

Article

Methods for Overcoming Dormancy in *Eryngium foetidum* Propagules

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Abstract

Eryngium foetidum is classified as a Non-Conventional Food Plant (NCFP) with socio-economic importance in the Amazon region, whose propagation is limited by low and irregular germination. This study aimed to evaluate methods for overcoming dormancy and promoting the germination of propagules of this species. Treatments consisted of chemical scarification with sulfuric acid (1, 2, and 3 min), mechanical scarification with sandpaper, soaking in water at room temperature, soaking in hot water, and an untreated control. Germination and seed vigor parameters were evaluated. Based on the analyses performed, chemical scarification treatments provided the best results, promoting higher germination and germination speed index, as well as a lower percentage of hard propagules and reduced mean germination time compared with the other treatments. Furthermore, the reduction in the number of hard propagules and their negative association with germination and vigor suggest the possible occurrence of physical dormancy in this species. Therefore, it can be concluded that chemical scarification with sulfuric acid is the most effective method for overcoming dormancy in *Eryngium foetidum*, contributing to the development of more efficient propagation protocols.

Keywords: physical dormancy; chemical scarification; mechanical scarification

1. Introduction

Eryngium foetidum L. (Apiaceae) is a perennial vegetable species belonging to the group of Non-Conventional Food Plants (NCFPs). In Brazil, it is commonly known as Amazon chicory, coentrão, and wild coriander [1], while in other countries it is referred to as culantro, cilantro, and alcapate [2]. This species is widely used as a culinary seasoning due to its distinctive aroma and flavor [3]. In addition, it has demonstrated pharmacological potential owing to the presence of compounds with antibacterial, antioxidant, antifungal, and anti-inflammatory properties [4].

Despite the socioeconomic importance of this species, its reproductive behavior and the production patterns of its propagules remain poorly understood [5]. Current knowledge regarding *E. foetidum* seeds indicates the presence of dormancy, which results in low germination rates, non-uniform seedling emergence, and delays in seedling production [6–8]. The causes of dormancy in this species are not yet fully understood, and only a limited number of studies have been conducted to investigate the mechanisms involved [7,8].



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Studies suggest that the presence of coumarin in the seeds, fruits, and leaves of this species may be associated with germination inhibition [6]. Coumarin is a well-known phenolic compound that acts as a germination inhibitor, and its effects on seeds can be mitigated through the application of gibberellic acid [9]. However, even with the use of gibberellic acid (GA₃), Ref. [7] did not obtain promising results in overcoming dormancy in *E. foetidum*, as the maximum GA₃ concentration tested resulted in a germination percentage of only approximately 3.35%.

Native to tropical regions of Central and South America [4], *E. foetidum*, like many other species from these regions, may exhibit adaptive mechanisms in response to high rainfall and humidity. Given that restrictions to water uptake caused by impermeable compounds such as waxes, suberin, cutin, and lignin, as well as by specific seed coat structures, represent a common adaptive strategy among tropical species [9,10], the hypothesis of physical dormancy in *E. foetidum* seeds deserves consideration.

In view of this, chemical and mechanical scarification methods, widely used to promote seed coat permeability and favor water imbibition [9], may contribute to overcoming dormancy in the propagules of this species, considering the possibility of physical dormancy. Therefore, this study aimed to evaluate methods for overcoming dormancy and promoting germination of *Eryngium foetidum* L. propagules.

2. Materials and Methods

Eryngium foetidum produces schizocarpic fruits (dry and indehiscent), composed of subunits known as mericarps or carpidia [11]. Therefore, the term propagules will be used throughout this study, since the material evaluated does not correspond to the seed itself [12].

Capitula from the first to the fifth order were harvested manually at different times as they reached maturity, characterized by a dark brown to black coloration [13] and the onset of seed shedding, during April 2023 in Jaboticabal, São Paulo State, Brazil.

The capitula were spread on paper and allowed to dry on a laboratory bench at the Seed Analysis Laboratory, FCAV/UNESP, at 24 ± 2 °C for one week. Subsequently, the carpidia (propagules) were extracted by gently rubbing the capitula between the fingers.

