



Seed dormancy and germination in *Sophora secundiflora* (Fabaceae)

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Abstract. Given the ecological and horticultural significance of *Sophora secundiflora* (Ortega) DC., effective dormancy-breaking techniques are crucial for enhancing its cultivation and ensuring successful establishment in both natural and managed environments. Seeds have an extremely hard and water impermeable testa. This study evaluated the effects of mechanical scarification, sulphuric acid soaking for 30, 60 and 90 minutes and hydrogen peroxide soaking for 10 and 20 minutes on final germination percentage (FGP), mean germination time (MGT), time to 50% germination (T50) and coefficient of velocity of germination (CVG). Mechanical scarification and 60-minute sulphuric acid treatments were the most effective, achieving FGPs of 95% and 93%, respectively, and showing efficient germination processes as indicated by T50 and CVG metrics. Mechanical scarification resulted in the fastest and most consistent germination. Sulphuric acid treatments showed time-dependent efficacy, with the 60-minute treatment optimising both germination speed and percentage, whereas the 90-minute treatment caused potential seed damage, reflected in a poorer FGP. Hydrogen peroxide treatments were less effective overall, with a maximum FGP of 33% for the 20-minute soaking. Statistical analyses highlighted significant differences among

treatments, particularly for FGP ($p < 0.0001$), T50 ($p = 0.0020$) and CVG ($p = 0.0348$). These findings support the role of physical and chemical scarification in breaking dormancy in Fabaceae seeds, offering valuable insights to optimise germination protocols for *S. secundiflora* and similar species.

Keywords: coat dormancy, germination, mescal-bean, *Sophora secundiflora*, scarification.

Introduction

Dormancy and germination are crucial seed traits that are integral to the plant life cycle (Kildisheva *et al.*, 2020; Nautiyal *et al.*, 2023). Research indicates that 15 families of angiosperms display physical dormancy, notably within the three subfamilies of Fabaceae: Mimosoideae, Papilionoideae, and Caesalpinoideae (Baskin *et al.*, 2000). Seed coat dormancy, or physical dormancy, is characterised by a hard, impermeable seed coat that inhibits the penetration of water and gases, thereby preventing the embryo from accessing the conditions necessary for germination (Bhatla and Lal, 2023).

Seed dormancy poses a considerable challenge in cultivating *Sophora secundiflora* (Ortega) DC. (Fabaceae), also known as *Dermatophyllum secundiflorum* or *Calia secundiflora*, commonly referred to as mescal-bean. This species is known for its ornamental and ecological benefits (Fu *et al.*, 2016). *S. secundiflora* is an evergreen shrub or small tree native to western Texas, New Mexico, and northern Mexico (Aly *et al.*, 2020). Mescal-bean typically reaches a height of up to 4 m. The plants flower in March or April, producing attractive light purple petals. The fruit is a woody pod containing several seeds. These seeds are bright orange to scarlet-red and possess very hard seed coats (Jordan, 2014). Although mescal bean can be a noxious plant on rangelands, it is frequently cultivated as an ornamental and is highly effective for rehabilitating degraded soils in arid and semi-arid regions due to its symbiotic relationship with *Rhizobium* bacteria (Correll and Johnston, 1970; Taylor and Ralphs, 2019; Oono *et al.*, 2021). The genus *Sophora* (Papilionaceae) comprises 30 species with a worldwide distribution. *S. secundiflora* is considered as an excellent native plant for landscaping in Texas due to its tolerance to alkaline soils and moderate drought conditions (Niu *et al.*, 2011). However, there is limited research on the seed biology of *S. secundiflora* during germination, and protocols for pretreating seeds to break seed coat dormancy are scarce. Previous studies have indicated that natural regeneration

of *S. secundiflora* is very poor, with several extrinsic and intrinsic constraints identified (Kildisheva *et al.*, 2013; Ihtisham *et al.*, 2021). Therefore, breaking this dormancy is essential for the successful propagation and cultivation of this species.

