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Pre-germination techniques for "faveira" in lots of different provenances as subsidy to standardization and commercialization

Técnicas de pré-germinação de faveira em lotes de diferentes procedências como subsídio para a padronização e comercialização

Tecnicas de pregerminación de faveira em lotes de diferentes procedencias como subsidio para la estandarización y comercialización

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Abstract
The native seed market in Brazil is recent, requires organization, and its governance is important, to approve resources' quality and origin from taken resources. Widely ranged, *Enterolobium schomburgkii* was chosen, to sign use of simple techniques to evaluate seeds production. For the assessment of initial growth (germination and emergency), packages of three different provenances seeds (Alta Floresta, Sinop and Xingu-Araguaia) were evaluated, in two environmental conditions (controlled/Mangelsdorf/30 °C and seedbed/open area/room temperature) and four dormancy breaks (CTEA18, CTEA4, ELX, EA18), four samples with 25 seeds, in factorial design. Variables as germination/plant growth and GSR were evaluated with non-parametrical tests. Provenances were different: germination/plant growth was affected by all studied characters; meanwhile GSR, except environment. Both these characters are applied to evaluate plant vigour, important information to packages availability; or else, bigger will be GSR, higher will be seed growth speed, more environmentally adaptable will be the seed pack. Concerning dormancy, ELX, although laborious, was more consistent than other techniques, factor connected to provenances, due to variability of species distribution. Considering its wide range, physical dormancy break by sandpaper scarification was efficient. Thus, new studies are needed, in order to ensure robustness to the currently known recommendations.
Keywords: Dormancy; Native seeds; Governance; Quality control; Variability.

Resumo
O setor de sementes nativas no Brasil é recente, necessita de organização, e a sua regulamentação é importante, ao garantir qualidade e origem dos recursos utilizados. De ampla distribuição, *Enterolobium schomburgkii* foi escolhida, a fim de evidenciar o uso de técnicas simples na avaliação da produção de sementes. Para os estudos de crescimento inicial (germinação e emergência), foram utilizados lotes de três procedências (Alta Floresta, Sinop e Xingu-Araguaia), duas condições (controlado/Mangelsdorf/30 °C e canteiro/aberto/temperatura ambiente) e quatro quebras de dormência (CTEA18, CTEA4, ELX, EA18), com quatro repetições de 25 sementes, em delineamento fatorial. Variáveis como

germinação/emergência e IVG foram estudadas, usando testes não paramétricos. Houve variação entre as procedências: a germinação/emergência é afetada por todos os caracteres avaliados; enquanto o IVG, exceto pelo ambiente. Estas duas variáveis normalmente são utilizadas para expressar o vigor do material, informação importante para a avaliação dos lotes; ou seja, quanto maior o IVG, maior a velocidade de germinação, mais adaptável ambientalmente será o lote. Com relação à dormência, ELX foi consistente com relação às outras metodologias, fator também conectado às próprias procedências, por questões de variabilidade da distribuição da espécie. Considerando a ampla distribuição, a superação física de dormência por meio da escarificação com lixa se mostrou eficiente. Porém, novos estudos são necessários, a fim de garantir robustez às recomendações atualmente conhecidas.
Palavras-chave: Controle de qualidade; Dormência; Regulamentação; Sementes nativas; Variabilidade.

