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Dormancy-break and germination of *Zornia gibbosa* seeds by chemical and mechanical methods

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Abstract
In present study various chemical and mechanical pretreatment methods were tested to determine their effect on seed germination in *Zornia gibbosa*. The pretreatments included different concentrations and durations of H_2SO_4, KNO_3, exposure to microwave radiation, hot water treatments, and mechanical scarification. The highest germination was observed in mechanical scarification followed by soaking in water for 30 minutes, which achieved 100% seed germination by day 6, with a germination index of 10 and a germination rate of 16.67% seeds/day. In addition, acid treatment (20% H_2SO_4 for 10 min), hot water treatment (80°C for 10 min) and exposure to microwave radiation (for 2 min) were also significantly increase the seed germination in *Zornia gibbosa*. These findings provide valuable insights for improving germination protocols for *Zornia gibbosa* and potentially other leguminous species with similar seed dormancy characteristics.

Keywords: *Zornia gibbosa*, mechanical scarification, seed germination and acid treatment

Introduction

The Aravalli range represents the hilliest part of the Rajasthan. If we talk about the oldest hills in the world, the Aravalli hills secure a separate and important place. In India, it is considered to be the most ancient range of Fold Mountains (Roy, 1990) [9]. The Aravalli ranges have deciduous forests with prickly grasses, shrubs, and trees. The Aravalli ranges are segmented into 3 parts: northern Aravalli range, central Aravalli range and southern Aravalli range. The central Aravalli range includes whole Ajmer district, Sambhar basin in north Jaipur, north Rajsamand district and west Tonk district. The Central Aravalli Range possess several economically important and nitrogen fixer native legumes genera like *Aeschynomene, Abrus, Albizia, Acacia, Alysicarpus, Butea, Crotalaria, Clitoria, Dalbergia, Indigofera, Medicago, Mimosa, Mucuna, Pongamia, Pterocarpus, Prosopis, Rhynchosia, Trigonella, Tephrosia* and *Zornia* (Otaghvari *et al*., 2015) [7]. These plants use rhizobia to fix nitrogen, which increases soil fertility (Sankhla *et al*., 2015; 2017; 2018; Yadav *et al*., 2022) [10, 11, 12, 17]. The plant *Zornia gibbosa* is an annual herb having an erect or ascending stem (Sharma *et al*., 2024) [24]. The plant is found commonly in the wastelands and forests having wet and shaded habitat, specially in the grassy areas. Flowering and fruiting occur after monsoon in August to October month (Shetty and Singh, 1987) [14]. Like other Leguminous plants the seeds of *Zornia gibbosa* possess a hard seed coat, which acts as a barrier and prevents easy germination. Consequently, breaking down this barrier is necessary to improve germination rates. Therefore, the aim of the present study was to investigate the effect of various pretreatments on the germination of *Zornia gibbosa* seeds. By exploring different methods to soften or penetrate the hard seed coat, this research seeks to determine the most effective techniques for enhancing seed germination and supporting the successful cultivation of *Zornia gibbosa.*

Material and methods

The fresh and healthy seeds of *Zornia gibbosa* were collected from Amer hills (part of Aravalli region) after monsoon season in month of October. Seeds were kept with silica gel and brought to the laboratory for their germination study.

Experimental design

The collected seeds of *Zornia gibbosa* were pretreated by different concentrations and durations of H_2SO_4, KNO_3, exposure to microwave radiation, hot water treatments, and mechanical scarification pretreatments to break their dormancy and improve germination. A summary of 18 pretreatments and their process are presented in table 1.