In the Fabaceae family, seed coat dormancy is commonly addressed through physical and chemical scarification techniques (Kildisheva *et al.*, 2020; Jara-Peña and Marín-Bravo, 2023). Without pretreatment, germination can be erratic and prolonged, sometimes extending over many years (Rehmani *et al.*, 2022). Therefore, artificial or natural dormancy-breaking treatments are employed to improve germination of such hard-coated seeds (Baskin and Baskin, 2020). Previous research has demonstrated the effectiveness of sulphuric acid treatments and mechanical scarification in enhancing germination by breaking down the hard seed coat. Sulphuric acid treatments chemically erode the seed coat, making it more permeable, while mechanical scarification physically disrupts the seed coat, allowing water and gases to penetrate more easily (Kheloufi *et al.*, 2018; Kheloufi *et al.*, 2019; Kheloufi *et al.*, 2020; Mansouri and Kheloufi, 2021; Kheloufi, 2022; Mansouri and Kheloufi, 2023; Kheloufi, 2024). Another method involves the use of hydrogen peroxide to enhance the internal conditions of the seed, promoting the metabolic processes necessary for germination (Bhatla and Lal, 2023).

This study aims to evaluate the effectiveness of these pretreatment methods in breaking the dormancy of *S. secundiflora* seeds. By evaluating the relative effectiveness of these methods, We aim to provide practical recommendations to enhance the germination and propagation of *S. secundiflora*. The results from this study will contribute to the existing body of knowledge on seed dormancy in Fabaceae and offer insights into optimising germination protocols for *S. secundiflora*.

Materials and methods

Seed harvest and morphometry

Mature pods of *S. secundiflora* were collected on January 2024, from 9 shrubs located in the municipal park of Oran in North-West of Algeria (35°4'12" N, 0°38'42" W; 111.8 m a.s.l.) (Fig. 1). Seeds were stored in paper bags under standard laboratory conditions until they were used on March 2024. Seed morphological characteristics of *S. secundiflora* used in this study are presented in Table 1. The seed sample for this experiment was obtained by mixing all the collected seeds. The 1000 seeds weighed 785.7 g.



Figure 1. *Sophora secundiflora* flowers, leaves, pods, and seeds.

Table 1. Morphometric characteristics of *Sophora secundiflora* seeds (n=100).

Parameters	Mean \pm SD	Minimum	Maximum
Length (cm)	1.33 \pm 0.18	1.18	1.52
Width (cm)	1.11 \pm 0.07	1.02	1.22
Thickness (cm)	0.96 \pm 0.06	0.87	1.06
Weight (g)	0.74 \pm 0.08	0.39	1.01

Experimental design

Different pretreatments were given to freshly collected seeds of *S. secundiflora* (4 replicates of 25 seeds) to break seed coat-imposed dormancy. The treatments were 1) control (untreated seeds), 2) chemical scarification of intact seeds with concentrated sulphuric acid (H₂SO₄) for 30, 60 and 90 minutes followed by washing in tap water for 5 minutes, 3) chemical scarification of intact seeds with hydrogen peroxide (H₂O₂) for 10 and 20 minutes followed by washing in tap water for 10 minutes, 4) mechanical scarification (making a small and shallow cut of the endocarp using the corner of nail clippers) (Kheloufi, 2024).

Seeds from each treatment were germinated in plastic container between two layers of moist filter paper in total darkness under 25 °C (\pm 2 °C) for 17 days. Germination was defined as the emergence of radicle from the seed coat. As part of the experiment, it was essential to maintain a certain level of humidity for the seeds. A complete randomised design was used to conduct the germination test.

Germination Parameters

Final germination percentage (FGP): The FGP represents the total number of seeds germinated out of the total seeds. This germination parameter was calculated using the formula:

$$\text{FGP (\%)} = \frac{\sum ni}{N} \times 100 \quad (1)$$

where FGP is the final germination percentage, ni is the number of germinated seeds on the last day of the test, and N is the total number of seeds incubated per test (Côme, 1970).

