

Effect of Sulphuric Acid Scarification on Seed Germination of 11 Wild Legume Species from the Senegal River Delta

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Abstract

Seed dormancy is a major constraint that limits the use of wild legumes as green manures. This study assessed the effect of sulphuric acid scarification on the germination of 11 wild legume species collected from the Senegal River Delta. The experiment was conducted under laboratory conditions using a completely randomised factorial design with three replicates. Seeds were subjected to two treatments: an untreated control and chemical scarification with concentrated H₂SO₄ (98%). The measured parameters included final germination rate (FGR), average daily germination rate (ADGR), dormancy rate (DR), and mortality rate (MR). Overall, chemical scarification had a negative effect on germination: treated seeds exhibited lower FGR and ADGR (27.6% and 1.4 seeds day⁻¹) than the controls (53.9% and 2.7 seeds day⁻¹; P < 0.01), along with higher mortality (28.2% vs. 4.0%). However, improved germination was observed in *Rhynchosia minima* (+13.4%), *Macroptilium lathyroides* (+6.0%), and *Aeschynomene indica* (+0.7%). These findings suggest that sulphuric acid scarification is suitable only for these three species, while gentler dormancy-breaking methods should be considered for the others.

Keywords

Seed Dormancy, Wild Legumes, H₂SO₄, Chemical Scarification, Senegal River Delta

1. Introduction

Stretching from the Sahara Desert in the north to the tropical savannahs in the

south, the Sahel is a vast transition zone with an arid to semi-arid tropical climate, characterised by a short rainy season of three to four months and a long dry season of eight to nine months [1]. The vegetation, dominated by therophytes and phanerophytes, is strongly influenced by rainfall variability. For several decades, this region has been undergoing progressive land degradation, exacerbated by declining rainfall [2] [3], loss of biodiversity and a reduction in vegetation cover, leading to a significant decline in soil fertility [4]. These phenomena, amplified by the effects of climate change, make sub-Saharan Africa particularly vulnerable due to its low adaptive capacity [5]. This fragility poses a major threat to rural populations, nearly 70% of whom depend directly on agriculture for their livelihoods [6] [7].

In the face of these challenges, land restoration and sustainable land management are priorities for strengthening the resilience of Sahelian agricultural systems. The use of legumes appears to be a promising way to improve soil fertility, reduce dependence on mineral nitrogen fertilisers and restore degraded ecosystems [8]-[10]. Indeed, numerous studies have shown that integrating legumes into crop rotations contributes to biological nitrogen fixation, improves the physical and chemical properties of the soil, and increases the yields of subsequent crops [11] [12]. However, the use of wild herbaceous legumes, particularly those native to sub-Saharan Africa (SSA), remains limited [13]. One of the major constraints to their use is seed dormancy, which hinders germination and complicates their domestication as green manure.

Seed dormancy, defined as the temporary inability of a viable seed to germinate under favourable conditions, can be physiological, physical or physio-physical in nature [14] [15]. Physiological dormancy (PD) results from a hormonal imbalance between abscisic acid (ABA) and gibberellic acid (GA), while physical dormancy (PY) results from a water-impermeable seed coat formed of rigid palisade cells that provide mechanical protection for the embryo [16]. A mixed form, known as physio-physical (PY + PD), combines these two mechanisms. Among these types, physical dormancy is most frequently observed in legumes [17]-[19].

Various methods can be used to break this dormancy, including stratification, leaching of inhibitory metabolites, chemical or hormonal treatments, and scarification [14] [20]. Among these, scarification, particularly mechanical, thermal, or chemical, remains the most commonly used method for species with hard seed coats, as it facilitates water imbibition and triggers germination [19] [21].

It is in this context that the present study aims to evaluate the effect of chemical scarification with concentrated sulphuric acid (98%) on the germination parameters of seeds from 11 species of wild herbaceous legumes found in the Senegal River delta. More specifically, the aim is to analyse, for each species treated or not treated with sulphuric acid (H_2SO_4), the final germination rate (FGR), dormancy rate (DR), mortality rate (MR), average daily germination rate (ADGR) and germination kinetics.

2. Materials and Methods

2.1. Presentation of the Study Area

The study was conducted at the laboratory of the Agricultural Research Department of the Senegalese Sugar Company (SSC), located in the Senegal River delta in the north of the country (16° 48'N, 15° 57'W). The region has a Sahelo-Saharan climate, characterised by a short rainy season of about three months (July to September) alternating with a long dry season of about nine months. **Figure 1** illustrates the average evolution of temperature, insolation, evaporation and rainfall in the study area over the last 22 years (2000-2022).