Resumen
El mercado de semillas nativas en Brasil es reciente, requiere organización y su gobernanza es importante para aprobar la calidad de los recursos y el origen de los recursos tomados. Se eligió *Enterolobium schomburgkii*, de amplia distribución, para señalar el uso de técnicas simples para evaluar la producción de semillas. Para la evaluación del crecimiento inicial (germinación y emergencia) se evaluaron paquetes de semillas de tres procedencias diferentes (Alta Floresta, Sinop y Xingu-Araguaia), en dos condiciones ambientales (controlada/Mangelsdorf/30 °C y semillero/área abierta/cuarto temperatura) y cuatro rupturas de latencia (CTEA18, CTEA4, ELX, EA18), cuatro muestras con 25 semillas, en diseño factorial. Variables como germinación/crecimiento de plantas y GSR fueron evaluadas con pruebas no paramétricas. Las procedencias fueron diferentes: la germinación/crecimiento de la planta se vio afectada por todos los caracteres estudiados; mientras tanto GSR, excepto medio ambiente. Ambos caracteres se aplican para evaluar el vigor de la planta, información importante para la disponibilidad de paquetes; o bien, mayor será el GSR, mayor será la velocidad de crecimiento de la semilla, más adaptable ambientalmente será el paquete de semillas. En cuanto a la latencia, ELX, aunque laborioso, fue más consistente que otras técnicas, factor relacionado con las procedencias, debido a la variabilidad en la distribución de las especies. Teniendo en cuenta su amplio rango, la ruptura de la latencia física por escarificación con papel de lija fue eficiente. Por lo tanto, se necesitan nuevos estudios para garantizar la solidez de las recomendaciones actualmente conocidas.
Palabras clave: Control de calidad; Dormencia; Regulación; Semillas nativas; Variabilidad.

1. Introduction

Several technical, scientific and legal advances have occurred in Brazil for organization of seed and native seed industry, especially in last 20 years (Ribeiro-Oliveira & Ranal, 2014). In the technical and legal field, this should be given to the Normative Instruction n. º 17/2017 (Brasil, 2017), published to regulate production, commercialization and use of seeds and seedlings of forest species, among others, for the purpose to guarantee origin, identity and quality of these resources. In order to evaluate quality of native seeds in Brazil in compliance with the new legislation, several specific normative instructions were published (Brasil, 2010; 2011; 2012) and standardized methods for forest seeds analysis (Brasil, 2013). Together, these publications bring initial information to more than 300 native species, based on data from recently published research. Summed up to manuals that emerged in recent years, with recommendations for conducting quality tests with native seeds and technical recommendations for collecting, processing, storage and propagation (Campos Filho, 2012; Carrero et al., 2014; Campos Filho & Sartorelli, 2015; Oliveira et al., 2016).

Enterolobium schomburgkii (Benth.) Benth. (Fabaceae) is an ecological and forestry important species (Ramos & Ferraz, 2008) and commercial (Muñiz et al., 2012; d'Oliveira et al., 2013), due to its hard wood, with good resistance and moderately durability (Lorenzi, 1998), also significant on many mammals' species eating habits (Izawa, 1979; Defler & Defler, 1996; Fragoso & Huffman, 2000). Pinzón et al. (2012) reported this species contributed to aerial biomass in their studies at Panamá. In Amazonian biome, it's distributed widely ranged, including frontier regions (Mews et al., 2012; Borges et al., 2014). Species with wide range distribution previously studied, as ours, shows variation among populations, resulting from individuals adaptation to the environmental conditions in areas they occur (Noronha et al., 2018). As characters that reflect adaptation to environment, dormancy degree and pre-germinating test responses influence seedlings production equality, and, therefore, their quality and market costs to implementation.

Production of *E. schomburgkii* seeds is generous and frequent, with physical dormancy caused by tegument water impermeability (Souza & Varela, 1989; Linhares et al., 2020). As in other Fabaceae, breaking dormancy pre-germinative methods, as mechanical scarification (Souza & Varela, 1989) and cutting integument (Sautu et al., 2006), or even passing through mammals digestive tract (Fragoso & Huffman, 2000), has been efficient in breaking dormancy. Nevertheless, these techniques were tested in only one individual or provenance, and are difficult to apply in commercial seedlings production.

Considering the presented, the study aims to evaluate if simple techniques are efficient to break dormancy and if different provenances of *E. schomburgkii* in different environments, controlled or not, improves seeds germination efficiency.