Manual cleaning was performed to remove empty propagules and plant debris, obtaining pure propagules for the application of the following treatments: (A) control—untreated propagules; (B) H₂O 24 ± 2 —propagules immersed in water at room temperature (22 °C) for 16 h; and (C) hot water—propagules immersed in water for 16 h, with an initial temperature of 86 °C and a final temperature of 22 °C. The propagules were immersed in hot water and maintained in open containers on a bench in a climate-controlled laboratory, allowing gradual cooling of the water; (D) mechanical scarification—propagules rubbed against No. 180 sandpaper for 10 s, and (E) chemical scarification with H₂SO₄ for 1, 2, and 3 min—propagules immersed in 95% sulfuric acid (36 N) for the specified periods.

At the end of each chemical scarification treatment, the propagules were transferred to plastic sieves and washed under running water with gentle manual rubbing to remove acid residues from their surface. Personal protective equipment, including plastic gloves and a face mask, was used during handling of the acid. Treatments B, C, and E were completed by drying the propagules on paper sheets placed on a laboratory bench at 24 ± 2 °C.

After treatment application, the propagules were evaluated using the following tests and measurements:

Germination Test: Four subsamples of 50 propagules were placed on two sheets of Germitest® (J.Prolab, São José dos Pinhais, PR, Brazil) paper towel moistened with distilled water at a ratio of 2.5 times the dry paper weight. The propagules were then placed in transparent acrylic boxes with lids (11.0 × 11.0 × 3.5 cm). After setup, the boxes

were enclosed in plastic bags and incubated in a B.O.D. (Biochemical Oxygen Demand) germination chamber (EL141, Eletrolab, São Paulo, SP, Brazil) [14]. The test was conducted at 25 °C under an 8-h photoperiod [14] for 28 days, as seedling emergence had stabilized by this period. At the end of the test, the percentages of normal seedlings, abnormal seedlings, dead propagules, and hard propagules were determined.

First-Count Germination was performed together with the germination test, calculating the percentage of normal seedlings on the 14th day after sowing, when the count reached 50% of the final germination, based on results reported by Ref. [15].

Germination Speed Index (GSI) and Mean Germination Time (MGT) were determined in conjunction with the germination test through daily counts of germinated propagules until the end of the evaluation period, according to the methodologies described by Ref. [16] and Ref. [17], respectively. Results were expressed as a dimensionless index for GSI and in days for MGT.

Relative Germination Frequency (RGF) was determined in conjunction with the germination test from the daily counts of germinated propagules and calculated according to the method proposed by Ref. [18].

The experiment was conducted using a completely randomized design (CRD) with four replications. Prior to analysis, data were evaluated for ANOVA assumptions; residual normality was assessed using the Shapiro–Wilk test, a Q-Q plot, and a density function, while homogeneity of variances was verified using Bartlett’s test.

Due to the non-normality and heteroscedasticity of the residuals for the studied variables, a Spearman correlation network was constructed using the packages tidyverse version 2.0.0 [19], ggraph version 2.2.2 [20], and PerformanceAnalytics version 2.1.0 [21]. The Spearman correlation coefficients were tested using Student’s *t*-test at significance levels of 1% and 5%.

In addition, a clustered heatmap analysis was performed using the ComplexHeatmap package version 2.26.1 [22]. The two variables that did not meet the assumptions of normality and homoscedasticity were removed (abnormal seedlings and dead propagules). Subsequently, a dissimilarity matrix was obtained using standardized mean Euclidean distance, which was then subjected to cluster analysis using the UPGMA method (Unweighted Pair Group Method Using Arithmetic Averages), implemented in the MultivariateAnalysis package version 0.4.4 [23]. The optimal number of groups was determined using the function “fviz_nbclust” from the factoextra package version 1.0.7 [24], where the criteria used for cluster formation and for defining the cut-off point are based on multiple indices that collectively indicate, through a voting approach, the most appropriate number of groups for the analyzed data.

Differences among treatments were evaluated using the Kruskal–Wallis test implemented in the stats package version 4.5.2 [25]. When significant differences were detected, Dunn’s test was performed for multiple pairwise comparisons among treatments using the rstatix package version 0.7.3 [26], with no adjustment of *p*-values. All analyses were conducted using RStudio (version 2026.05.0 + 218).

3. Results

The germination pattern of Amazon chicory (*Eryngium foetidum* L.) propagules varied over time as a function of the dormancy-breaking treatment applied (Figure 1).