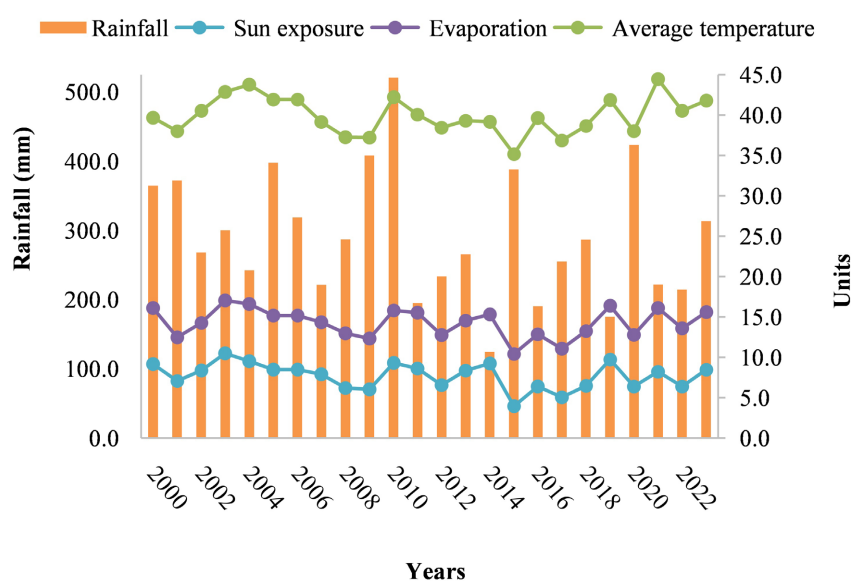


Figure 1. Some climatic characteristics (2000-2022) of the study area (Source: Author's data, Diokhané, 2025).

2.2. Species Used and Seed Collection

The biological material used in this study consists of seeds from 11 species of wild herbaceous legumes: *Aeschynomene indica*, *Cassia occidentalis*, *Crotalaria retusa*, *Cassia obtusifolia*, *Indigofera tinctoria*, *Indigofera hirsuta*, *Macroptilium lathyroides*, *Rhynchosia minima*, *Sesbania pachycarpa*, *Sesbania rostrata* and *Sesbania sesban*. The seeds were harvested at maturity from sugar cane fields and surrounding areas in the Senegal River delta immediately after the end of the rainy season. After harvesting, they were dried indoors at room temperature for about a month, then shelled to separate the seeds from their pods. A preliminary sorting process eliminated empty, damaged or immature seeds, followed by careful sorting with a binocular magnifying glass to remove any seeds that were unlikely to germinate.

2.3. Seed Dormancy Breaking

The seeds were treated with concentrated sulfuric acid (H₂SO₄, 98%) for five minutes to break dormancy of the seed coat. This protocol was chosen because

previous studies have shown that it is effective in disrupting seed coat impermeability while preserving embryo viability [22]-[24]. However, longer exposure times have been reported to cause embryo injury, whereas shorter durations result in incomplete or uneven germination. Furthermore, concentrations of acid lower than 98% have been shown to provide insufficient scarification for effective dormancy release [23] [25].

2.4. Seed Pre-Treatment

The seeds were surface sterilised using a 1% sodium hypochlorite solution for 10 minutes and then rinsed thoroughly three times with distilled water to remove any residual disinfectant traces [23]. After rinsing, the seeds were divided into two batches. The control batch was placed directly in Petri dishes lined with two layers of absorbent tissue paper (Kleenex), which had been moistened. The treated batch was soaked in concentrated sulphuric acid (98%) for five minutes, as described by [20]. Then, it was washed thoroughly three times with distilled water before being placed in conditions identical to those of the control batch.

The seeds were evenly distributed in the Petri dishes so as to avoid contact between them. The pre-moistened dishes were placed on laboratory benches at room temperature. Regular re-moistening was carried out to maintain optimal humidity levels throughout the germination test.

2.5. Experimental Design

The germination test involved 300 seeds for each of the 11 species of wild herbaceous legumes studied. For each species, the seeds were divided into two batches of 150 seeds, one corresponding to the control batch not subjected to chemical scarification and the other to the batch treated with concentrated sulphuric acid (H₂SO₄). Each batch of 150 seeds was then randomly subdivided into three sub-samples of 50 seeds, representing the three replicates of the experimental design. A total of 66 Petri dishes were thus prepared for all the tests. The experimental design was organised according to a 2 × 11 factorial plan, in complete randomised blocks with three replicates. Two factors were considered: the first corresponded to the type of legume species, with 11 modalities (*Aeschynomene indica*, *Cassia occidentalis*, *Crotalaria retusa*, *Cassia obtusifolia*, *Indigofera tinctoria*, *Indigofera hirsuta*, *Macroptilium lathyroides*, *Rhynchosia minima*, *Sesbania pachycarpa*, *Sesbania rostrata* and *Sesbania sesban*); the second factor corresponded to the type of treatment, with two modalities (with or without scarification with sulphuric acid). This setup allowed for rigorous evaluation of the effect of chemical treatment on the germination of the different species studied.

2.6. Parameters Evaluated

Germination was monitored daily, at the same time, over a period of 30 days, in order to count the number of germinated seeds for each treatment and each species. Each seed considered to have germinated, *i.e.* with a radicle emerging from

the seed coat by approximately 2 mm [20] [26], was immediately recorded and then removed from the Petri dish. Similarly, dead seeds (mouldy or rotten) were identified, counted and removed to prevent contamination spreading to other seeds.

This rigorous daily monitoring made it possible to determine the main germination parameters for each of the 11 species studied, namely the final germination rate (FGR), dormancy rate (DR), mortality rate (MR), average daily germination rate (ADGR) and germination kinetics over the entire observation period.

2.6.1. Final Germination Rate (FGR)

The final germination rate was calculated for each species by dividing the total number of seeds germinated during the experimental period by the total number of seeds placed in germination. This rate was determined by taking into account the results of all repetitions using the following formula.

$$FGR(\%) = \frac{\sum ni \times 100}{N}$$

where *FGR* is the final germination rate, $\sum ni$ is the cumulative number of seeds germinated during the various germination counting dates *i*, and *N* is the number of seeds placed in germination.