2. Methodology

Lots of *E. schomburgkii* seeds, from Mato Grosso state, of two different biomes provenances were obtained at: Sinop, located in a Cerrado-Amazon biomes transition zone, collected in 2011; in a region between Xingu and Araguaia valley rivers, in Cerrado biome areas, collected in 2014; and Alta Floresta region, Amazon biome, collected in 2014. Seeds packages were stocked in cold chamber until test conduction, at 10-15 °C and medium relatively humidity at 50%. After collecting, a hundred seeds weight (HSW) from each stock was determined. The absence of a control treatment in this study is justified by records of germination tests, pointed both in literature (Souza & Varela, 1989; Ramos & Ferraz, 2008), and in an official document, known as Seed Analysis Rules (or Regras para Análise de Sementes, in Portuguese), issued by Brazil's Ministry of Livestock, Agriculture and Supply (Brasil, 2013), which notes the species definitely has physical dormancy (integumentary).

To evaluate the germinative responses between lots, the seeds were tested in two environments with different conditions: (a) controlled environment in germination chamber (“Mangelsdorf”), over filter paper, white and dark light conditions, rotated in 12-hour breaks, at 30 °C constantly, to get germination rate (%G); and (b), uncontrolled environment, in sand seedbeds, kept shadowed at 30%, which seeds were set in at depths equivalent to their diameters, and periodical irrigation with sprinklers and natural rainfall, to get emergency rate (%E).

Four dormancy breaking methods in both environments were tested: five-minute 60 °C heat shock, plus 18-hour water soaking (CTEA18); five-minute 60 °C heat shock, plus 4-hour water soaking (CTEA4); sandpaper scarification (ELX); and 18-hour water soaking (EA18). Four samples, with 25 seed each were applied for each treatment.

Both trials were followed through 56 days: between September and October 2015, to the germination trial (a); and between October and December 2015, in seedbeds, for emergency trial (b). In each monitoring, ordinary seedlings, according to Ramos and Ferraz (2008), were counted and discarded. At trials’ end, growth speed rates (GSR) were calculated, according to Fogaça (2015), as germination and emergency ending rates from each pack, as in Brasil (2013).

Accumulated ordinary seedlings percentage along days after sowing (DAS) was measured by data described analysis, applying mean standard error. For germination/emergency rates (%G/%E) and germination speed rates (GSR), a Kolmogorov-Smirnov (KS) test, was applied to test data normality, for each essay. Non-normal distributed data were transformed in $\log_{10}(x+1)$ and submitted to variance analysis, on F test, at 5% error probability. Comparisons between means from different seed packages and dormancy break methods were tested by Tukey’s. Joint within provenances, environments, and dormancy break treatments were considered as random and tested for germination, emergency and GSR at joint variance analysis, in R software (R Core Development Group, 2008).

3. Results and Discussion

General information as temperature (minimum, average and maximum) and year on provenances can be assessed in Table 1 (INMET, 2019). While running the experiment in seedbeds (b), medium temperature and humidity were, respectively, 27,0 °C (min.: 19.53 °C; max.: 39.15 °C) and 79% (min.: 17.1%; max.: 96.4%), 357,90 mm total and accumulated 606.3 mm rainfall.

Table 1. Physical characteristics of seed stocks tested for each study source in Mato Grosso region.

Provenance	Rain precipitation (mm/year)	Minimum temperature (°C)	Average temperature (°C)	Maximum temperature (°C)	Collection year	Hundred seeds weight (g)	Seed number/kg
Alta Floresta	1867,3	18,7	25,9	36,3	2014	54.8	17,887
Sinop	2155,3	18,0	25,9	38,5	2011	66.5	15,038
Xingu-Araguaia	1956,0	17,4	24,5	36,4	2014	56.1	20,438

Source: Authors (2021).

