Seed Germination of Native Species of Atlantic Forest (Bahia, Brazil)

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The objective of this study was to evaluate the seed germination of native species of the Atlantic Forest biome. We evaluated sucupira *Bowdichia virgilioides* Kunth, jatobá *Hymenaea courbaril L.*, monkfish *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae), guava *Psidium guajava L.* (Myrtaceae) and jequitibá-rosa *Cariniana legalis* Kuntze (Lecythidaceae), through germination rate (GR), germination speed index (GSI), and mortality rate (MR), after pre-germination treatment stoovercome dormancy and subsequent production of seedlings to be used in Degraded Area Recovery Plans. Sets of 100 seeds were divided into four groups of 25 for the treatments, one physical by scarification (wall sandpaper number 120) and two chemicals by immersion in water at room temperature for 48 hours and acetic acid for 15 minutes. These treatments were compared with the control of untreated seeds, subsequently grown in washed sand at room temperature in the shade. The indexes GR, GSI, and MR were calculated and analyzed. In general, scarification was more efficient for *H. courbaril* (GR=48%) and *E. contortisiliquum* (GSI=0.75) but increased the MR of this in other species. Immersion in water favored new seeds of sucupira *B. virgilioides* (GR=52%, GSI=0.69), and immersion in acetic acid showed a varied and less effective performance overall. Scarification with sandpaper and immersion in water at room temperature for 48 hours were the most effective methods to increase the seedling production of the studied species.
Keywords: Overcoming Dormancy. Scarification. Water Immersion. Acetic Acid.

In native forests, viable seeds that do not germinate are typical, although the environmental conditions are favorable. These dormant seeds require exposure to some environmental factor that overcomes their dormancy to make them germinate. Numbness is a natural mechanism of resistance to environmental factors and can manifest itself in three ways: dormancy imposed by the integument, embryonic dormancy, and dormancy by the imbalance between germination-promoting and inhibiting substances [1].

Dormancy has important ecological significance as an adaptive trait that ensures the perpetuation and survival of species. Germination retardation keeps the species in the seed stage, the phase of the plant's cycle when its resistance to unfavorable conditions is most excellent. Late or prolonged dormancy can be induced in mature seeds of native tropical species to reduce the risk of adaptation and extend their permanence in the environment in response to unfavorable environmental conditions in the dry and rainy weather seasons [2].

Seeds of many native forest species have slow, irregular, or no germination, even under favorable environmental conditions or after proper harvest and storage [3]. This situation results from the impermeability of the integument associated with several botanical species, being more frequent in Fabaceae [4]. Numbness is a problem for cultivation due to irregular germination, affecting the seedling uniformity and seedling production time [5,6]. To overcome dormancy, it is necessary to use physical and/or chemical methods that favor germination.

The method includes scarification and the effect of temperature, while the chemical method can use water (distilled or running), hydrogen peroxide, acetic acid, hydrochloric acid, sulfuric acid, sodium hydroxide (caustic soda), acetone, and alcohol [7].

Restoring native ecosystems is crucial for environmental conservation and recovering degraded areas. There are still gaps in the scientific

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literature on germination in seedling production, so the objective of this work was to evaluate the germination of seeds of native species of the Mata biomeAtlantica through germination rate (GR), germination speed indices (GSI) and mortality rate (MR), after pre-germination treatments to overcome dormancy and subsequent production of seedlings to be used in Degraded Area Recovery Plans at Reunidas Gravatá farm and surrounding areas in Teodoro Sampaio, Bahia (Brazil).

The objective of this study was to evaluate the seed germination of five native species of the Atlantic forest biome through germination rate (GR), germination speed index (GSI), and mortality rate (MR), after pre-germination treatment stoovercome dormancy and subsequent production of seedlings to be used in Degraded Area Recovery Plans.

Materials and Methods

This germination study was carried out at the Reunidas Gravatá farm, Teodoro Sampaio municipality, Bahia (Brazil), from September 2023 to August 2024 (Figure 1).

The species chosen were sucupira *Bowdichia virgilioides* Kunth (Fabaceae), jatobá *Hymenaea courbaril L*. (Fabaceae), monkfish *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae), guava *Psidium guajava L*. (Myrtaceae) and pink jequitibá *Cariniana legalis* Kuntze (Lecythidaceae) (Figure 2).

The seeds were collected from matrices in municipalities in the center and north of the state of Bahia. In their processing, the peels and pulp were removed with scissors, hammers, and sieves. Then, they were stored in transparent polyethylene packages, sealed, and kept at room temperature for months in the seed house for further sowing.