2.6.2. Average Daily Germination (ADG)

This corresponds to the average number of seeds that germinated each day [27]. It is determined by the following formula, $\sum ni$ total number of seeds that germinated during the monitoring period; *T*, number of monitoring days.

$$ADG = \frac{\sum ni}{T}$$

2.6.3. Seed Dormancy Rate (DR)

In this study, we considered all non-germinated seeds that remained intact throughout the germination test without any change, despite the favourable conditions in which they were placed [23], to be dormant seeds. The dormancy rate was therefore determined using the following formula, where *N* is the total number of seeds placed in germination.

$$DR(\%) = \frac{\sum dormancy\ seeds}{N} \times 100$$

2.7. Statistical Analysis

The normality of the data was verified using the Shapiro–Wilk test. When this condition was met, the data were subjected to analysis of variance (ANOVA) followed by Tukey’s multiple comparison test to identify significant differences between the treatment means. However, when the data did not follow a normal distribution, the non-parametric Kruskal-Wallis test was applied. All statistical analyses were performed using Statistix software, version 10, and differences

were considered significant at a probability threshold of $P < 0.05$ [28]. In addition, germination curves over time were plotted using Microsoft Excel 2013 software.

3. Results

The results of the analysis of variance performed on the various parameters studied (Table 1) show that, apart from the variable percentage of dormant seeds (DS), all other treatments of the two factors studied, namely sulphuric acid pre-treatment and legume, as well as their interaction, reveal a highly significant difference ($P < 0.001$). This result indicates that pre-treating the seeds of the 11 legumes with concentrated sulphuric acid significantly influences the germination rate, mortality rate and average daily germination. On the other hand, the acid treatment has no significant influence on the rate of dormant seeds.

Table 1. Analysis of variance of the different parameters.

Source	D.F.	Mean square (MS)			
		GR (%)	MR (%)	DR (%)	ADG
Legumes	10	2695.8**	937.5**	2009.0**	0.75**
Pre-treatment	1	11415.5**	13963.6**	128.2ns	3.17**
Legumes*Pre-treatment	10	1775.4**	1072.2**	2679.4**	0.49**
Error	44	37.6	16.2	69.6	0.01

* $P < 0.05$; ** $P < 0.01$, ns: not significant at $P < 0.05$, GR: germination rate, DR: dormancy rate, MR: mortality rate, ADG: average daily germination, DF: degrees of freedom.

3.1. Effect of Acid Treatment on Seed Germination

Figure 2 shows that the germination rate of seeds not treated with sulphuric acid is significantly higher than that of treated seeds ($P < 0.001$). This indicates that soaking the seeds of these 11 legumes in sulphuric acid does not improve their germination. In fact, with the exception of *R. minima*, *M. lathyroides* and *A. indica*, concentrated sulphuric acid has an adverse effect on the germination of all other species.

Considering seeds not treated with acid, Figure 2 shows that *S. pachycarpa* and *I. tinctoria* have the highest germination rates, reaching 98.6% and 95.3% respectively. They are followed by *M. lathyroides*, *S. rostrata*, *C. retusa* and *C. occidentalis*, with germination rates of 70.6%, 67.3%, 66.6% and 62.0% respectively. In contrast, the lowest germination rates were observed in *S. sesban* and *C. obtusifolia*.

With regard to seeds treated with sulphuric acid, a significant difference was observed between the germination rates of the different species ($P < 0.05$).

Among the 11 species studied, only *R. minima*, *M. lathyroides* and *A. indica* showed a slight improvement in germination rates compared to the untreated

control, with gains of +13.4%, +6.0% and +0.7% respectively. In contrast, the other eight species showed a significant decrease in germination rates. The highest germination rates after treatment were observed in *M. lathyroides* (76.6%) and *C. retusa* (58.6%), which remain statistically superior to the other species. Conversely, the lowest rates were recorded in *S. sesban* (6.7%), *I. tinctoria* (3.3%), *C. occidentalis* (4.0%) and *C. obtusifolia* (4.0%).

Furthermore, **Figure 2** shows that sulphuric acid treatment was particularly unfavourable for the species *I. tinctoria*, *S. pachycarpa*, *C. occidentalis* and *S. rostrata*, which recorded respective decreases in their final germination rates of -92.0%, -67.3%, -58.0% and -47.3% compared to their untreated control.