Table 1: Pretreatment methods and processes for enhancing germination of *Zornia gibbosa* seeds

S.N.	Pretreatment methods	Processes
1	Control	No treatment (Seeds were placed in a petri dish moistened with distilled water)
2	20% H_2SO_4 (10 min.)	Seeds were soaked in 20% sulfuric acid for 10 minutes and subsequently washed with fresh water
3	30% H_2SO_4 (10 min.)	Seeds were soaked in 30% sulfuric acid for 10 minutes and subsequently washed with fresh water
4	2% KNO_3 (30 min.)	Seeds were soaked in 2% potassium nitrate for 30 minutes and subsequently washed with fresh water
5	5% KNO_3 (30 min.)	Seeds were soaked in 5% potassium nitrate for 30 minutes and subsequently washed with fresh water
6	10% KNO_3 (30 min.)	Seeds were soaked in 10% potassium nitrate for 30 minutes and subsequently washed with fresh water
7	20% KNO_3 (30 min.)	Seeds were soaked in 20% potassium nitrate for 30 minutes and subsequently 8washed with fresh water
8	Microwave (30 Sec.)	Seeds were exp9osed to microwave radiation for 30 sec
9	Microwave (1 min.)	Seeds were exposed to microwave radiation for 1 min
10	Microwave (2 min.)	Seeds were exposed to microwave radiation for 2 min
11	Hot water (65°C for 10 min.)	Seeds were soaked in hot water (65°C) for 10 min
12	Hot water (80°C for 10 min.)	Seeds were soaked in hot water (80°C) for 10 min

13	Hot water (100°C for 10 min.)	Seeds were soaked in hot water (100°C) for 10 min
14	Scarification + Water (30°C for 30 min.)	Seeds were scarified with sand paper and soaked in water (30°C) for 30 min
15	Scarification + Water (50°C for 30 min.)	Seeds were scarified with sand paper and soaked in water (50°C) for 30 min
16	Scarification + Water (60°C for 10 min.)	Seeds were scarified with sand paper and soaked in water (60°C) for 10 min
17	Scarification + Water (60°C for 30 Min.)	Seeds were scarified with sand paper and soaked in water (60°C) for 30 min
18	Scarification + Water (70°C for 10 min.)	Seeds were scarified with sand paper and soaked in water (70°C) for 30 min

The 20 pretreated seeds for each pretreatment were plated on moist filter papers in petri plates and placed in a seed germinator set to a temperature of 28°C for 15 days. Each day, the plates were checked to count the number of germinated seeds. Germination counts were specifically recorded on days 3, 6, 9, 12, and 15. The experiment was conducted in triplicate to ensure the reliability and accuracy of the results.

Data analysis

Germination parameters including germination percentage, germination rate and germination index were studied by using the following equations (Ranal *et al*., 2009) [8]:

$$\text{Germination Percentage (GP)} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \text{ X100}$$

$$\text{Germination Rate (GR)} = \frac{\text{Germination Percentage}}{\text{Number of days taken for germination}}$$

$$\text{Germination Index (GI)} = \frac{\text{Germination Percentage in treatment}}{\text{Germination Percentage in control}}$$

Results

The germination study of *Zornia gibbosa* seeds under various pretreatment conditions showed diverse results. Figure 1 shows a part of the experimental setup, collected seeds, and plant *Zornia gibbosa*, that is growing in the Central Aravalli Region, Jaipur.

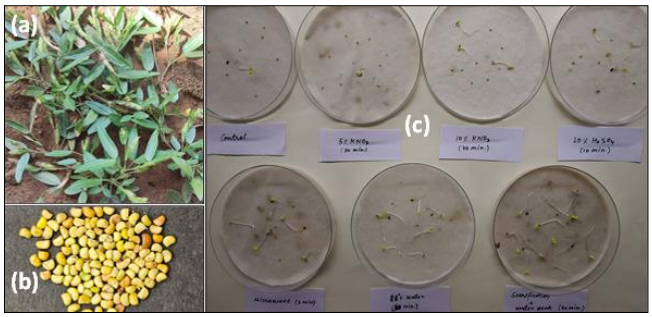

Fig 1: *Zornia gibbosa* plant (a), seeds (b) and a part of the experimental setup (c)

The control group (T1) exhibited minimal germination, with only 10% of seeds germinating by day 15, resulting in a germination index of 1 and a germination rate of 0.67% seeds/day (Fig. 2, Fig. 3 and Fig. 4). Seeds treated with 20% sulfuric acid (T2) showed a significant improvement, achieving a 50% germination, with a germination index of 5 and a germination rate of 3.33% seeds/day. The higher concentration 30% sulfuric acid (T3) not showed good results however still better than control.

