

# Mechanical Scarification Influence on *Gleditsia assamica* Bor Water Uptake and Germination

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**Abstract.** Physical dormancy is typically found in Fabaceae species, including *Gleditsia assamica*. This type of dormancy can be alleviated, by mechanical scarification. Previous studies on the mechanical scarification effect on *G. assamica* seed focus only on its germination parameter without regard to its effect on seed water uptake. This study aims to understand the treatment effect on both seed water uptake and germination parameters. The tetrazolium dyeing test is conducted by immersing treated and untreated *G. assamica* seeds in 1% of tetrazolium solution for up to 72 hours. Cut test for the seeds was conducted at 24, 48, and 72 hours after immersion. Seed weight measurement trials were conducted by put treated and untreated *G. assamica* seeds in a petri dish with moist straw paper for up to 48 hours. Seeds were weighted at 24 and 48 hours to measure their water uptake rate. Lastly, a germination test for *G. assamica* seeds was conducted by germinating seeds for ten days in a completely randomized design of 25 seeds per replication for treated and untreated seeds. Germination is counted for each day and used to calculate germination parameters. This study result shows that mechanical scarification can enhance *G. assamica* seed water uptake. The treatment also significantly improved the seed's final germination percentage and germination speed index. This study result gives us a more precise understanding of the effect of mechanical scarification on alleviating *G. assamica* dormancy and germination. This study result will be advantageous to the species conservation and domestication efforts.

**Key words:** dormancy; Fabaceae; hard seed coat; physical scarification; tetrazolium

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## INTRODUCTION

Germination is an essential chapter of plant life history as it is the first step in plant development (Wolny et al., 2018). Numerous factors, either external or internal, influence seed germination. Seed dormancy is an example of an element that affects seed germination. Seed dormancy is defined as a viable seed's inability to germinate under favorable conditions (Soppe and Bentsink, 2020). Despite its advantage as a seedlings adaptation strategy in its natural habitat, seed dormancy is negatively impacted plant and stand recruitment, as dormant seeds may produce low, delayed, erratic germination and fragile stands (Kimura and Islam, 2012; Kildisheva et al., 2020).

Several kinds of dormancy, such as morphological dormancy, physiological dormancy, morphophysiological dormancy, physical dormancy, and a combination of physical and physiological dormancy, are present in plant species (Kildisheva et al., 2020). A particular trait produces each kind of dormancy in plant seed, and each will requires a different alleviation treatment. Among these kinds of seed dormancy, physical dormancy is a kind of dormancy in which a hard seed coat is impermeable to water and inhibits seed

water uptake (Mira et al., 2017; Wyse and Dickie, 2018; Jaganathan, 2020). As seed germination is triggered by water uptake, commonly known as imbibition (Harb, 2013; Smýkal et al., 2014), any disruption in this process will cause seed germination to be delayed, and seed dormancy is induced. Thus, the water uptake is crucial for seed germination (Zhu et al., 2021).

Physical dormancy is the most common kind of dormancy in Fabaceae (Wyse and Dickie, 2018; Carruggio et al., 2020). The notion is backed up by the fact that two Fabaceae genera, *Cassia* and *Astragalus*, have been physically dormant (Rodrigues-Junior et al., 2020; Soltani et al., 2020). Physical dormancy is also present in the majority of Australian *Acacia* species (Burrows et al., 2019)

Various methods such as heat shock, chemical scarification using an acid solution, and mechanical scarification are utilized to alleviate physical dormancy in Fabaceae species, with acid treatment being the most effective (Kimura and Islam, 2012; Rusdy, 2017). Numerous studies have shown that these treatments can alleviate many Fabaceae plants' physical dormancy. Mechanical, chemical, and heat treatments, for example, overcome

physical dormancy in two *Bituminaria* species, *Bituminaria bituminosa* and *B. basaltica* (Carruggio et al., 2020). Meanwhile, heat treatment is said to break *Bauhinia winitii* seeds' physical dormancy (Lestari and Firdiana, 2021).