Mean Germination Time (MGT): The MGT index showed that how fast the seeds emerged in a population. This was calculated using the following formula:

$$\text{MGT (days)} = \frac{\sum(ti.ni)}{\sum ni} \quad (2)$$

where MGT is the mean germination time, ti is the number of days since the beginning of the test, ni is the number of germinated seeds recorded at time $t(i)$, and $\sum ni$ is the total number of germinated seeds (Orchard, 1977).

Time to 50% germination (T_{50}): The T_{50} was developed to find out the time required for 50% seed germination. This is reported through the following formula:

$$T_{50} \text{ (days)} = \frac{ti + (N/2 - ni)(tj - ti)}{(nj - ni)} \quad (3)$$

where N final number of seeds emerged, n_j and n_i are the cumulative numbers of seeds emerged after adjacent counts during t_j and t_i , when $n_i < N/2 < n_j$ (Coolbear *et al.*, 1984).

Coefficient of the velocity of germination (CVG): The CVG represents the velocity of germination of seeds in an experiment, which will increase with an upsurge in the frequency of germinated seeds. The highest theoretical CVG value will be obtained when all sown seeds grow on the first day. This is calculated using the formula:

$$\text{CVG (\%)} = \frac{N_1 + N_2 + N_3 \dots N_x}{100} \times N_1 T_1 \dots N_x T_x \quad (4)$$

in which N is the frequency of seeds germinating every day and T represents the time from sowing to germination of seed N (Khan *et al.*, 2019).

Statistical analyses

The effects of different pretreatments on the four variables studied were tested by one-way analysis of variance (ANOVA). Differences between treatments following ANOVA were made by means comparison. Multiple comparisons of means were carried out using Tukey's test ($p < 0.05$). All statistical analyses were performed using SAS software Version 9.0 (Statistical Analysis System) (2002).

Results

Germination kinetics

Figure 2 presents an analysis of the daily cumulative germination percentages of *S. secundiflora* seeds subjected to various pretreatments aimed at breaking dormancy and enhancing germination. The treatments include different durations of soaking in sulphuric acid (SA), soaking in hydrogen peroxide (HP), and mechanical scarification (MS). The results show distinct patterns of germination responses over a 17-day period. The germination kinetics of *S. secundiflora* seeds can be interpreted by examining the cumulative germination curves over time for different pretreatments. These curves typically exhibit three distinct stages: latency, exponential growth, and plateau. Understanding these stages provides insights into the effectiveness of the dormancy-breaking treatments and the overall germination process.

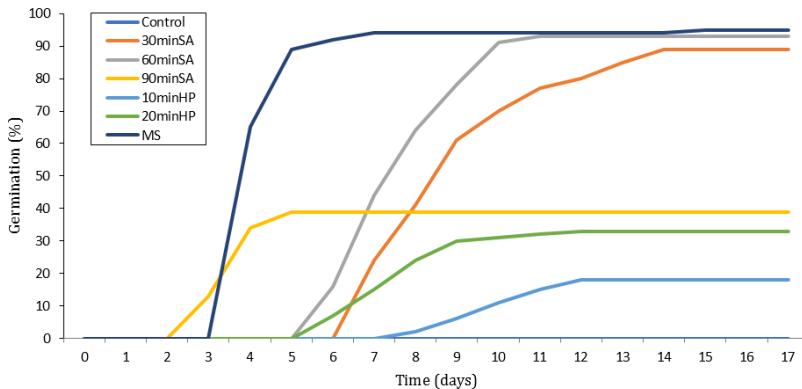


Figure 2. Cumulative germination percentages of *Sophora secundiflora* seeds after different pretreatments for 17 days. Control (untreated seeds); 30minSA (30 minutes soaking in sulphuric acid); 60minSA (60 minutes soaking in sulphuric acid); 90minSA (90 minutes soaking in sulphuric acid); 10minHP (10 minutes soaking in hydrogen peroxide); 20minHP (20 minutes soaking in hydrogen peroxide); MS (Mechanical scarification).