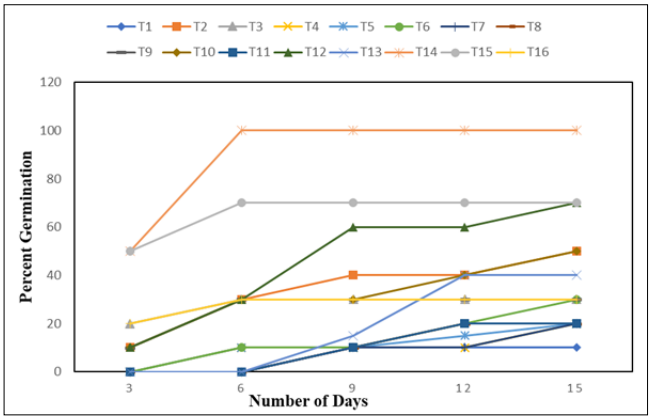

Fig 2: Effect of different pretreatments on seed germination speed in *Zornia gibbosa*

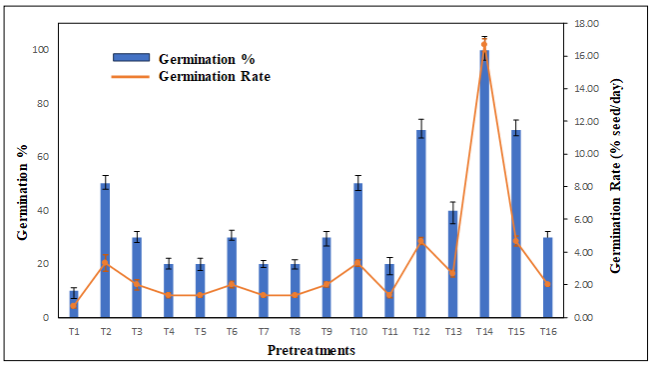

Fig 3: Germination percentage and germination rate in seeds of *Zornia gibbosa* under different pretreatments

Potassium nitrate treatments varied in effectiveness. T4 (2% KNO_3 for 30 minutes) and T5 (5% KNO_3 for 30 minutes) both reached 20% germination. T6 (10% KNO_3 for 30 minutes) had a 30% seed germination with germination index of 3, and germination rate of 2.0% seeds/day. T7 (20% KNO_3 for 30 minutes) and T8 (microwave for 30 seconds) both showed a 20% seed germination, with germination index of 2 and rates of 1.33% seeds/day. Microwave treatments for 1 minute (T9) and 2 minutes (T10) achieved seed germination of 30% and 50% respectively by day 15. The T10 showed the same effectiveness as T2. Hot water treatment at 65°C for 10 minutes (T11) resulted in a 20% seed germination with a germination index of 2 and a rate of 1.33 % seeds/day. Hot water treatment at 80°C for 5 minutes (T12) showed a higher efficacy, reaching 70% seed germination, with a germination index of 7 and germination rate of 4.67% seeds/day.

The highest germination rate was observed in T14 (scarification followed by soaking in water 30°C for 30 minutes), which achieved 100% seed germination by day 6,

with a germination index of 10 and a germination rate of 16.67% seeds/day. T15 (scarification followed by soaking in 50°C water for 30 minutes) also showed a high seed germination (70%) by day 15, with a germination index of 7 and a rate of 4.67% seeds/day.

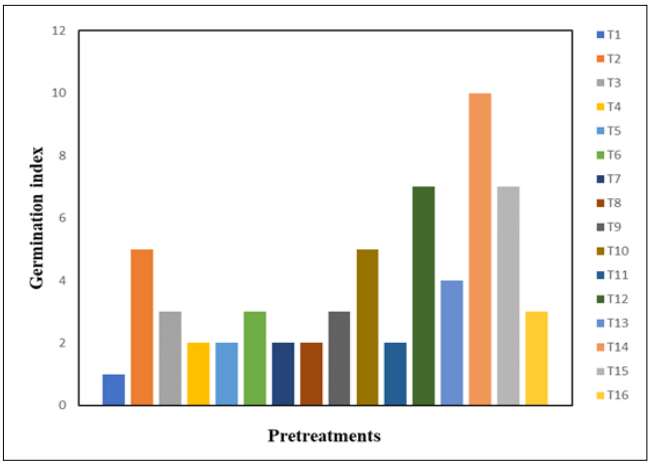

Fig 4: Germination index under different pretreatments

Treatments T16 (scarification followed by soaking in 60°C water for 10 minutes) resulted in a 30% seed germination Treatments T17 (scarification followed by soaking in 60°C water for 30 minutes) and T18 (scarification followed by soaking in 70°C water for 10 minutes) showed no germination, resulting in germination index and rates of 0.