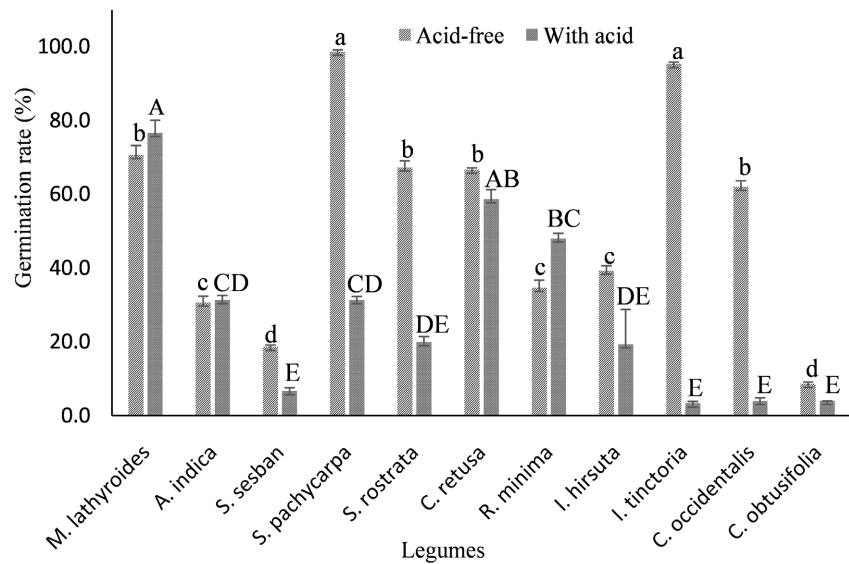


Figure 2. Final germination rate of seeds treated or untreated with concentrated sulphuric acid. Different lowercase letters indicate significant differences ($P < 0.05$) between the germination rates of legumes not treated with acid. Different capital letters indicate significant differences ($P < 0.05$) between the germination rates of legumes treated with sulphuric acid.

3.2. Mortality Rates of Seeds Treated or Untreated with Acid

Analysis of variance reveals a significant difference between the mortality rates of seeds of different species, whether treated or untreated with concentrated sulphuric acid ($P < 0.001$). Treatment with H_2SO_4 resulted in a significant increase in the mortality rate of legume seeds (**Figure 3**).

In the batch of acid-treated seeds, the highest mortality rates were observed in *C. occidentalis* (86.0%) and *C. obtusifolia* (74.0%), followed by *A. indica* (38.6%), *S. pachycarpa* (32.6%), *S. sesban* (31.3%) and *S. rostrata* (26.6%). In contrast, the lowest rates were recorded in *M. lathyroides* (8.7%) and *I. tinctoria* (6.7%).

Among seeds not treated with acid, the mortality rate remained low. Only *C. retusa* (16.0%) and *S. rostrata* (14.6%) had significantly higher mortality rates than the other species studied. The latter all had mortality rates below 4.0% (**Figure 3**).

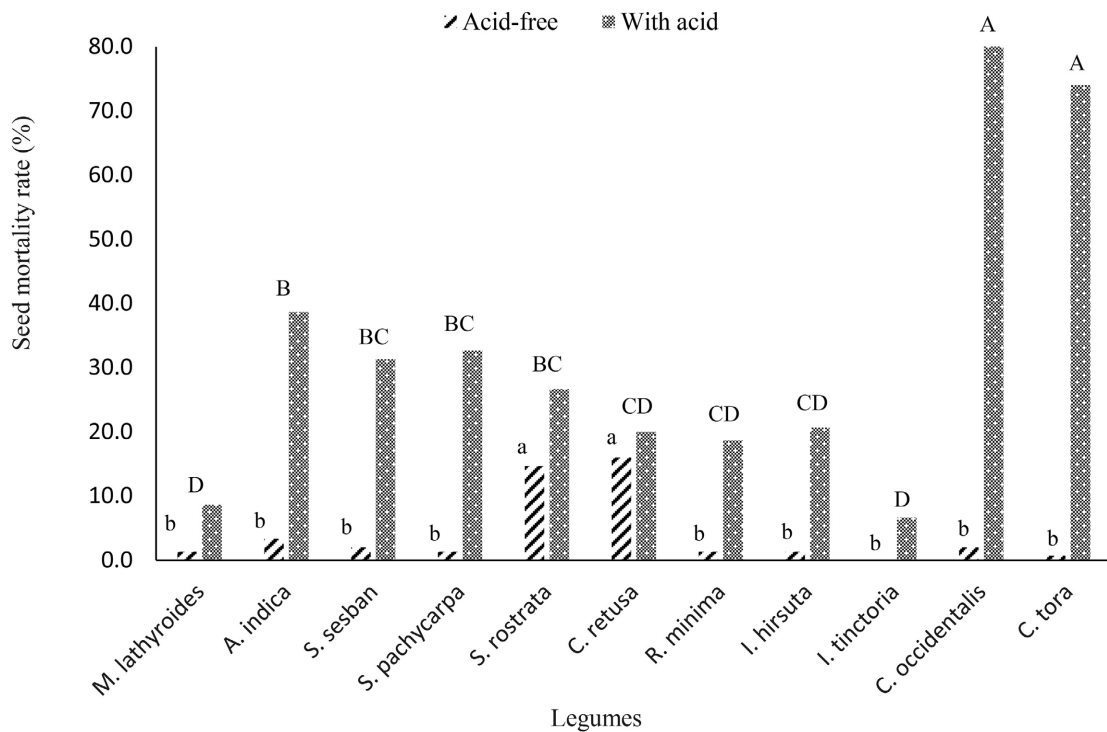


Figure 3. Mortality rates of seeds treated or untreated with concentrated sulphuric acid. Different lowercase letters indicate significant differences ($P < 0.05$) between the mortality rates of legumes not treated with acid. Different capital letters indicate significant differences ($P < 0.05$) between the mortality rates of legumes treated with sulphuric acid.