The initial latency stage is characterised by low to no germination. During this period, seeds are absorbing water and initiating internal biochemical processes necessary for germination but have not yet begun to sprout. The latency period varies depending on the pretreatment method. In untreated seeds (control), the latency period extends indefinitely, as no germination is observed throughout the experiment. In contrast, seeds treated with 30minSA and 60minSA show a latency period of about 6-7 days before germination begins. Mechanical scarification (MS) significantly reduces the latency period, with germination starting as early as day 4-5.

During the exponential stage, germination occurs rapidly. The seeds that have broken dormancy begin to germinate, and the cumulative germination percentage increases quickly. For the 60minSA and 30minSA treatments, this stage begins around day 7-8, with a rapid increase in germination observed until day 11-12. The MS treatment enters the exponential phase earlier and reaches near-complete germination by day 7-8, indicating a very effective dormancy-breaking process. The hydrogen peroxide treatments (10minHP and 20minHP) enter the exponential growth stage later and exhibit a slower rate of increase, reflecting their reduced efficacy in breaking seed dormancy.

The final plateau stage is characterised by a leveling off of the cumulative germination curve, where the rate of new germination decreases and the curve approaches an asymptote. This stage indicates that most viable seeds have germinated, and further increases in germination percentage are minimal. For the 60minSA treatment, the plateau of germination is reached around day 12-13 with an FGP of 93%. Germination under the 30minSA treatment reaches a plateau around day 15, with an FGP of 89%. On the other hand, germination under the MS treatment reaches its plateau the earliest, by days 9-10, with an FGP of 95%. However, germination under the 90minSA treatment reaches its plateau much earlier, around day 6, but at a reduced FGP of 39%, suggesting potential seed damage due to overexposure to sulfuric acid. Germination under the HP treatments plateaus at decreased FGPs, with 33% for the 20minHP treatment and 18% for the 10minHP treatment, by days 14-15.

Germination traits

The statistical analysis of the pretreatments for *S. secundiflora* seeds, indicated by F-values and p-values, reveals significant differences in their effectiveness. The final germination percentage (FGP) exhibited highly significant differences among treatments (F-value = 181.60, $p < 0.0001$), indicating that the pretreatments had a substantial impact on breaking seed dormancy and promoting germination. While the mean germination time (MGT) showed no significant differences (F-value = 2.46, $p = 0.0725$), the time to 50% germination

(T₅₀) displayed significant variation (F-value = 6.00, p = 0.0020), suggesting differences in how quickly 50% of the seeds germinated across treatments. The coefficient of velocity of germination (CVG) also showed significant differences (F-value = 3.13, p = 0.0348), reflecting variability in the speed of germination among the treatments. These statistical results highlighted the importance of choosing the right pretreatment method to optimise germination, with mechanical scarification and sulphuric acid treatments being particularly effective (Tab. 2).

The control group, consisting of untreated seeds, showed no germination (FGP = 0%), highlighting that *S. secundiflora* seeds have intrinsic dormancy that requires intervention for germination to occur. Consequently, mean germination time (MGT), time to 50% germination (T₅₀), and the coefficient of velocity of germination (CVG) were not calculated for this group (Tab. 2).

Seeds treated with 30minSA achieved a high FGP of 89%, which was statistically similar to other high-performing treatments. The mean germination time (MGT) was 6.14 days, with the time to 50% germination (T₅₀) at 9.06 days. The coefficient of velocity of germination (CVG) was 16.3%, indicating a relatively efficient germination process (Tab. 2).

Table 2. Effects of different pretreatment on the final germination percentage (FGP), mean germination time (MGT), time to 50% germination (T₅₀) and coefficient of velocity of germination (CVG) of *Sophora secundiflora*.