Immersion of propagules in hot water resulted in a relatively constant germination pattern throughout the evaluation period. In contrast, chemical scarification with H₂SO₄ for 1 and 3 min produced a single peak in the relative germination frequency distribution, with germination concentrated between the 8th and 18th days after sowing. Chemical

scarification for 2 min resulted in a bimodal distribution, whereas mechanical scarification with sandpaper and the control treatment exhibited three germination peaks.

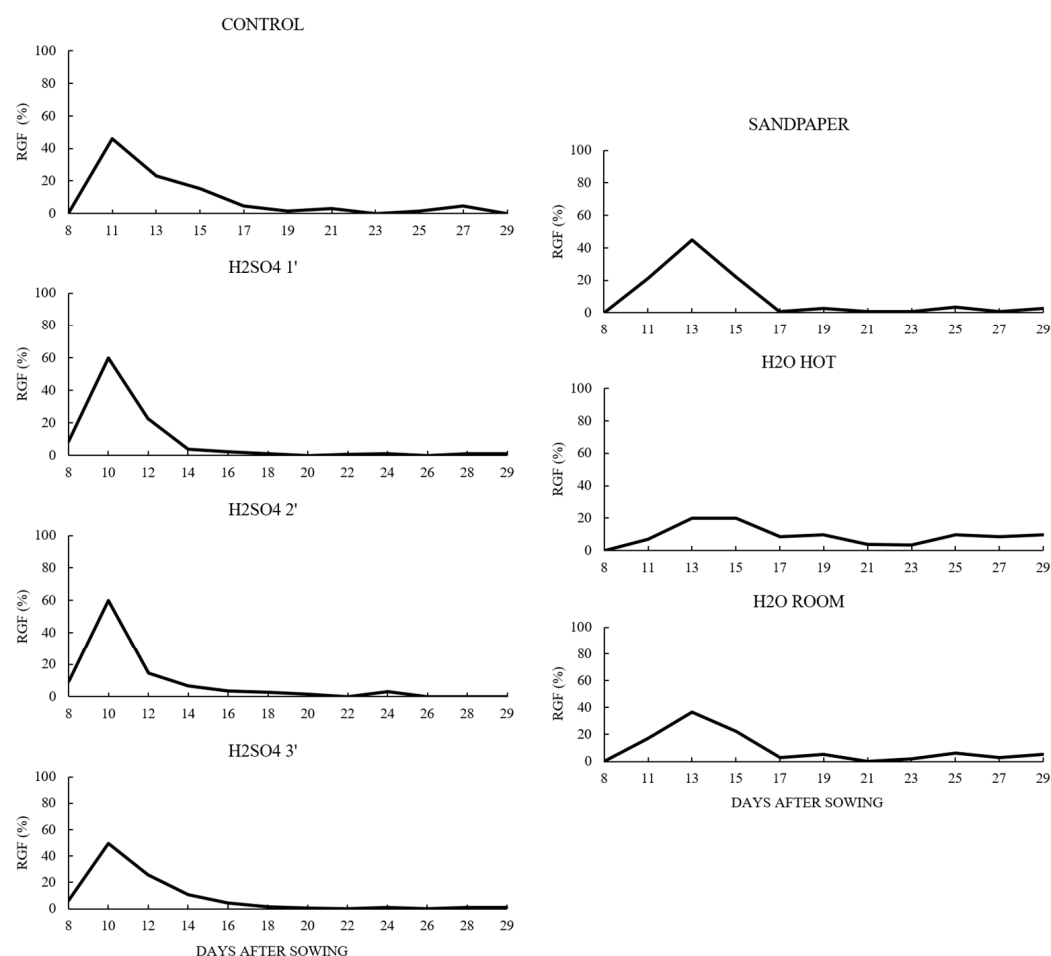


Figure 1. Relative germination frequency (RGF) of *Eryngium foetidum* propagules subjected to different dormancy-breaking treatments.

A clustered heatmap analysis was used to evaluate the effects of the treatments on dormancy overcoming in *Eryngium foetidum* by relating the main variables to the percentage of hard propagules (H), which was considered an auxiliary variable (Figure 2).

Based on the dissimilarity matrix, the hierarchical clustering displayed in the heatmap separated the treatments into two distinct groups: Group I, comprising the H₂SO₄ scarification treatments (1, 2, and 3 min), and Group II, consisting of the control, hot water, room-temperature water, and sandpaper scarification treatments (Figure 2).