3.3. Dormant Seed Rates for Seeds Treated or Not Treated with Acid

Factorial analysis did not reveal any statistically significant effect of sulphuric acid pre-treatment on seed dormancy ($P < 0.05$). In other words, pre-treatment does not significantly influence seed dormancy rates compared to untreated controls. However, when each batch of seeds was analysed individually, a statistically significant difference was observed in both the treated and untreated seeds (Table 2). In the batch of seeds treated with sulphuric acid, the highest dormancy rates were observed, in descending order, in *I. tinctoria* (90.0%), followed by *S. sesban* (62.0%), *I. hirsuta* (60.0%) and *S. rostrata* (53.3%). Conversely, the lowest rates were recorded in *C. occidentalis* (10.0%) and *M. lathyroides* (14.6%). For untreated seeds, the highest dormancy rates were observed in *C. obtusifolia* (90.6%) and *S. sesban* (79.3%), with both species showing statistically higher values than the others. They were followed by *A. indica* (66.6%), *R. minima* (64.0%) and *I. hirsuta* (59.3%). In contrast, the lowest rates were recorded in *S. pachycarpa* (0.0%) and *I. tinctoria* (4.6%).

Table 2. Dormancy rates of seeds treated or untreated with H_2SO_4 .

Legumes	Control	H_2SO_4 scarification
	DR	DR
<i>M. lathyroides</i>	28.0 ± 5.8cd	14.7 ± 8.9D
<i>A. indica</i>	66.0 ± 4.3b	30.0 ± 4.3BCD

Continued

<i>S. sesban</i>	79.3 ± 0.9a	62.0 ± 1.6AB
<i>S. pachycarpa</i>	0.0 ± 0.0e	36.0 ± 2.8BCD
<i>S. rostrata</i>	18.0 ± 3.3d	53.3 ± 6.6BC
<i>C. retusa</i>	17.3 ± 2.5d	21.3 ± 3.8CD
<i>R. minima</i>	64.0 ± 4.9b	33.3 ± 6.8BCD
<i>I. hirsuta</i>	59.3 ± 3.4b	60.0 ± 24.2AB
<i>I. tinctoria</i>	4.6 ± 0.9e	90.0 ± 2.8A
<i>C. occidentalis</i>	36.0 ± 3.3c	10.0 ± 5.7D
<i>C. obtusifolia</i>	90.6 ± 1.9a	22.0 ± 8.6CD
Average	42.1	39.3
P-value	<0.00	<0.00
CV	37.9	16.5

Different lowercase letters indicate significant differences ($P < 0.05$) among the dormancy rates of seeds untreated with H_2SO_4 . Different capital letters indicate significant differences ($P < 0.05$) among the dormancy rates of seeds treated with H_2SO_4 .

3.4. Average Daily Germination (ADG) of Seeds Treated or Untreated with Acid

The analysis of variance reveals a significant difference ($P < 0.01$) in the effect of pre-treatment on the average daily germination rate (ADGR) of seeds from different legumes.

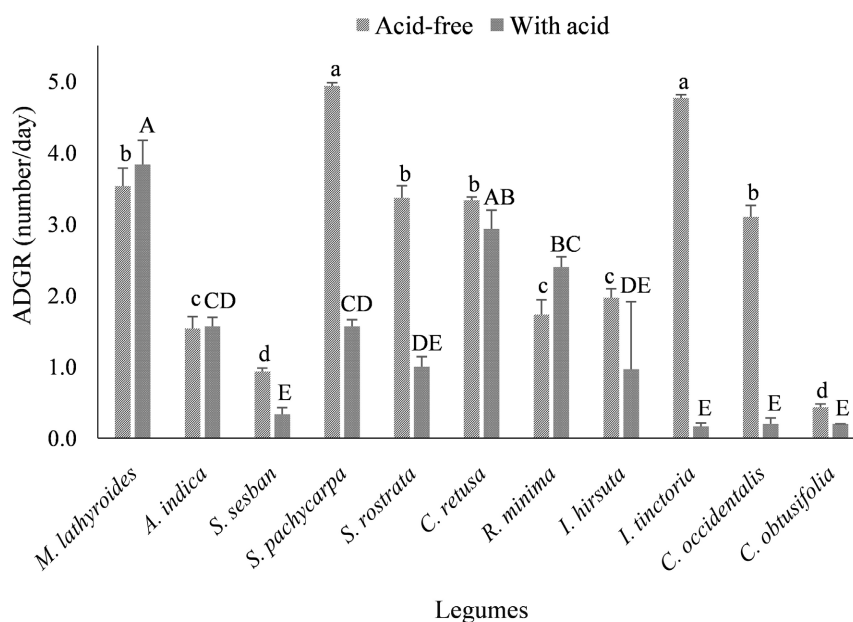


Figure 4. ADGR of seeds treated or untreated with concentrated sulphuric acid. Different lowercase letters indicate significant differences ($P < 0.05$) between the germination rates of legumes not treated with acid. Different capital letters indicate significant differences ($P < 0.05$) between the germination rates of legumes treated with sulphuric acid.

Among the pretreated seeds, *M. lathyroides* had the highest ADGR (3.8 seeds/day), significantly higher than the other species (Figure 4). It was followed by *C. retusa* and *R. minima*, with ADGR of 2.9 and 2.4 seeds/day, respectively. In contrast, the lowest ADGR were observed in *S. sesban*, *I. tinctoria*, *C. occidentalis* and *C. obtusifolia*, with values below 0.8 seeds/day.