Pretreatments	FGP (%)	MGT (days)	T ₅₀ (days)	CVG (%)
Control	0.00 ^d	NC	NC	NC
30minSA	89 ± 4.83 ^a	6.14 ± 0.53 ^a	9.06 ± 0.46 ^a	16.3 ± 1.46 ^b
60minSA	93 ± 3.03 ^a	7.05 ± 0.86 ^a	8.26 ± 0.80 ^{ab}	14.3 ± 1.82 ^b
90minSA	39 ± 4.87 ^b	4.78 ± 0.58 ^a	4.15 ± 0.58 ^c	21.4 ± 2.63 ^{ab}
10minHP	18 ± 5.16 ^c	3.87 ± 1.29 ^a	5.29 ± 1.99 ^{abc}	27.7 ± 6.75 ^a
20minHP	33 ± 6.83 ^b	7.66 ± 0.33 ^a	8.12 ± 0.86 ^{ab}	13.1 ± 0.66 ^b
MS	95 ± 3.83 ^a	5.32 ± 0.28 ^a	4.75 ± 0.15 ^{bc}	18.8 ± 1.03 ^{ab}
F-value	181.60	2.46	6.00	3.13
p-value	<0.0001	0.0725	0.0020	0.0348

The different letters in the same column indicate a significant difference at p < 0.05, as evaluated by Tukey's test. NC (not calculated); Control (untreated seeds); 30minSA (30 minutes soaking in sulphuric acid); 60minSA (60 minutes soaking in sulphuric acid); 90minSA (90 minutes soaking in sulphuric acid); 10minHP (10 minutes soaking in hydrogen peroxide); 20minHP (20 minutes soaking in hydrogen peroxide); MS (Mechanical scarification).

A similar pattern was observed for seeds treated with 60minSA, which had a slightly higher FGP of 93%. However, the MGT was slightly longer at 7.05 days, and the T₅₀ was 8.26 days. The CVG for this treatment was 14.35%, slightly

decreased than the 30-minute treatment, suggesting a marginal decrease in germination speed despite the higher overall germination percentage. However, seeds treated with 90minSA showed a significantly poorer FGP of 39%. Despite this reduced final germination rate, these seeds had a faster germination process with an MGT of 4.78 days and a T_{50} of 4.15 days. The CVG was 21.43%, indicating a rapid initial germination phase, possibly due to a more aggressive scarification. However, the poorer FGP suggests potential seed damage from prolonged sulphuric acid exposure (Tab. 2).

Soaking seeds in 10minHP resulted in an FGP of 18%, with an MGT of 3.87 days and a T_{50} of 5.29 days. This treatment had the highest CVG of 27.7%, indicating a quick germination onset. However, the overall germination percentage remained low, signifying that while this treatment may accelerate the initiation of germination, it does not sufficiently break dormancy for a large proportion of seeds. Extending the soaking time in 20minHP improved the FGP to 33%, with an MGT of 7.66 days and a T_{50} of 8.12 days. The CVG was reduced to 13.1%, indicating a slower germination process compared to the 10-minute treatment (Tab. 2).

Mechanical scarification was the most effective treatment, achieving the highest FGP of 95%, which was statistically similar to the 60minSA treatment. The MGT was 5.32 days, and the T_{50} was 4.75 days. The CVG was 18.8%, indicating a relatively efficient germination process. Mechanical scarification effectively breaks seed dormancy by physically damaging the seed coat, allowing for rapid and high-percentage germination (Tab. 2).

Discussion

Seed surface morphology reveals that a hard and impermeable testa is the primary barrier to imbibition, consequently delaying germination (Lamont and Pausas, 2023). Studies on *S. secundiflora* seeds demonstrate the effectiveness of various pretreatments in breaking seed dormancy and improving germination. These findings are consistent with general observations in the Fabaceae family, where physical and chemical scarification are commonly used to enhance germination (Kheloufi, 2020; Jaganathan and Biddick, 2021). Pretreatments such as soaking in sulphuric acid, mechanical scarification, and soaking in hydrogen peroxide have shown varying degrees of success in breaking seed coat dormancy and promoting germination (Nautiyal *et al.*, 2023).