Studied packages presented variation among environments in their physical characters (Table 1), however, they are placed in range observed in other Amazon regions, with mean 18,749 seeds/kg for two seed packages (Ramos & Ferraz, 2008) and also in Souza and Varela (1989), which collected seeds from only one individual, containing 21,607 seeds/kg. This highlights the importance of sampling and harvesting seeds in more than one mother tree.

The germination environment, the seed provenance and the dormancy break method affect the seeds germination/emergence of *E. schomburgkii* ($p < 0.05$), while GSR was affected only by provenance and dormancy break method ($p < 0.02$) (Table 2). However, it was decided to split triple interaction for both variables and seed stocks obtained at different times and provenances differed in terms of germination/emergence and germination speed (Tables 3 and 4), being lower than control treatment with mechanical scarification (ELX) (Figures 1 and 2). Thus, the stocks, harvested in years and different provenances, showed distinct germinative potential, except when impediment resulted from impermeable tegument with mechanical scarification was removed (Tables 3 and 4).

Table 2. Analysis of variance (ANOVA) for controlled (germinator) and uncontrolled environments (sand seedbeds) and unfolding of degrees of freedom (DF) of treatments between provenances (P) and dormancy break method (SD) for germination / emergence percentage (G / E) and germination speed index (GSR).

FV	DF	G/E		Log_{10} (GSR+1)	
		MQ	p	MQ	P
Environment (E)	1	32.67	0.687	0.003	0.393
Provenances (P)	2	3876.17	0.002	0.223	0.000
Dormancy Break (DB)	3	16464.67	0.000	1.008	0.000
E x P	2	561.17	0.121	0.010	0.137
E x DB	3	242.44	0.351	0.012	0.104
P x DB	6	489.50	0.128	0.026	0.016
E x P x DB	6	182.94	0.034	0.004	0.261
Mean		40.92		0.27	
CV		21.23		19.69	

FV: variation source; CV: coefficient of variation; MQ: medium square; p: p-value (at 5% significance). Source: Authors (2021).

Table 3. Percentage of germination, emergence and rate of germination (GSR) of *Enterolobium schomburgkii* seed stocks from different sources in Mato Grosso state, submitted to treatments for breaking dormancy under controlled conditions of seeding of temperature, light and humidity.

Provenances	Controlled environment (germination chamber)				Means
	Heat shock + 18-hour in water	**Heat shock + 4-hour in water**	**Mechanical scarification**	**18-hour in water**	
	Germination/Emergency (%)				
Alta Floresta	16.00 Bb[1]	32.00 Bb	79.00 Aa	16.00 ABb	35.75
Sinop	25.00 Bb	21.00 Bbc	82.00 Aa	7.00 Bc	33.75
Xingu-Araguaia	41.00 Abc	51.00 Ab	84.00 Aa	30.00 Ac	51.50
Means	27.33	34.67	81.67	17.67	40.33
	GSR[3]				
Alta Floresta	0.28 Bb	0.53 Bb	2.80 Aa	0.29 ABb	0.98
Sinop	0.27 Bb	0.21 Bb	2.91 Aa	0.07Bb	0.87
Xingu-Araguaia	0.77 Ab	1.04 Ab	2.98 Aa	0.69 Ab	1.37
Means	0.44	0.59	2.90	0.35	1.07