Seeds of sucupira *B. virgilioides* were collected manually from the trees of UNEB Campus II in Alagoinhas in January/2023 for the first test in September/2023. For the second test, the seeds were collected manually from a tree from the Reunidas Gravatá farm in January/2024, with the help of pruning shears. The light, orange, and red seeds were used in the processing stage; the immature, broken, and greenish-brown seeds were discarded [8]. The seeds of jatobá *H. courbaril* were collected manually from a tree on the farm Morrinhos in Lajedinho in September/2023. For processing, a hammer was used to break the fruit, and a knife was used to break the fruit and extract the pulp. In May/2024, at the Reunidas Gravatá farm. Monkfish seeds *E. contortisiliquum* and guava *P. guajava* were collected manually from a single tree on the property with scissors pruning. In processing the seed, a sieve was used to remove the pulp. The seeds of the pink jequitibá *C. legalis* were collected manually with the help of a pruner of a single individual from the farm Santo Antônio in Teodoro Sampaio in October/2022, being transported in the pyxis itself and stored for about 15 months until its use in January/2024. The wings were removed at the moment of sowing during processing.

The seeds, chosen for their good visible appearance of size, standard coloration of each species, and integrity of the integument, were submitted to pre-germination treatments to overcome dormancy and, subsequently, cultivated in washed sand with a depth of 1 cm and a distance of 5 cm. Seeds were cultivated in the sowing protected by 100-micron diffuser film to reduce competition between seedlings. In monitoring, the seeds were watered twice daily (morning and afternoon) with a sprinkler system for 15 minutes.

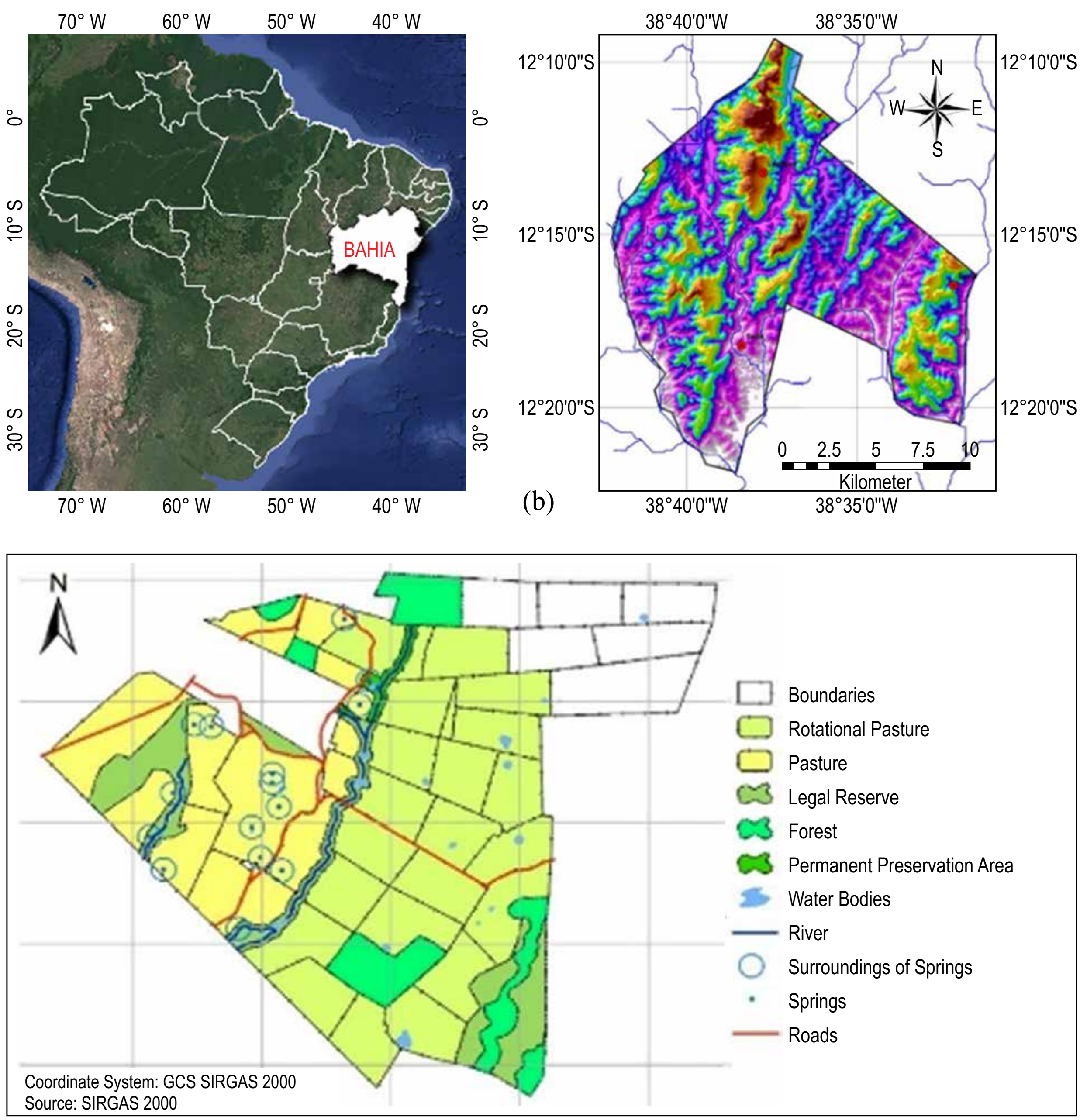
Data on the germination time and general morphological aspects of the seedlings in three days/weeks for ninety days. Invasive seedlings were removed to avoid competition. The following were considered normal seedlings with complete essential structures and abnormal seedlings that did not show visible potential to continue their development even under favorable conditions [9,10].