Sulphuric acid treatments significantly improved germination rates, with the 60-minute treatment showing the highest final germination percentage at 93%. This method is effective because sulphuric acid breaks down the hard seed coat, allowing water to penetrate and initiate the germination process. The time-dependent nature of SA is evident, as the 90-minute treatment resulted in

a reduced FGP (39%), possibly due to overexposure causing seed damage. These results are consistent with other studies on Fabaceae species. Kheloufi (2022) showed similar improvements in germination with sulphuric acid treatments. Additionally, Baskin and Baskin (1998) noted that sulphuric acid is a commonly used method to overcome physical dormancy in seeds with hard seed coats. Kheloufi *et al.* (2018) reported that treating seeds with sulphuric acid removed some or all of the cuticular layer, resulting in rapid germination.

Mechanical scarification was the most effective overall, achieving an FGP of 95%. This method directly damages the seed coat, facilitating water uptake and germination. The rapid beginning and high percentage of germination align with findings in other Fabaceae species, where physical scarification has been shown to effectively break seed dormancy (Tang *et al.*, 2022).

Treatments with hydrogen peroxide were less effective than SA and MS. The 10-minute soaking resulted in a low FGP of 18%, while extending the soaking to 20 minutes increased FGP to 33%. This method appears to be less efficient in breaking seed dormancy, likely due to insufficient physical or chemical disruption of the seed coat. Similar observations have been made in other Fabaceae species, where hydrogen peroxide treatments alone were less effective compared to chemical or mechanical scarification (Sirkeck and Singh, 2023). Moreover, Kheloufi (2022) found that hydrogen peroxide soaking did not significantly enhance germination in *Acacia* species, highlighting the necessity for more aggressive scarification methods.

According to Wang (1991), untreated fresh seeds of *S. secundiflora* required 10 weeks to achieve 50% germination, whereas untreated one-year-old seeds exhibited only 8% germination by the end of the experiment. Additionally, mechanical scarification did not significantly enhance the germination of fresh seeds, with a germination rate of only 42%.

The development of a very hard seed coat in its fully desiccated state is typically attributed to the substantial presence of heavily thickened galactomannan or mannan polymers lining the endosperm cell walls (Steinbrecher and Leubner-Metzger, 2017). Within the endosperm, the presence of hydrophilic galactomannan leads to a mucilaginous transformation upon imbibing and subsequent hydrolysis (Zandi *et al.*, 2015). While impermeability of the seed coat and its mechanisms for delaying germination are common traits among legumes, they serve to delay seed germination under adverse environmental conditions (Naik and Deshpande, 2021). The strength of the seed coat and also the endocarp helps in safeguarding the seeds against mechanical harm, facilitating their survival in arid soils during droughts, or enabling natural dispersion and recolonization following fire events (Fenner, 2017; Shiferaw *et al.*, 2018; Dalling *et al.*, 2020; Kheloufi, 2024).

Conclusions

This study demonstrates that mechanical scarification (MS) is the most effective pretreatment for breaking seed dormancy and enhancing germination in *S. secundiflora*, achieving a final germination percentage (FGP) of 95%. This method outperformed all other treatments, including various durations of sulphuric acid soaking and hydrogen peroxide treatments, in both germination percentage and efficiency metrics. Sulphuric acid treatments, particularly the 60-minute soak, also showed high effectiveness with an FGP of 93%, but the mechanical scarification provided the fastest and most consistent results. These findings highlight the critical role of seed coat disruption in overcoming dormancy for *S. secundiflora*. The statistical significance of the results enhances the reliability of these methods, highlighting their practical applicability in improving germination outcomes. Researchers and horticulturists working with *S. secundiflora* or similar hard-seeded species in the Fabaceae family should consider mechanical scarification as the primary method for optimising germination.

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