In the untreated seed batch, *S. pachycarpa* (4.9 seeds/day) and *I. tinctoria* (4.8 seeds/day) showed significantly higher ADGR than the other species. They were followed by *S. rostrata*, *C. retusa*, *M. lathyroides* and *C. occidentalis*, which occupied an intermediate position. In contrast, the lowest ADGR were recorded for *S. sesban* and *C. obtusifolia*.

3.5. Germination Kinetics

Figure 5 and Figure 6 illustrate the germination kinetics of seeds treated and untreated with concentrated sulphuric acid, respectively. These graphs clearly show that treated seeds not only germinate faster, but also have a shorter germination time.

Comparing the acid-treated seeds with each other (Figure 5), we observe that the majority of species reached their peak germination between the 2nd and 3rd day after sowing (DAS). This is particularly the case for *C. retusa* (53.3%), *M. lathyroides* (50.0%), *R. minima* (36.6%), *S. pachycarpa* (22.0%), *S. rostrata* (17.3%), *I. hirsuta* (15.3%) and *S. sesban* (5.3%). Only *A. indica* reached its peak germination on the 1st DAS. Some species, such as *M. lathyroides*, *C. retusa* and *A. indica*, still showed some germination beyond the 24th DAS. The species *M. lathyroides* was particularly notable for having two germination peaks, notably on the 2nd and 4th DAS, with respective rates of 40% and 16%.

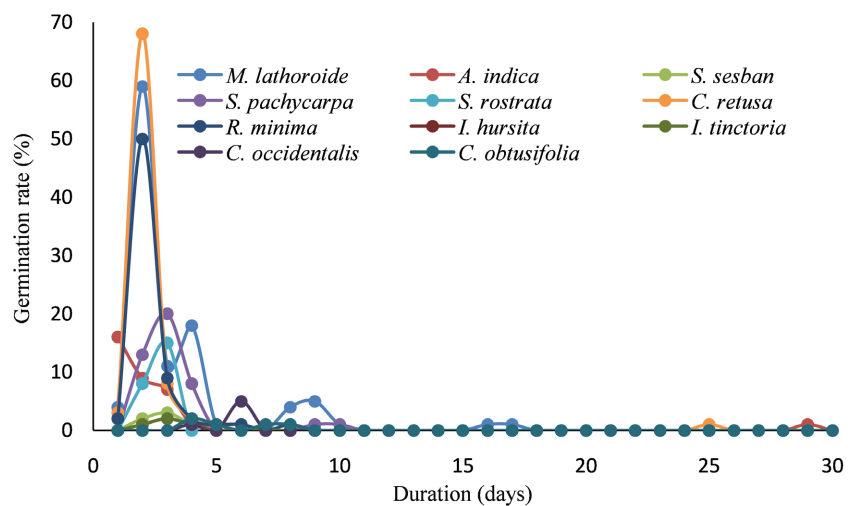


Figure 5. Evolution of germination of legumes treated with H_2SO_4 over time.

With regard to untreated seeds (Figure 6), with the exception of *C. occidentalis* and *C. retusa*, which germinated mainly during the first three DAS with respective rates of 58.0% and 32.6%, all other species showed prolonged germination beyond

25 DAS. However, two overall germination trends were observed. A first group, comprising *C. occidentalis*, *C. retusa*, *A. indica*, *I. hirsuta* and *C. obtusifolia*, reached its peak germination during the first three DAS. In contrast, other species, such as *M. lathyroides*, *S. pachycarpa* and *I. tinctoria*, reached their peak germination on the 8th DAS. Among the latter, *M. lathyroides* and *S. pachycarpa* stood out due to the presence of two germination peaks, between the 2nd and 3rd DAS and then on the 8th DAS, respectively.

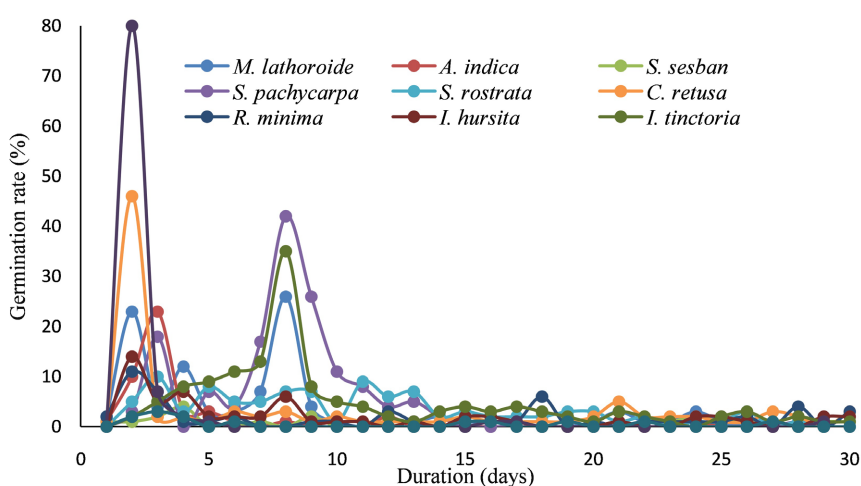


Figure 6. Evolution of germination of legumes not treated with H_2SO_4 as a function of duration.

4. Discussion

The objective of this study was to evaluate the effect of chemical scarification with concentrated sulphuric acid (98%) for 5 minutes on the germination of seeds from 11 wild native herbaceous legume species from the Senegal River delta. The parameters analysed included the final germination rate (FGR), the dormancy rate (DR), the mortality rate (MR) and the average daily germination rate (ADGR). Analysis of variance revealed highly significant differences ($P < 0.001$) for all parameters except DR, reflecting the effects of sulphuric acid pre-treatment, species and their interaction.