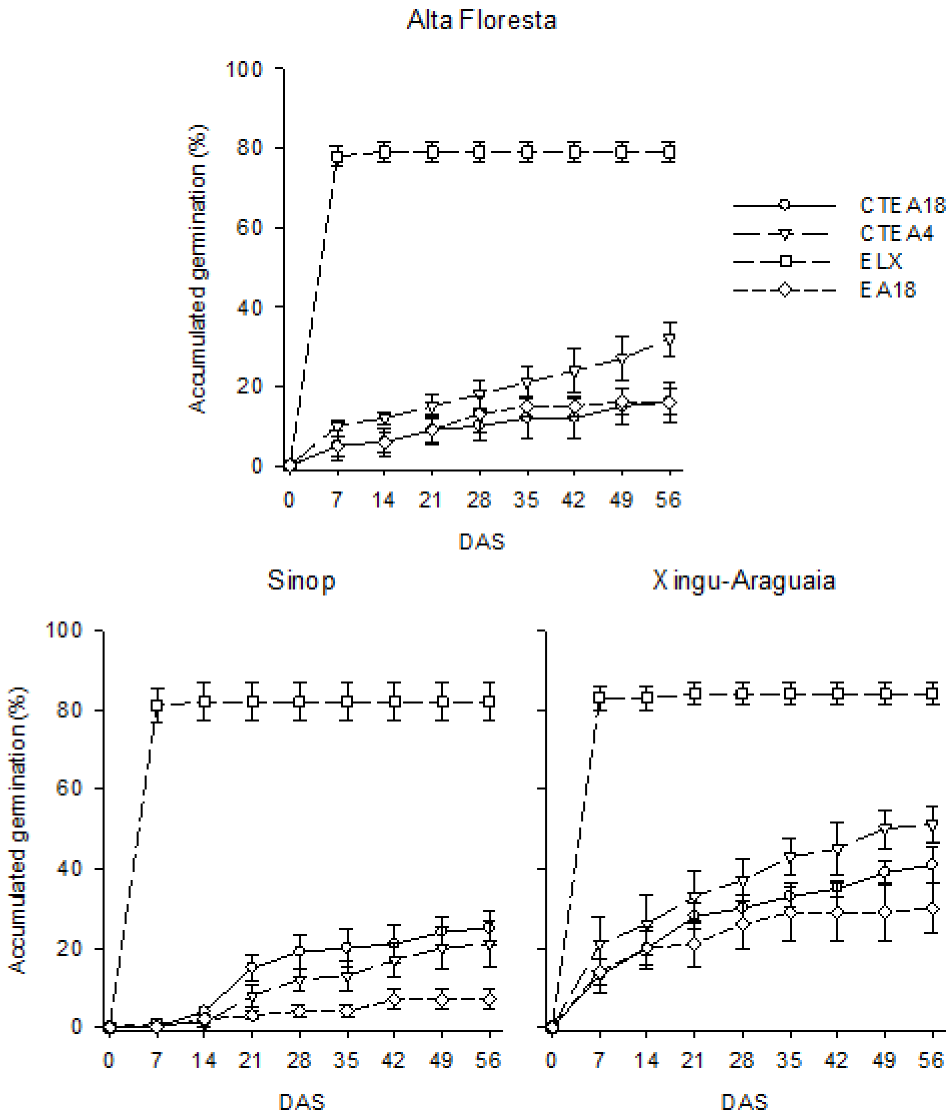
1) Means followed by the same capital letter in the column and lowercase in the line do not differ from each other (Tukey, 5%); 2) ↑ and ↓ indicate significant difference greater or lesser (Tukey, 5%) of the sand seedbed in relation to the growth chamber within each source and breakage of dormancy, and 3). For GSR the means test was performed with the data transformed into log10 (x + 1) and the means presented without transformation. Source: Authors (2021).

Table 4. Percentage of germination, emergence and rate of germination (GSR) of *Enterolobium schomburgkii* seed stocks from different sources in Mato Grosso state, submitted to treatments for breaking dormancy under non-controlled conditions of seeding of temperature, light and humidity.