*Gleditsia* is a woody shrub and tree genus in the Fabaceae plant family that was initially distributed in subtropical and temperate regions (Cerino et al., 2018). Some *Gleditsia* species, such as *Gleditsia sinensis* and *G. triacanthos* are medicinal plants (Zhang et al., 2015; Gao et al., 2016), whereas *G. amorphoides* are employed as timber and industrial plant species (Cerino et al., 2018). *G. assamica* is a *Gleditsia* species with vulnerable conservation status (World Conservation Monitoring Centre, 1998). In India, parts of this plant have long been utilized for its medicinal properties (Banerjee et al., 2019). *G. assamica* is a possible plant species for reforestation efforts (Peneng and Priyadi, 2013).

According to Peneng and Priyadi (2013), *G. assamica* seed shows physical dormancy, and mechanical scarification is the most effective treatment for increasing the species' germination performance. However, to our knowledge, no previous studies describe the treatment effect on *G. assamica* seed water uptake. As the effectiveness of physical dormancy alleviation treatment is connected with its capacity to enable seed water uptake, the current study aims to determine the effect of mechanical scarification on *G. assamica* seed water uptake and germination parameters. This research will help us better understand the effect of mechanical scarification on *G. assamica* germination biology and will be helpful for the species' future conservation and domestication efforts.

## METHODS

This research is being carried out at the Bali Botanic Garden Seed Bank Laboratory. The laboratory lies in Bedugul, Bali, approximately 2000 meters above sea level.

### Seeds Collection

*G. assamica* seeds utilized in this research were obtained from the botanic garden collection under code number VII.D.17. The seeds were collected from pods that abundantly fell below the trees living specimens. In the laboratory, *G. assamica* seeds were removed from their pods, washed with running tap water, rinsed, and dried against a room dehumidifier at ambient temperature for roughly two hours. Mechanical scarification is performed by nicking the seeds with a nail clipper on one side. In this research, unscarified seeds are used as

control.

### Seed Water Uptake Experiments

Two trials were conducted to understand the mechanical scarification effect on *G. assamica* water uptake. The first trial was a tetrazolium penetration test (Vishwanath et al., 2013), which was carried out by immersing 15 seeds for each treated and untreated *G. assamica* in a 10 ml of 1% tetrazolium solution at the dark room temperature. Cut tests were done on five seeds for each treated and untreated seed at 24 hours, 48 hours, and 72 hours following imbibition.

The second trial compared the proportion of treated and untreated *G. assamica* seed water uptake. Before the experiments, the weights of treated and untreated *G. assamica* seeds were measured using an analytical scale. Afterward, the seeds were put in a Petri dish with moist straw paper as media, then incubated under ambient conditions. The seeds' weight was then measured 24 and 48 hours following seed sowing. Seed weight was measured by temporarily removing the seeds from their media, drying them on dry tissue paper, and then weighing them on an analytic scale. The seeds were returned in their original condition following the weight measurement (Nizam, 2011). In this trial, four replications are employed, each with ten seeds.

### Seed Germination Trial

The germination trial is carried out by sowing treated and untreated *G. assamica* seed in a Petri dish with straw paper as germination media. The seeds were then incubated for ten days at room temperature. Germination is when the radicle emerges from the seed and is observed every day until the last incubation day. For each treatment, a completely randomized design of four replications with 25 seeds per replication was employed in this experiment.

### Data analysis

The tetrazolium penetration test data were analyzed descriptively. Meanwhile, *G. assamica* seeds water uptake data is estimated according to Nizam (2011), Silva et al. (2018), and Ali (2021) as follows:

$$\text{Water Uptake Percentage (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

W1 is the initial weight of the seeds and W2 is the weight of the seeds after they have absorbed water for a specific amount of time.

Meanwhile, nine germination parameters, namely Final Germination Parameter (FGP), Germination Speed Index (GSI), T10 (Time needed

to reach 10% of germination), T50 (Time needed to reach 10% of germination), T90 (Time needed to reach 10% of germination), Mean Germination Time (MGT), Mean Germination Rate (MGR), Germination Speed Coefficient (CVG) and Uniformity of Germination (UnifG) using GermCalc function in SeedCalc germination parameters calculation software for R (Silva et al., 2019). We exclude four parameters in the function, namely the Variance of Germination Time, Coefficient of Variation of Germination Time (CVT), Germination Synchrony (Sinc), and Germination Uncertainty (UNC), because they produce "NaN" and zero values in the calculation result or some replications. An independent t-test was also performed using SPSS 26 software to assess statistical differences in the germination parameters of *G. assamica* seeds with and without physical scarification.