While the overall effect of the acid treatment on dormancy rate was not significant, the interaction between species and pre-treatment was highly significant ($P < 0.001$), indicating that the response to chemical scarification is strongly species-dependent. Some species tolerated the 5-minute acid soak and responded positively, whereas others were more sensitive or unaffected. These variations are due to differences in seed coat structure and chemistry, such as thickness, palisade cell layering, which affect permeability and susceptibility to acid [24] [25]. These findings emphasise that scarification protocols should be tailored to the specific characteristics of each species rather than being applied uniformly.

The obtained results highlight a highly significant negative impact of soaking the seeds in concentrated sulphuric acid (98%) on FGR, MR and ADGR. However,

no significant effect of the acid treatment was observed on DR. The treated seeds showed dormancy rates comparable to those of the untreated seeds.

The study of the effect of scarification with concentrated sulphuric acid on germination revealed that the final germination rate (FGR) of untreated seeds is significantly higher than that of treated seeds ($P < 0.001$). This indicates that chemical scarification by soaking in concentrated sulphuric acid does not improve the overall germination rate. Indeed, the average germination rate of seeds treated with H_2SO_4 is 27.6%, compared to 53.9% for untreated seeds.

With the exception of *R. minima*, *M. lathyroides* and *A. indica*, sulphuric acid had an adverse effect on the germination of all other species. This result shows that only these three species among the 11 studied tolerate a 5-minute soak in 98% concentrated sulphuric acid. This variation in response between species to chemical scarification could be attributed to structural differences in seed coats, resulting in a harder shell in some species than others [19] [22] [24]. This variation in response to chemical scarification is likely due to structural and chemical differences in the seed coat, such as thickness, palisade cell arrangement, the presence of a waxy cuticle, and deposits of suberin and phenolic compounds. These factors influence permeability and acid sensitivity. Therefore, a thicker, more sclerified seed coat is generally more resistant and may require a longer treatment period, or may be damaged more easily, whereas a thinner seed coat can be scarified more easily [24] [29].

Comparing only the batch of species pre-treated with acid, the highest germination rates were recorded for the species *M. lathyroides* (76.6%) and *C. retusa* (58.6%), which remain statistically better than the other species. The lowest germination rates, however, were observed in the species *S. sesban* (6.7%), *C. occidentalis* (4.0%), *C. obtusifolia* (4.0%) and *I. tinctoria* (3.3%). This result indicates that these last four species do not tolerate scarification with sulphuric acid for 5 minutes very well. For these species, it would be advisable to explore alternative and less aggressive dormancy-breaking methods, particularly those targeting seed coat impermeability, such as mechanical scarification, hot water treatment, or prolonged soaking in water.

In the batch of seeds not treated with acid, *S. pachycarpa* and *I. tinctoria* showed significantly higher germination rates than the other species, reaching 98.6% and 95.3% respectively. They are followed, in descending order, by *M. lathyroides* (70.6%), *S. rostrata* (67.3%), *C. retusa* (66.6%) and *C. occidentalis* (62.0%). In contrast, the lowest germination rates were observed in *S. sesban* (18.7%) and *C. obtusifolia* (8.7%).

These results indicate that *S. pachycarpa* and *I. tinctoria* do not require any special dormancy-breaking techniques, as simple soaking in water is sufficient to ensure germination rates of over 95%. For the other species, however, it would be necessary to explore other dormancy-breaking techniques in order to improve their germination rates.

Our data also reveal a highly significant difference between the mortality rate

of legume seeds treated with concentrated sulphuric acid and that of control seeds ($P < 0.001$). Scarification of seeds with concentrated sulphuric acid resulted in a significant increase in the mortality rate of all legume seeds. The average MR of acid-pre-treated seeds is 28.2% compared to 4.0% for the untreated seed batch.

The highest mortality rates for species treated with acid were recorded in *C. occidentalis* and *C. obtusifolia*, with 86.0% and 74.0% of seeds dying, respectively. These were followed by species such as *A. indica* (38.6%), *S. pachycarpa* (32.6%), *S. sesban* (31.3%) and *S. rostrata* (26.6%). The lowest mortality rates were recorded in the species *M. lathyroides* (8.7%) and *I. tinctoria* (6.7%). These results on seed mortality correlate with those obtained on germination rates. In fact, soaking the seeds for 5 minutes caused the destruction of the seed coat in most species, resulting in direct contact between the acid and the seed embryo [28] [30] [31]. This resulted in high mortality among acid-treated seeds, with the exception of species such as *R. minima*, *M. lathyroides*, and *A. indica*, which appear to have a harder seed coat.

The study also showed that pre-treatment with sulphuric acid had no significant influence ($P > 0.05$) on the dormancy rate of seeds treated with concentrated sulphuric acid compared to untreated seeds. However, when comparing only the batch of seeds treated with acid, the highest dormancy rate was observed in the species *I. tinctoria* (90%), which remains significantly different from the other species ($P < 0.05$). This was followed by the species *S. sesban* (62.0%), *I. hirsuta* (60.0%) and *S. rostrata* (53.3%). The lowest dormancy rates were observed in the species *C. occidentalis* (10.0%) and *M. lathyroides* (14.6%).

