the chances of successful germination but also

support the ecological restoration and conservation of *P. cineraria*, furthering its role in sustainable land management.

Due to the constraints on sample size (n=5 per treatment), the findings of this study should be interpreted with caution. A larger sample size is typically necessary to ensure the reliability and statistical power required to draw definitive conclusions. As the current sample may not provide sufficient evidence to definitively compare the effectiveness of the priming methods, future research with larger samples is recommended. Further research with proper statistical analysis is needed to confirm these findings and establish their reliability. In this study, hydropriming followed by mechanical scarification showed the highest observed germination percentage. However, further research with larger sample sizes and statistical analysis is needed to confirm its effectiveness.

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