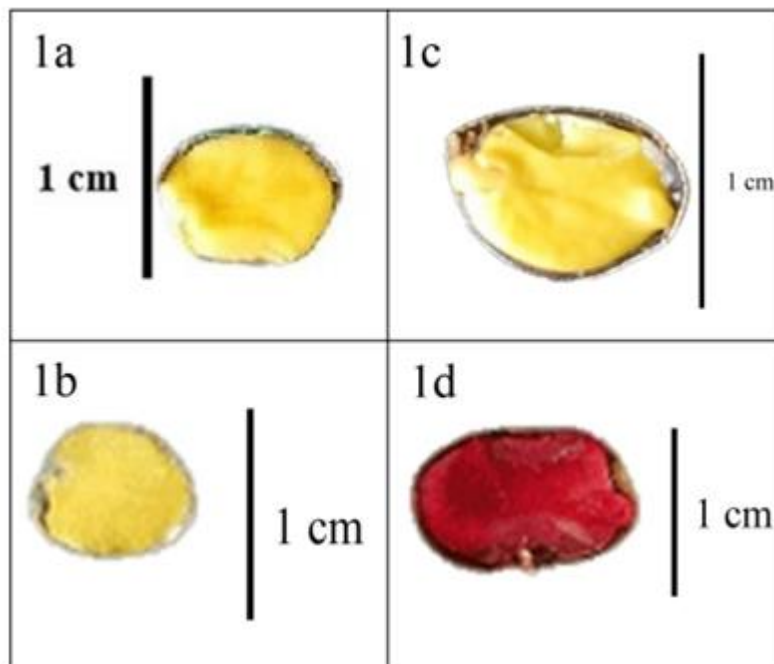
## RESULTS AND DISCUSSION

This research results show that only one non-scarified *G. assamica* seed displayed red coloration after submerging in tetrazolium solution. Meanwhile, following the same treatment, all scarified seeds turn red. Figure 1 depicts the tetrazolium coloring of treated and untreated *G. assamica* seed in this research. The increased frequency and intensity of red coloring in scarified seeds compared to unscarified seeds indicated the

ability of mechanical scarification to facilitate water penetration into the seed, as evidenced by red color-induced tetrazolium in its inner part after seed immersion.

Tetrazolium salt will transform into a red chemical complex known as formazans when in contact with a live seed cell (Vishwanath et al., 2013; Anghinoni et al., 2019; Zhu et al., 2021). The formazans will then be displayed as red coloration in the seeds. As a result, tetrazolium solution is commonly used to assess seed quality (França-Neto & Krzyzanowski, 2019). Moreover, because tetrazolium dissolves in water, the solution might also be used to study seed water uptake. Tetrazolium solution has already been used to study the water uptake of certain plant species, including wild and mutant *Arabidopsis* (Vishwanath et al., 2013). Tetrazolium has also been used to investigate seed water uptake in another *Gleditsia* species, *G. sinensis* (Zhu et al., 2021), as well as other Fabaceae species such as *Stylosanthes humilis* (Chaves et al., 2017) and *Parkia multijuga* (Costa et al., 2018).

The red color in the tetrazolium penetration test will appear first in the seed water uptake site region. This assertion is consistent with Zhu et al. (2021) that tetrazolium dyeing on *G. sinensis* seed shows red coloring is initially visible on the seed radicle tip next to the micropyle, which is then confirmed by a magnetic resonance imaging test as the seed's



**Figure 1.** *Gleditsia assamica* seed coloration at 48 hours post immersion in tetrazolium solution. 1a: untreated seeds before immersion; 1b: untreated seeds after 48 hours immersion; 1c: treated seeds before immersion; 1d: treated seeds after 48 hours immersion. Treated seeds indicate mechanical scarification by nicking the seeds with a nail clipper.

initial water uptake location. Dye tracking investigations on other hard seed coat Fabaceae species *Cercis chinensis* show dye coloration with aniline blue initially found only in the initial water uptake point (Wang et al., 2019) support this assertion.