For seeds not treated with acid, the highest dormancy rates were recorded in *C. obtusifolia* (90.6%) and *S. sesban* (79.3%), followed by *A. indica* (66.6%), *R. minima* (64.0%) and *I. hirsuta* (59.3%). The lowest dormancy rates, on the other hand, were observed in the species *S. pachycarpa* (0.0%) and *I. tinctoria* (4.6%). As the species *S. pachycarpa* has no dormant seeds, it does not require any dormancy-breaking techniques to improve germination. This result is similar to that obtained by [32], who highlighted a very high variability ($P < 0.01$) in dormancy among several legume species belonging to the genus *Sesbania*.

Analysis of variance performed on our data shows that the ADGR of seeds not treated with acid is significantly higher than that of pre-treated seeds ($P < 0.001$). The ADGR of seeds not treated with acid is 2.7 ± 1.4 compared to 1.4 ± 1.2 for seeds treated with concentrated sulphuric acid. This result could be explained either by a relatively long exposure time of the seeds to acid or by an acid concentration that is not suitable for most of the legume seeds studied. Indeed, [33] showed that acid concentration and exposure time are very critical and must be quantified for each species, as seeds exposed for a long period of time are easily damaged.

Among the species in the batch of acid-treated seeds, *M. lathyroides* had a significantly higher ADGR (3.8 seeds/day) than all other legume species. It was followed by *C. retusa* and *R. minima*, which recorded ADGR of 2.9 and 2.4 seeds/day,

respectively. The lowest ADGR were recorded in the species *S. sesban*, *I. tinctoria*, *C. occidentalis* and *C. obtusifolia*, with ADGR of less than 0.8 seeds/day. This result indicates that the different species studied respond differently to acid scarification. This result corroborates the thesis that two species belonging to the same subfamily may differ in their responses to several dormancy-breaking treatments [34].

With regard to the untreated seed batch, the ADGR of the species *S. pachycarpa* (4.9 seeds/day) and *I. tinctoria* (4.8 seeds/day) appear to be significantly better than those of the other species. The species *M. lathyroides* (3.5 seeds/day), *S. rostrata* (3.4 seeds/day), *C. retusa* (3.3 seeds/day) and *C. occidentalis* (3.1 seeds/day) came in second place. The lowest ADGR were recorded for the species *S. sesban* and *C. obtusifolia*, with 0.9 and 0.4 seeds/day, respectively.

The study of the evolution of the percentage of germinated seeds over time revealed that H₂SO₄ pre-treatment accelerates germination, concentrating seedling emergence over a shorter period. However, this advantage is offset by significantly higher mortality, resulting in a lower final germination rate (FGR). Therefore, although surviving seeds germinate faster, overall germination success is reduced, indicating that concentrated sulphuric acid selectively affects seed populations. [24] [35]. Indeed, a comparison of acid-treated seeds reveals that almost all species reached their peak germination during the first 3 DAS. For seeds not treated with acid, on the other hand, almost all species saw their germination prolonged beyond 25 DAS with two clearly identified germination peaks between the 2nd and 3rd DAS and on the 8th DAS, respectively. This result is consistent with that obtained by [36], who showed that prolonged exposure of legume seeds to sulphuric acid significantly improves the germination rate of certain legume species.

5. Conclusions

This study demonstrated that chemical scarification by soaking seeds in concentrated sulphuric acid (98%) for 5 minutes had a significantly adverse effect ($P < 0.01$) on several germination parameters of 11 species of native wild herbaceous legumes, including *Aeschynomene indica*, *Cassia occidentalis*, *Crotalaria retusa*, *Cassia obtusifolia*, *Indigofera tinctoria*, *Indigofera hirsuta*, *Macroptilium lathyroides*, *Rhynchosia minima*, *Sesbania pachycarpa*, *Sesbania rostrata*, and *Sesbania sesban*.

Acid treatment significantly decreased the final germination rate (FGR), increased seed mortality rate (MR), and reduced average daily germination rate (ADGR) compared to untreated seeds. The average FGR of acid-treated seeds was 27.6%, compared to 53.9% for untreated seeds, while the ADGR dropped from 2.7 ± 1.4 seeds/day to 1.4 ± 1.2 seeds/day after treatment.

With the exception of *R. minima*, *M. lathyroides*, and *A. indica*, acid scarification negatively affected germination in the remaining eight species, with particularly high mortality in *C. occidentalis* (86%) and *C. obtusifolia* (74%). This highlights that chemical scarification is strongly species-dependent. For these eight

sensitive species, alternative dormancy-breaking methods, such as soaking in water, mechanical scarification, or hot water treatment, are recommended to reduce mortality and improve germination.

From a practical perspective, these findings have direct implications for using wild herbaceous legumes as green manure or cover crops. High mortality and reduced germination after acid treatment make most species unsuitable for field establishment protocols relying on chemical scarification. Only *R. minima*, *M. lathyroides*, and *A. indica* can be reliably sown following a 5-minute sulphuric acid treatment. For the other species, gentler dormancy-breaking techniques must be employed to ensure adequate seedling establishment and the subsequent benefits of soil enrichment, nitrogen fixation, and biomass production. These results underscore the need to tailor scarification protocols to individual species to optimize their use in sustainable agroecosystems.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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