The formation of the observed groups is related to the similarity in responses among the treatments within each group. Overall, Group I treatments showed high germination percentage, abnormal seedlings, and first-count germination, as well as a high germination speed index, together with a lower mean germination time (Figure 2).

Although the 1-min sulfuric acid treatment differed from the other treatments in Group I with respect to the percentage of abnormal seedlings, this difference was not sufficient to justify its exclusion from the group (Figure 2), given that significant differences between this treatment and the control were observed in germination percentage, hard propagules, germination speed index, and first-count germination (Table 1).

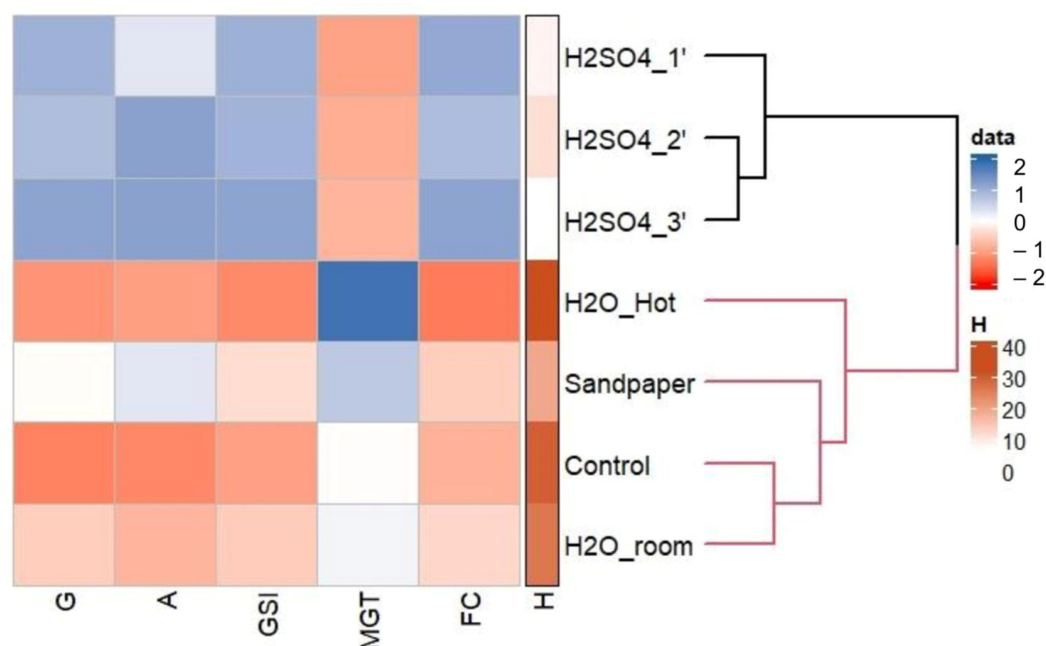


Figure 2. Heatmap generated from hierarchical cluster analysis based on dormancy-breaking treatments applied to Amazon chicory (*Eryngium foetidum*) propagules. Rows represent the evaluated variables, and columns represent the treatments. The orange color scale indicates values below the variable mean, whereas the blue color scale indicates values above the mean for germination (G), abnormal seedlings (A), germination speed index (GSI), mean germination time (MGT), and first-count germination (FC). For the auxiliary variable, hard propagules (H), increasing shades of purple indicate increasing values from zero.

Table 1. Mean values of germination percentage (G), abnormal seedlings (A), dead propagules (D), hard propagules (H), germination speed index (GSI), first-count germination (FC), and mean germination time (MGT) of *Eryngium foetidum* propagules subjected to different dormancy-breaking treatments.