According to our findings, two scarified *G. assamica* seeds evaluated 24 hours after immersion in tetrazolium solution show red coloration only around the scarified zone (Figure 2). The condition shows that mechanical scarification of the seed coat serves as a water uptake route for the seeds. This situation also suggests that mechanical scarification capacity to promote *G. assamica* seed water uptake is owing to its ability to provide a water uptake site for the seed.

This study also reveals that the water content of *G. assamica* seeds increases more rapidly in scarified seeds than in non-scarified seeds. Scarified seeds have water absorption rates of 41.77 % and 88.42 % at 24 hours and 48 hours after sowing, respectively, compared to just 8.86 % and 13.24 % on non-scarified seeds in the same time interval. Figure 3 depicts the complete data of *G. assamica* seed water uptake measured during this research.

The faster water uptake in scarified seed relative to non-scarified seed found in this study is also observed in other hard seed coat Fabaceae species, particularly *Cercis chinensis* (Wang et al., 2019). This finding suggests that mechanical scarification might help the seed absorb water more effectively. This result also confirms the ability of mechanical scarification to increase seed water uptake, as previously observed in other *Gleditsia* species, namely *G. sinensis* (Zhu et al., 2021), and other Fabaceae species such as *Abrus precatorius* (Prakash et al., 2013), *Ormosia paraensis* (Silva et

al., 2018) and six Fabaceae species from Argentina; *Crotalaria incana*, *C. pumila*, *C. Stipularia*, *Desmanthus virgatus*, *Galactia texana* and *Senna aphylla* (Galíndez et al., 2016).

### **Gleditsia assamica Seed Germination**

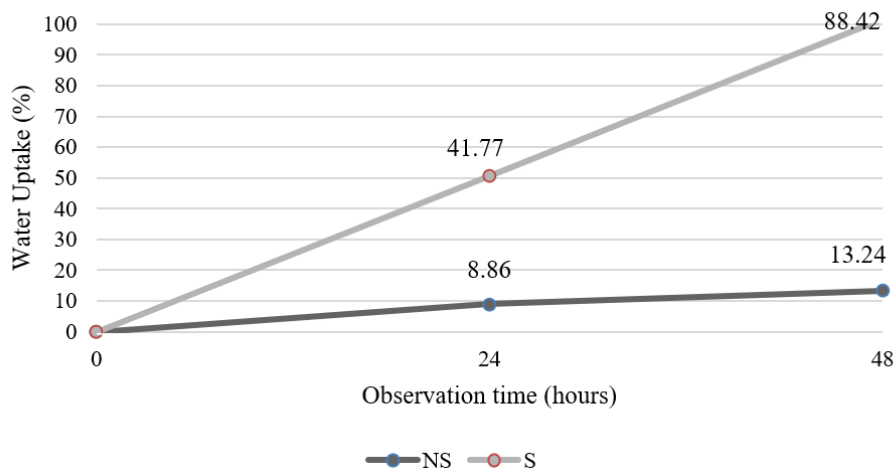
This study reveals that mechanical scarification can significantly improve three *G. assamica* two germination parameters: FGP and GSI. However, the treatment could not significantly increase seven other germination metrics, namely T10, T50, T90, UnifG, MGT, MGR, and CVG. Table 1 shows the complete list of *G. assamica* germination characteristics observed in this research.

FGP is an index that describes the amount of germinated seed relative to sown seed solely on the final germination day, without considering seed germination speed and uniformity. A higher FGP indicates that a more significant number of germinated seeds are detected in the seed population (Kader, 2005). Our findings demonstrate that scarified seeds had a higher FGP value, implying that scarified seeds germinated more than unscarified seeds on the last germination day. The results are congruent with Peneng and Priyadi (2013), who discovered that mechanical scarification was the most efficient method for increasing *G. assamica* seed germination. Physical scarification and sulfuric acid treatment were also thought to be the most effective treatment for raising the germination percentage of other *Gleditsia* species such *G. caspica* (Nourmohammadi et al., 2019).