Provenances	Uncontrolled environment (sand seedbed)				Means
	Heat shock + 18-hour in water	**Heat shock + 4-hour in water**	**Mechanical scarification**	**18-hour in water**	
	Germination/Emergency (%)				
Alta Floresta	35.00 Bc ↑[2]	42.00 Ab	78.00 Aa	26.00 Bd	45.25
Sinop	11.00 Cc ↓	13.00 Bb	77.00 Aa	5.00 Cd	26.50
Xingu-Araguaia	55.00 Ab ↑	36.00 Ad ↓	77.00 Aa	43.00 Ac ↑	52.75
Means	33.67	30.33	77.33	24.67	41.50
	GSR[3]				
Alta Floresta	0.62 Bb ↑	0.57 Ab	2.67 Aa	0.47 Bb	1.08
Sinop	0.14 Cb	0.13Bb	2.62 Aa	0.05 Cb	0.74
Xingu-Araguaia	1.25 Ab ↑	0.85 Ab	2.57 Aa	1.01 Ab ↑	1.42
Means	0.67	0.51	2.62	0.51	1.08

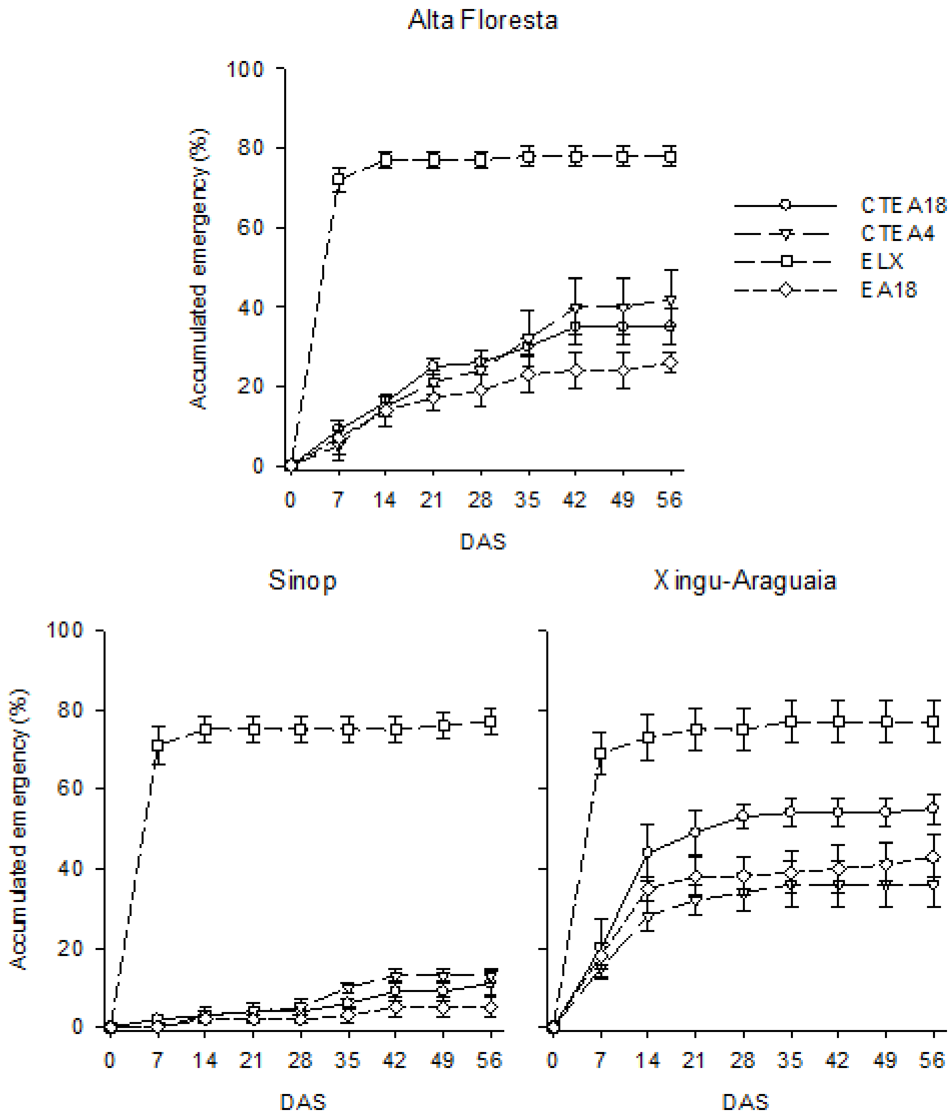
1) Means followed by the same capital letter in the column and lowercase in the line do not differ from each other (Tukey, 5%); 2) ↑ and ↓ indicate significant difference greater or lesser (Tukey, 5%) of the sand seedbed in relation to the growth chamber within each source and breakage of dormancy, and 3). For GSR the means test was performed with the data transformed into log10 (x + 1) and the means presented without transformation. Source: Authors (2021).