Six experiments were carried out with 100 seeds, two of which were *B. virgilioides* and four other species, *H. courbaril*, *E. contortisiliquum*, P. *guajava*, and *C. legalis*. The sets were divided into four groups of 25 to 3 treatments: one physical through scarification, with wall sandpaper (number 120) in the region opposite the embryonic axis; and two chemical by immersion, in room temperature water for 48 hours and acetic acid for 15 minutes—the control of untreated seeds. Scarification was considered only the mechanical action of scraping the seed with sandpaper to cause superficial fissures with more or less pronounced scars in the seed coat. All the values of germinated or ungerminated seeds until the end of each experiment were used to calculate the germination rate (GR), germination speed index (GSI), and mortality rate (MR).

The equation obtained the germination rate (GR):

$$\%GR = (\sum ni \cdot N\text{-}1) \cdot 100$$

Figure 1. The state of Bahia (Brazil) (a), Teodoro Sampaio (b) and the study area at the Reunidas Gravatá farm (c).



(c) Source: José Gabriel Ferreira dos Santos, 2023; Marcos de Oliveira Dias, 2017; ML Tavares Engenharia e Construções Ltda., 2010.

Figure 2. Fruits and seeds collected from 2023 to 2024.

1. sucupira *Bowdichia virgilioides* Kunth (Fabaceae); **2.** jatobá *Hymenaea courbaril L.* (Fabaceae); **3.** monkfish *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae); **4.** guava *Psidium guajava L.* (Myrtaceae); **5.** jequitibá-rosa *Cariniana legalis* Kuntze (Lecythidaceae).

where ∑ni is the total number of seeds germinated relative to the number of seeds arranged to germinate (N).

The germination speed index (GSI) was calculated pela equação [11]:

$$GSI = G\,1/N1 + G2/N2 + ...\ Gn/Nn$$

where G1, G2, ... Gn is the number of germinated seeds; E1, E2, ... En is the number of normal seedlings in each observation; N1, N2, ... Nn is the number of days after sowing.

The mortality rate was calculated as a percentage, following the equation:

$$\%MR = (N-1)/N$$

N-1 is the number of individuals killed, and N is the number of individuals planted for each species and treatment.

All GR, GSI, and MR values of the 5 species were submitted to statistical analysis ($p<0.5$) by Statistica 7.0.

Results and Discussion

Comparing pre-germination treatments and native species, scarification allowed to achieve better GR results (48%) for jatobá *H. courbaril* and GSI (0.75) for monkfish *E. contortisiliquum* but increased the MR of this and the other Species. Immersion in water favored the GR (52%) of the new seeds of sucupira B. virgilioides in experiment 2. Immersion in acetic acid (15 min) performed poorly overall when compared to the other treatments (Table 1).

Considering each treatment and species separately, between the seeds of sucupira *B. virgilioides* used in both experiments, immersion in water for 48 hours favored GR (52%) and the GSI (0.69) probably by hydration controlled by the hardness of the resistant seed coat of new seeds in experiment 2, high impediment MR due to embryo drowning (Table 1).