In summary, scarification combined with water soaking (T14) proved to be the most effective pretreatment method for enhancing the germination of *Zornia gibbosa* seeds. Other effective treatments included exposure to sulfuric acid, potassium nitrate, and microwave radiation, with varying degrees of success. The results highlight the importance of pretreatment in overcoming seed dormancy and improving germination rates.

Discussion

Common pretreatments utilized to overcome seed coat dormancy include soaking in concentrated acid, hot water, and mechanical scarification (Baatuuwie *et al*., 2019; Akpalu *et al*., 2019; Tang *et al*., 2022; Lamichaney *et al*., 2024) [2, 5, 15].

The results from the present study indicate that the germination of *Zornia gibbosa* seeds is significantly influenced by pretreatment methods. Scarification combined with water soak proved to be the most effective method, achieving 100% germination within 6 days with a germination index of 10 and a germination rate of 16.67% seeds/day. This method aligns with research by Teketay (1996) [16], who found that mechanical disruption of the seed coat, followed by hydration, greatly enhances germination rates in leguminous seeds by breaking the hard seed coat and allowing water uptake and gas exchange. Similarly, Tang *et al*. (2022) [15] reported Mechanical scarification as the best pretreatment to break the seed dormancy in *Mimosa* species.

Chemical treatments with H_2SO_4 and KNO_3 also enhanced germination compared to the control, but their effectiveness varied with concentration and exposure duration. For instance, 20% H_2SO_4 for 10 minutes led to a maximum of 60% germination, whereas 30% H_2SO_4 was less effective, indicating a possible inhibitory effect at higher concentrations. Similar results were also observed by Zare *et al*. (2011) [18] in various *Prosopis* species. In contrast to this Ghosh and Maiti (2014) [4] achieved 100% seed germination in *Abrus precatorius* by using concentrated H_2SO_4 for 2 hr as a pretreatment. In our study, moderate concentrations of KNO_3 (10-20%) showed better results compared to lower or higher concentrations, implying an optimal range for promoting seed germination. Asci *et al*. (2011) [1] similarly reported enhanced germination in nitrate-treated seeds, likely due to nitrate's role in seed metabolism.

Thermal treatments, including hot water and microwave radiation, exhibited mixed results. Lower temperatures (65°C) and shorter microwave exposures (30 seconds) were less effective compared to higher temperatures (80°C) and longer exposures (1-2 minutes). Nelson (1976) [6] and Baskin and Baskin (1998) [3] and Lamichaney *et al*. (2024) [5] also observed improved germination rates in seeds treated with heat or microwave radiation suggesting that a precise thermal shock can break dormancy without damaging the seed.

Overall, this study demonstrates that *Zornia gibbosa* seeds have specific requirements for breaking dormancy and initiating germination. Mechanical scarification, especially when combined with water soak, appears to be the most reliable method, while chemical and thermal treatments require careful optimization to balance effectiveness and seed viability. These findings provide valuable insights for improving germination protocols for *Zornia gibbosa* and potentially other leguminous species with similar seed dormancy characteristics.

Conclusion

The germination study of *Zornia gibbosa* seeds under various pretreatment conditions revealed significant differences in germination effectiveness. The control group (untreated seeds) exhibited minimal germination (10% by day 15), emphasizing the need for effective pretreatment methods to overcome seed dormancy. The most effective pretreatment was scarification followed by soaking in water for 30 minutes, achieving 100% germination by day 6. This method demonstrated the highest germination index (10) and rate (16.67% seeds/day), indicating that scarification combined with water soaking is highly beneficial for *Zornia gibbosa* seed germination. Other pretreatments including sulfuric acid, potassium nitrate, hot water, and microwave radiation also improve germination rates, but their effectiveness varies. These findings underscore the importance of pretreatment in breaking seed dormancy and optimizing germination outcomes for *Zornia gibbosa*.