Treatments	G	A	D	H	GSI	FC	MGT
	%				Days		
	Group I						
H ₂ SO ₄ 1'	73 bc	3.5 ab	8.0 a	8 b	3.26 c	34.3 bc	11 c
H ₂ SO ₄ 2'	70 bc	5.5 b	2.5 abc	11 b	3.22 bc	31.3 bc	11 bc
H ₂ SO ₄ 3'	76 c	5.5 b	6.0 ac	7 b	3.40 c	34.8 c	12 bc
Mean	73	2.5	5.5	9	3.29	33.4	11
Standard error	4	0.2	1.6	2	0.78	1.6	0.23
Group II							
Control	33 a	0.0 a	8.5 a	30 a	1.28 a	13.3 a	14 abc
H ₂ O room (24 ± 2 °C)	46 ab	1.0 a	0.5 bc	26 a	1.73 ab	17.5 ab	14 ab
H ₂ O hot	36 a	0.5 a	0.0 b	32 a	1.07 a	7.3 a	19 a
Sandpaper	55 abc	3.5 ab	3.5 abc	19 ab	1.91 abc	16.5 ab	15 a
Mean	42	1.1	3.1	27	1.50	13.6	15
Standard error	3	0.2	1.4	2	0.11	1.3	0.57
Overall mean	55	2.8	4.1	19	2.27	22.1	14

Note. Means followed by the same lowercase letter within a column do not differ significantly according to Dunn's test ($p \leq 0.05$).

Among the treatments classified in Group I, the sulfuric acid treatment for 3 min showed the highest germination percentage both at the end of the test (76%) and in the first count (35%), with a statistically significant difference compared with the control treatment (33% and 13%, respectively).

In contrast, Group II predominantly comprised treatments showing lower-than-average overall performance (Table 1) for germination percentage, abnormal seedlings, germination speed index, and first germination count, together with higher mean germination time values (Figure 2).

Although the mechanical scarification treatment (sandpaper) did not differ statistically from the control treatment for all variables, it also did not differ from the chemical scarification treatment (3 min) for germination percentage, abnormal seedlings, dead and hard propagules, and germination speed index (Table 1). However, this was not sufficient to justify its inclusion in Group I, and the treatment therefore remained classified in Group II (Figure 2).

The auxiliary variable hard propagules also contributed to the separation between groups, with Group I treatments exhibiting the lowest mean values for this characteristic (7–11%) and differing significantly from the control treatment (30%) (Table 1 and Figure 2).

In the correlation network analysis (Figure 3), the variable hard propagules showed a strong negative correlation with germination percentage (−0.95), germination speed index (−0.95), and first-count germination (−0.90), as well as a moderate positive correlation with mean germination time (+0.64), helping to explain the results obtained for the treatments included in Group I. According to Ref. [27], correlation coefficients ranging from 0.10 to 0.30 are considered weak, from 0.40 to 0.60 moderate, and from 0.70 to 1.00 strong.