In the jatoba seed *H. courbaril*, immersion in acetic acid (15 min) yielded a GR and GSI response of about 50% lower than scarification but 150% higher than hydration (48 h) and MR

Table 1. Germination rates of seeds submitted to treatments (1 physical, 2 chemicals, 1 control) to overcome dormancy. September/2023 to August/2024. Reunidas Gravatá farm, Teodoro Sampaio, Bahia (Brazil).

Species	Scarification (wall sandpaper number 120			Immersion in water at room temperature (48h)			Immersion in acetic acid (15 min)			Control (not treated)		
	GR %	GI %	MR %	GR %	GSI	MR %	GR	GSI	MR %	GR %	GSI	MR %
B. virgilioides (E1)	36	0.60	64	12	0.11	88	8	0.07	92	12	0.08	88
B. virgilioides (E2)	32	0.24	68	52	0.69	48	28	0.18	72	16	0.13	84
H. courbaril	48	0.45	52	8	0.08	92	24	0.21	76	16	0.07	84
E. contortisiliquum	20	0.75	80	12	0.33	88	12	0.30	88	8	0.05	92
P. guajava	12	0.04	88	44	0.67	56	32	0.22	68	40	0.31	60
C. legalis	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0
Total	**148**	**2.08**	**352**	**128**	**1.88**	**372**	**104**	**0.98**	**396**	**92**	**0.64**	**408**
Average	**24.67**	**0.35**	**58.7**	**21.33**	**0.31**	**62**	**17.33**	**0.16**	**66**	**15.33**	**0.11**	**68**

(76%) lower than control and immersion in prolonged water, showing the need for further testing of treatments with the hydration period for this species. Water immersion (48 h) was contraindicated for overcoming integumentary dormancy of this species, and should the need to reduce immersion time be assessed, or associating immersion and temperature (Table 1).

Monkfish *E. contortisiliquum* seeds immersed in water (48 h) and acetic acid (15 min) also had low GR (12%) and high MR (88%), both similar to the control group. This showed inefficacy, probably due to not viable embryos due to drowning and intoxication, respectively. The beginning of germination of this species during immersion in water (48 h) was noted, but not the development of germinated seeds.

P. guajava seeds germinated after 48 hours of water immersion, achieving a germination rate (GR) of 44% and a germination speed index (GSI) 0.67. These results suggest the need to shorten the soaking period for a more efficient and simplified dormancy-breaking method. All seeds of *C. legalis* did not germinate until the end of the year. Experiment by the death of the embryo, although they are still stored in the capsular pixie with visible integrity (Table 1).

In the initial contact with water, the monkfish *E. contortisiliquum* seeds emit a structure that protrudes like a radicle, showing that perhaps the long period of immersion was detrimental (Figure 3). Other authors usually use 4, 8, and 12 hours, and the long period of 16 hours was considered a factor in increasing the mortality rate by [12].

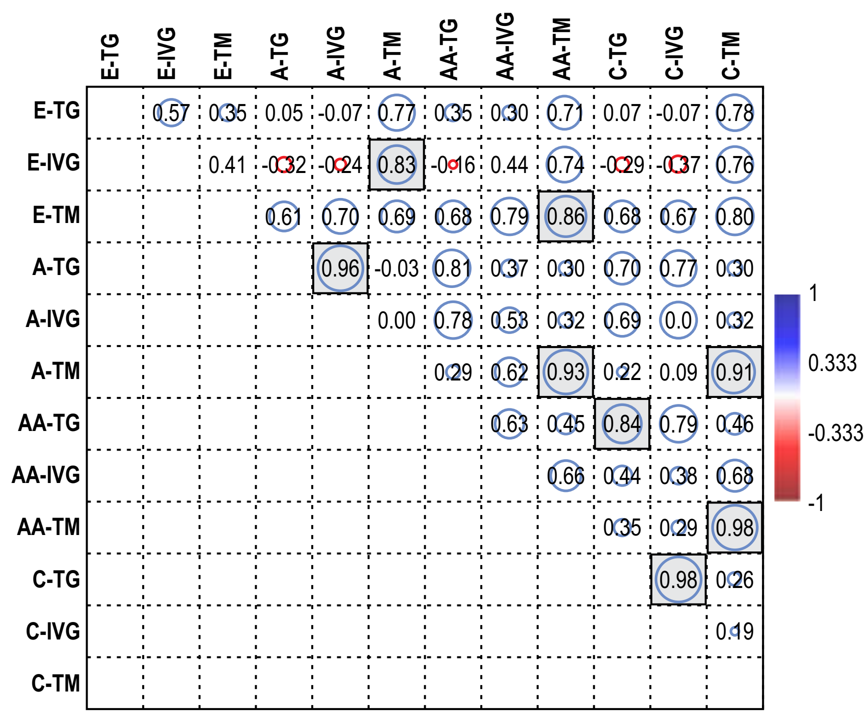
The test of variance showed a difference ($F=9.80$; $df=71$; $p=7.361$ E-10) between the treatments used to overcome seed dormancy, with correlations between GR and GSI of the treatment with immersion in water (0.96; $p<0.05$), the MR between the treatments with water and acetic acid (0.93; $p<0.05$) (Figure 4).