References

1. Asci OO, Acar Z, Ayan I, Basaran U, Mut H. Effect of pretreatments on seed germination rate of red clover (*Trifolium pratense* L.) populations. African Journal of Agricultural Research,2011:6(13):3055-3060.
2. Baatuuwie BN, Nasare LI, Smaila A, Issifu H, Asante WJ. Effect of seed pre-treatment and its duration on germination of *Detarium microcarpum* (Guill. and Perr.). African journal of environmental science and technology,2019:13(8):317-323.
3. Baskin CC, Baskin JM. Seeds: ecology, biogeography, and, evolution of dormancy and germination. Elsevier, 1998.

4. Ghosh PK, Maiti TK. Effect of seed scarification on *in vitro* seed germination of *Abrus precatorius* L. Plant Archives,2014:14(2):881-885
5. Lamichaney A, Naik SJ, Hazra KK, Datta D, Parihar AK, Aidbhavi R, *et al*. Overcoming seed coat–imposed dormancy in wild species of *Cajanus* and *Rhynchosia*. Crop Science,2024:64(1):386-398.
6. Nelson SO. Use of microwave and lower frequency RF energy for improving alfalfa seed germination. Journal of Microwave Power,1976:11(3):271-277.
7. Otaghvari AM, Yadav SR, Raina SN, Uniyal PL. Vegetational Wealth of Aravalli Ranges. Scientific Publishers, 2015.
8. Ranal MA, Santana DGD, Ferreira WR, Mendes-Rodrigues C. Calculating germination measurements and organizing spreadsheets. Brazilian Journal of Botany,2009:32:849-855.
9. Roy AB. Evolution of the Precambrian crust of the Aravalli Mountain range. In Developments in Precambrian Geology, Elsevier,1990:8:327-347.
10. Sankhla IS, Meghwal RR, Choudhary S, Rathi S, Tak N, Tak A, *et al*. Molecular characterization of microsymbionts associated with root nodules of *Crotalaria burhia* Buch.-Ham. ex Benth., a native keystone legume species from Thar Desert of India. Indian Journal of Experimental Biology,2018:56:373-384.
11. Sankhla IS, Meghwal RR, Tak N, Tak A, Gehlot HS. Phenotypic and molecular characterization of microsymbionts associated with *Crotalaria medicagenia*: a native legume of the Indian Thar Desert. Plant Archives,2015:15(2):1003-1010.
12. Sankhla IS, Tak N, Meghwal RR, Choudhary S, Tak A, Rathi S, *et al*. Molecular characterization of nitrogen fixing microsymbionts from root nodules of *Vachellia* (*Acacia*) *jacquemontii*, a native legume from the Thar Desert of India. Plant and Soil,2017:410:21-40.
13. Sharma G, Yadav A, Choudhary S, Sankhla IS. Biochemical Characterization of Microsymbionts Associated with *Zornia gibbosa* Span. in Central Aravalli Range,2024:12(1):190-201.
14. Shetty BV, Singh V. Flora of Rajasthan, Botanical Survey of India, Vol. I & II, Old Connaught Place, Dehradun, 1987.
15. Tang L, Baskin C, Baskin J, Luo K, Yu X, Huang W, *et al*. Methods of breaking physical dormancy in seeds of the invasive weed *Mimosa pudica* (Fabaceae) and a comparison with 36 other species in the genus. PeerJ,2022:*10*:e13567.
16. Teketay D. Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. Forest Ecology and Management,1996:*80*(1-3):209-223.
17. Yadav A, Solanki D, Sharma G, Dubey G, Sankhla IS. Phenotypic and Biochemical Characterization of Rhizobia Associated with *Medicago polymorpha* Growing in Rajasthan. Indian Journal of Advanced Botany,2022:2(2):5-11.
18. Zare S, Tavili A, Darini MJ. Effects of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis juliflora*. Journal of Forestry Research,2011:*22*:35-38.