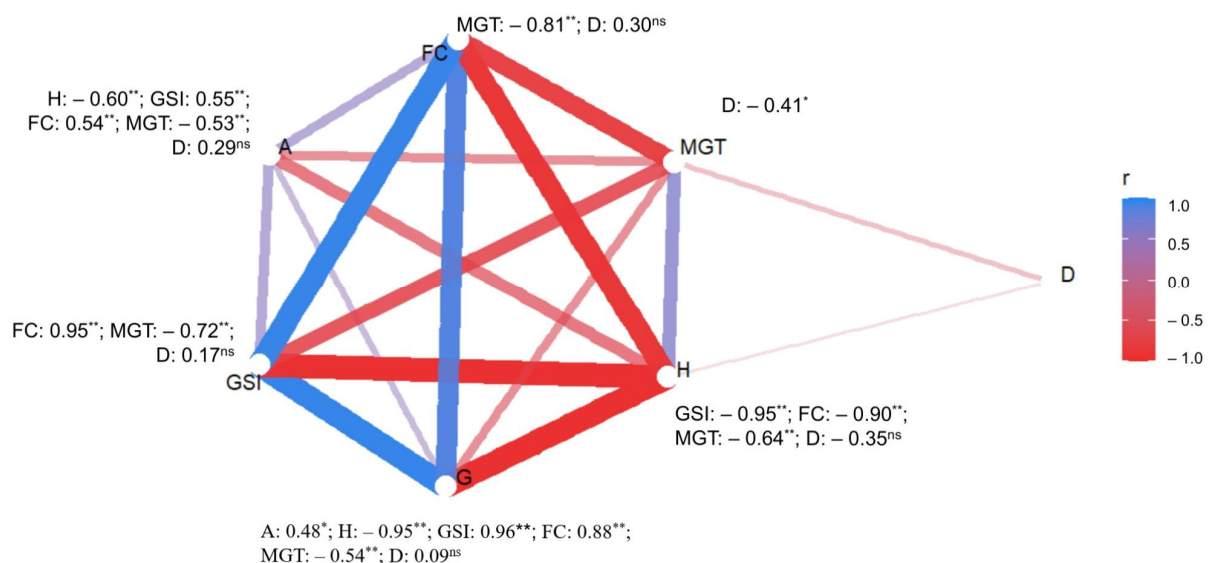


Figure 3. Spearman correlation network (r) among germination speed index (GSI), first-count germination (FC), germination percentage (GERM), hard propagules (H), dead propagules (D), abnormal seedlings (D), and mean germination time (MGT). *, **, and ^{ns} indicate significance at the 5% and 1% probability levels and non-significance, respectively, according to the t-test.

Regarding abnormal seedlings, the correlation network analysis revealed only moderate correlations with the other germination and vigor variables evaluated. In contrast, dead propagules showed limited relevance for explaining the results obtained in the present study, as they exhibited only a weak negative correlation with mean germination time (−0.41), while no significant correlations were observed with the remaining variables (Figure 3).

4. Discussion

Overall, chemical scarification treatments showed a positive performance across the evaluated germination and vigor variables, allowing clear differentiation from the other treatments (Figure 2), as they increased germination at the first count (FC) and final germination percentage (G), as well as the germination speed index, while reducing the number of hard propagules and promoting greater germination uniformity over time (Figures 1 and 2; Table 1). These results are consistent with the hypothesis of physical dormancy in the propagules of this species, since the reduction in the proportion of hard propagules after scarification treatments indicates changes in seed coat permeability, a feature commonly associated with this type of dormancy [28].

This interpretation is further supported by the correlation network analysis (Figure 3), in which hard propagules showed strong negative correlations with germination percentage, first-count germination, and germination speed index, indicating that reductions in the proportion of hard propagules were associated with improvements in germination performance.

Similar results were reported by Ref. [29] in *Sapindus saponaria* L., when seeds of this species were subjected to sulfuric acid immersion. The authors observed a reduction in dormancy associated with restricted water uptake into the seed, resulting from seed coat impermeability.

Specifically, chemical scarification for 1 and 3 min produced a single germination peak in the relative frequency distribution (Figure 1), indicating a more synchronized germination pattern. The concentration of germination within the first two weeks after sowing is particularly relevant for seedling production systems, as it reduces the overall germination period, shortens handling time, and consequently lowers production costs, while also minimizing losses of propagules under field or nursery conditions [12]. Therefore, germination synchrony emerges as an important criterion for evaluating dormancy-breaking treatments in future studies.

It is important to emphasize that the effectiveness of sulfuric acid scarification depends directly on the exposure time. For example, in seeds of *Cucumis anguria* L., immersion for 2 min was sufficient to reduce seed physiological quality due to damage to essential structures [30]. A similar pattern was observed in the present study for immersion times of 2 and 3 min in H₂SO₄, which showed an increase in abnormal seedlings (Figure 2).

However, despite the increase in abnormal seedlings in the chemically scarified treatments, it is important to consider their agronomic viability. Future studies should investigate whether these abnormalities compromise field development or represent only morphological deformities without functional impact. This interpretation is supported by the correlation network analysis (Figure 3), in which abnormal seedlings (A) showed moderate correlations with the evaluated traits (G = 0.48; GSI = 0.55; MGT = −0.53; FC = 0.54). The proper interpretation of these results is crucial for decision-making regarding the use of this technique at a commercial scale.

Regarding dead propagules, no significant differences were observed compared with the control treatment. Furthermore, according to the correlation network analysis (Figure 3), this variable did not contribute substantially to the interpretation of the results obtained in the present study. Therefore, although the chemical scarification treatments (2 and 3 min) increased the percentage of abnormal seedlings, there was no evidence that these treatments compromised embryo viability.

These results differ from those reported by Ref. [31] for seeds of royal poinciana (*Delonix regia* (Bojer ex Hook.) Raf.), who observed that sulfuric acid scarification increased the percentage of dead seeds, consequently reducing seed quality and vigor. However, the exposure time to acid scarification was not reported in that study, which may have

affected seed viability, since the duration of exposure to sulfuric acid can compromise embryo viability.