The jatobá *H. courbaril* had higher GR than the group between the sucupira *B. virgilioides* of experiment 1 and the monkfish *E. contortisiliquum*; all three species of the family Fabaceae had results close to GR and MR after scarification, despite the prominence of the monkfish *E. contortisiliquum* with greater GSI. The new seeds of sucupira *B.*

Figure 3. Seeds of monkfish *Enterolobium contortisiliquum* (Vell.) Morong in Reunidas Gravatá farm, Teodoro Sampaio, Bahia (Brazil), 2024.

1. Washed seed; 2. Sprouting structure in washing; 3. Germination in water immersion.

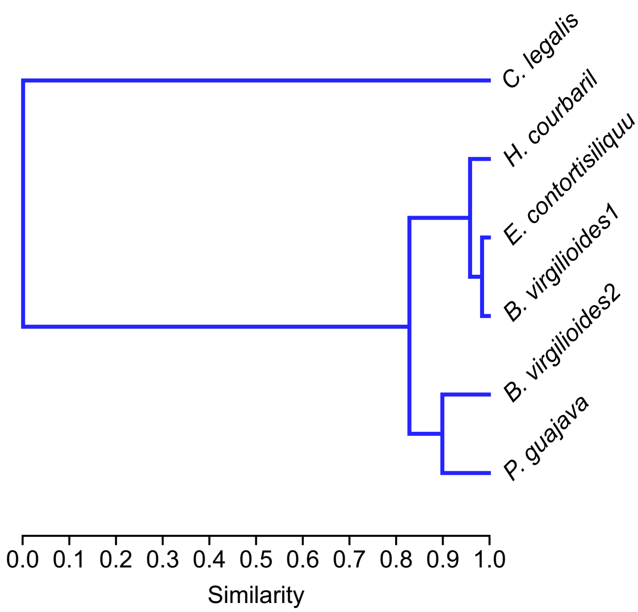
Figure 4. Correlation analysis (p<0.05) of germination and mortality rates of treated seeds. September/2023-August/2024. Reunidas Gravatá farm, Teodoro Sampaio, Bahia (Brazil).



virgilioides (Fabaceae) of experiment 2 and guava *P. guajava* (Myrtaceae) were close due to the average values of germination after immersion in water for 48 hours. The species *C. legalis* (Lecythidaceae) stood out from the other groups due to the total loss of 100 seeds treatment and control to overcome dormancy and germination (Figure 5).

When comparing the GR, GSI, and MR values between stored seeds of B. virgiliodes from experiment 1 and *C. legalis*, it is believed that native seeds lose their capacity when kept at room temperature in a dry environment for a prolonged period. On the other hand, in the native fragment, the humidity maintained in the leaf litter allows

Figure 5. Analysis of clusters by similarity between native species of the Atlantic Forest tested with pre-germination treatments to overcome seed dormancy, 2023-2024.



the seeds more time to overcome dormancy with regular germination throughout the phenological cycle in the natural environment.

The low values recorded for GR and GSI in the group controller, with untreated seeds, indicated the need for previous treatment to overcome numbness and increase inefficiency in the germination of the seeds of the selected native species, aiming at better results to reduce the MR.

This variation in results for the same species exposed to the same treatment was attributed to the storage time (8 months) of sucupira seed used in experiment 1 after the collection of the matrix tree, which may have acquired more excellent resistance due to integumentary hardening with maturation, being the more effective chiseling treatment for seeds stored as long as there is no contamination by fungi and consequent death of the embryo. On the other hand, the embryo of the new seed of sucupira used in experiment 2 was vulnerable to agents by chiseling after sowing, as the water had no effect in experiment 1, but reached the embryo of the new sucupira seed in experiment 2, causing it to germinate.