This research result also shows that scarified seeds produce significantly higher GSI values than unscarified seeds. The result indicated that the treatment enhanced the germination speed of *G. assamica*. This result follows Oliveira et al. (2017),



**Figure 2.** Two *G. Assamica* seeds display red coloring around the scarified location 24 hours post immersion in tetrazolium solution.



**Figure 3.** Water uptake value by *G. assamica* seeds (NS=non-scarified seeds and S=scarified seeds).

who reported that mechanical scarification significantly enhances the GSI value of Fabaceae species *Acacia aruculiformis*.

T10, T50, and T90 reflect the time required for the seed lot to achieve 10%, 50%, and 90% of the total germinated seed in each seed lot. The absence of significant difference between all three parameters in scarified and unscarified seeds indicated that mechanical scarification could not affect the time needed for the seed lot to attain the respective germination percentage. The same condition is also recorded from UnifG, MGT, MGR, and CVG, showing no significant difference between the scarified and unscarified seeds. No difference in UnifG value indicates that mechanical scarification treatment does not affect the uniformity of seed germination. MGT is an index to measure the average time length required by a seed lot to obtain maximum germination, whereas CVG is the reciprocal of MGT and is used to measure germination rate along with the MGR (Ranal and Santana, 2006). No significant difference in this parameter value observed during this study means that mechanical scarification

cannot affect *G. assamica* germination rate and the average time needed to obtain optimum germination.

This research result agrees with previous studies that state mechanical scarification can improve hard seed coat Fabaceae species germination. As an example, Soltani et al. (2020) reported that along with sulfuric acid treatment, mechanical scarification is the best treatment to alleviate *Astragalus* spp. Physical dormancy. Mechanical scarification was previously reported as the best treatment to alleviate two forage Fabaceae species, *Calobota sericea* and *Lassertia frutescens* (Müller et al., 2017), and support restoring critical Fabaceae species, *Schizolobium parahyba* (Salazar and Ramirez, 2019). Furthermore, it was also reported to significantly enhance the germination of six physically dormant Fabaceae species, namely *Crotalaria incana*, *C. pumila*, *C. stipularia*, *Desmanthus virgatus*, *Galactia texana*, and *Senna aphylla* (Galíndez et al., 2016), and improve the germination of non-physiologically dormant *S. humilis* (Chaves et al., 2017).

**Table 1.** Germination ability of *G. assamica* seeds with and without mechanical scarification

Treatment	FGP	GSI	T10	T50	T90	UnifG	MGT	MGR	CVG
Scarification	96.80a	5.260a	3.120a	4.322a	5.808a	2.688a	4.896a	0.205a	20.782a
Non-scarification	11.20b	0.472b	3.80a	5.350a	7.120a	3.320a	5.854a	0.178a	17.886a

Notes: Values followed by different notations show a significant difference among the treatments based on the t-test ( $p < 0.05$ ). Final Germination Parameter (FGP), Germination Speed Index (GSI), T10 (Time needed to reach 10% of germination), T50 (Time needed to reach 50% of germination), T90 (Time needed to reach 90% of germination), Mean Germination Time (MGT), Mean Germination Rate (MGR), Germination Speed Coefficient (CVG) and Uniformity of Germination (UnifG)

The potential of mechanical scarification to offer a pathway for seed water uptake improves seed germination in physically dormant seeds, including *G. assamica*. The seed water uptake process stimulates seed metabolic activity to sustain germination (Rosental et al., 2014). Consequently, better water uptake by the treated seed will improve its germination, resulting in better germination parameters of treated seeds than untreated seeds.

## CONCLUSION

Mechanical scarification can significantly boost *G. assamica* water absorption, thus increasing seed germination. This result confirms the ability of mechanical scarification to alleviate *G. assamica* seed's physical dormancy. These findings enhance understanding of the mechanical scarification effect to alleviate seed dormancy and its influence on the germination biology of hard seed coat Fabaceae species, especially *G. assamica*. As scarification is proved able to improve *G. assamica* water uptake and germination further research on the treatment effect on its seedling development may be conducted in the future. Research on scarification treatment in combination with other treatments such as seed priming should be also considered.

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