In contrast, chemical scarification for 30 min in *Albizia pedicellaris* seeds did not result in the highest percentages of dead seeds or abnormal seedlings compared with other treatments, such as mechanical scarification followed by soaking [32]. These findings highlight the importance of investigating both dormancy-breaking methods and treatment duration, as their effects may directly influence seed germination and vigor.

Although chemical scarification proved effective in the present study, Ref. [33] reported that this method has several limitations, including high operational costs, the requirement for proper handling during application and disposal, and potential environmental impacts. Consequently, the choice of a dormancy-breaking method should be based not only on its efficacy but also on considerations of operational safety, economic viability, and environmental sustainability [34].

As a safer alternative, mechanical scarification, although less effective than chemical scarification, showed intermediate results (Figures 1 and 2; Table 1) and may be feasible under field conditions or in small-scale nurseries. The sandpaper scarification treatment did not differ significantly from the control or from the 3-min sulfuric acid treatment for most evaluated variables. These results indicate that mechanical scarification exhibited performance comparable to chemical scarification, highlighting its potential as an alternative method for overcoming dormancy in *Eryngium foetidum* propagules. However, further studies are required to evaluate the effect of different mechanical scarification durations on germination, since only a 10-s exposure period was assessed in the present study.

The effectiveness of mechanical scarification with sandpaper has been reported for seeds of *Enterolobium cyclocarpum* (Fabaceae) and *Juniperus procera* (Cupressaceae) [35,36]. It has also been reported for Amazonian species such as *Ipomoea cavalcantei* [37]. However, available studies do not specify the optimal duration of mechanical scarification for these species.

Scarification may promote rapid seed imbibition due to the water potential gradient between the seed interior and the surrounding environment [32]. Consequently, both the duration of exposure to agents that alter the seed coat structure and the length of friction applied during mechanical scarification are critical to avoid damage to the embryo.

Overall, hot water treatment of *Eryngium foetidum* propagules was the least effective method for overcoming dormancy, resulting in lower final and first-count germination, as well as increased mean germination time (MGT). Ref [38] reported similar results for *Chloroleucon acacioides* and *Senna macranthera*, both members of the Fabaceae family, whose seeds exhibited reduced germination after thermal treatment.

In addition, this treatment resulted in greater germination non-uniformity over time (Figure 1). The temperature of 86 °C may have caused physiological impairment to the embryo, delaying germination and contributing to the broader distribution of germination events. However, despite this effect, hot water treatment did not increase the percentage of abnormal seedlings (Table 1). These results suggest that the absence of abnormal seedlings alone is not sufficient to indicate treatment effectiveness, as germination percentage, speed, and synchrony should also be considered when evaluating dormancy-breaking methods.

In addition, the 16 h seed immersion period in water may have limited oxygen supply, consequently affecting seed physiological quality. Ref. [8] observed reduced germination and germination speed index, as well as increased mean germination time in *Eryngium foetidum* seeds immersed in water for 24, 36, and 48 h.

Collectively, the results indicate that dormancy in *Eryngium foetidum* propagules may be associated with barriers related to the propagule covering structures, although the exact dormancy mechanism remains to be elucidated. According to Ref. [39], in physically

dormant seeds, certain physical barriers prevent water imbibition, which justifies the need for scarification.

However, further studies on dormancy breaking in Amazon chicory propagules are required, since a moderate negative correlation was observed between abnormal seedlings and hard propagules (Figure 3). Future studies should evaluate seedling development in response to the treatments applied in this work and/or assess growth regulator treatments using GA₃ [7], aiming to reduce the percentage of abnormal seedlings, particularly if this factor reduces plant vigor.

5. Conclusions

Sulfuric acid scarification was the most effective method for overcoming dormancy in *Eryngium foetidum* propagules, increasing germination and vigor while reducing mean germination time. However, further studies are required to assess its practical applicability. Mechanical scarification showed intermediate performance and may represent a viable alternative under field conditions, although optimization of friction time is necessary to improve its effectiveness.

The results are consistent with the hypothesis of physical dormancy in the species, as evidenced by the response to scarification treatments and the reduction in the number of hard propagules, contributing to the understanding of the mechanisms limiting propagule germination.

Thus, this study provides support for the development of more efficient propagation protocols for *Eryngium foetidum*.

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