In both experiments with *B. virgilioides*, seeds were found that were still intact (hard) until the end and considered unviable (dead). This indicates that, despite the time of exposure to treatments to overcome dormancy, there were still those with integumentary resistance due to possible genetic variability.

Seeds that remain without absorbing water for a prolonged period and have an appearance that has not yet swollen after hydration are relatively common among species of the Fabaceae and Malvaceae families. This greater resistance, resulting from the impermeability of the seed coat to water, characterizes the seed's dormancy property [9,13].

Jatoba *H. courbaril* seeds present dormancy caused by the impermeability of the integument. Scarification proved highly effective, with a GR higher than that observed in the other treatments and control group, corroborating the studies [14-16]. Piña-Rodrigues and colleagues (2014) attributed the high MR of this species to the long period of immersion for 16 hours in cold water, considering that the standard used is 4, 8, and 12 hours [12]. It is recommended that seeds of this species be studied in detail regarding the period of immersion in water at room temperature to detect the optimal range of hydration without the embryo not viable by possible drowning, as may have been the case with immersion for 48 hours. Germination capacity indicates the total number of germinated seeds but does not consider the time it takes for seeds to reach the germination percentage [17]. The higher the germination speed, the more vigorous the seed [18].

Cruz-Silva and Rosa (2011) observed that scarification treatment with sandpaper was the most efficient for overcoming the dormancy and germination of monkfish *E. contortisiliquum* seeds [19]. Scarification also increased GSI and MR in seeds studied by Piña-Rodrigues and colleagues [12]. It can be inferred that rapid germination is characteristic of species pioneers with a rapid or opportune colonization strategy, taking advantage of favorable environmental conditions to develop seedlings [17]. On the other hand, high mortality rates in the germination phase indicate a limited potential for natural recomposition by a late colonization species, which explains its threat of extinction [8].

Alves and colleagues (2015) obtained better germination of guava seeds *P. guajava* after immersion in cold water [20]. The softened appearance of the pink jequitibá *C. legalis* seeds at the end of the experiment may indicate the action of the decomposing microbiota (fungi and bacteria) that resulted in the inviability of the seeds of this species. Thin-skinned or little-skinned seeds may be more susceptible to colonization by fungi that decompose the embryo and prevent germination. Death of seeds can also be caused by the action of enzymes or toxins released by microorganisms in the germination phase orseedling growth [21]. Species from humid tropical regions have developed mechanisms to prevent water absorption, such as integument impermeability, to prevent germination soon after germination dispersal for seed survival in the environment [12].

Conclusion

The results demonstrate the efficacy of different pre-germination treatments in overcoming seed dormancy of native species of the Atlantic forest.

Scarifying with sandpaper and immersion in water at room temperature for 48 hours were the most effective methods, especially for the seeds of jatoba *H. courbaril*, monkfish *E. contortisiliquum*, the new sucupira *B. virgilioides* seeds from experiment 2, and of the guava *P. guajava*. These treatments provided higher germination rates and germination speed indexes, confirming their relevance for the production of seedlings of these species.

In general, the scarification was more efficient for jatobá *H. courbaril* (GR=48%) and monkfish *E. contortisiliquum* (GSI=0.75) but increased the MR of this species and the others. The water immersion favored the new seeds of sucupira *B. virgilioides* (GR=52%, GSI=0.69) to the detriment of the others, suggesting the need for experiments with a well-soaking period that refutes the hypothesis of embryo non-viability by drowning. Also, immersion in acetic acid (15 min) showed varied ineffectiveness, perhaps due to its toxic effect on the embryo, highlighting the need for future research to optimize its application or seek more effective alternatives.

The high mortality observed in some species, especially the pink jequitibá *C. legalis*, with MR=100%, suggests that factors such as storage time and fungi contamination can negatively influence seed viability.

Therefore, the application of appropriate pre-germination treatments is essential to maximize the germination efficiency of native seeds, contributing to programs for the recovery of degraded areas, especially in the Atlantic forest.

Further studies should explore combinations of treatments and the influence of environmental conditions on germination to further improve the production of seedlings for ecological restoration.

The experiment offered significant results on the germination characteristics of the native species studied from the Atlantic forest for some dormancy-overcoming treatments, giving suggestions for further studies that include new treatments with immersion time and use of temperature for better and more effective results in producing seedlings for use in a plan to restore degraded areas.

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