Figure 1. Percentage of accumulated germination of *Enterolobium schomburgkii* seeds in the Mangelsdorf germination chamber through days after sowing (DAS), submitted to different methods of dormancy break (CTEA18: five minute-60 °C heat shock, followed by 18-hour water soaking, CTEA4: five-minute-60 °C heat shock, followed by 4-hour water soaking, ELX: sandpaper scarification, EA18: 18-hour water soaking for seed stocks with different provenances (Alta Floresta, Sinop, Xingu-Araguaia) Vertical bars at each point represent the mean standard errors.



Source: Authors (2021).

Figure 2. Percentage of accumulated emergency of *Enterolobium schomburgkii* seeds in the sand seedbed through days after sowing (DAS), submitted to different methods of dormancy break (CTEA18: five minute-60 °C heat shock, followed by 18-hour water soaking, CTEA4: five-minute-60 °C heat shock, followed by 4-hour water soaking, ELX: sandpaper scarification, EA18: 18-hour water soaking for seed stocks with different provenances (Alta Floresta, Sinop, Xingu-Araguaia) Vertical bars at each point represent the mean standard errors.



Source: Authors (2021).

In general, Xingu-Araguaia provenance was the one that obtained greatest germination and emergency in relation to alternative methods of mechanical scarification. This can be attributed to the lower integumentary dormancy of this provenance related to others. On the other hand, Alta Floresta plot presented variable responses, according to adopted method and environment. In 18-hour soaking, germination of Alta Floresta was similar to obtained in Xingu-Araguaia plot in controlled condition, being the same verified in relation to its emergence after heat shock plus 4-hour water soaking in seedbed condition. Although, for mechanical scarification, known as standard method, there are no differences between provenances, with an average germination and emergence rate of 81%.

Similar behavior was observed for GSR (Table 3), with ordinary seedlings appearing seven days after sowing, regardless of provenance, dormancy break treatment or even germination condition (Figures 1 and 2). The fact that germination/emergence curves did not show stabilization until 56 days of cumulative number of regular seedlings shows that, except for control treatment, other methods were not efficient in allowing uniform seed dormancy break within stocks and accelerate their germination (Figure 1) and emergence (Figure 2) in this time interval. The GSR of alternative methods for breaking dormancy is at least two times lower than mechanical scarification GSR.

Seeds from Sinop presented germination/emergency and GSR lowest values when water soaking was used for 18 hours to break dormancy. This result may represent vigor reduction in the oldest stock, collected in 2011 (Table 1), expressed by emergence rate and germination speed low rates related to other stocks (Tables 3 and 4). In practice, both emergence and GSR are indicators applied to express seed vigor, although the latter is influenced by mean time and germination speed (Santana & Ranal, 2004). The higher the emergence speed rate, the shorter the seed exposure time in relation to adverse environmental factors, which can cause damage and even seedlings deterioration, ie, the seed stock studied is more vigorous (Nakagawa, 1999; Gonçalves et al., 2013).

Tests comparing *Enterolobium contortisiliquum* (Vell.) Morong. (Fabaceae) sown in plastic bags and seedbed, submitted to different pre-germination treatments, showed there was no significant difference between sowing sites, but germinative pre-treatments were responsible for germination success of the species (Nodari et al., 1983). For *E. schomburgkii*, mechanical scarification was more effective, regardless of seeds provenance or sowing environment. However, alternative methods through soaking in water and heat shock plus soaking in water present distinct responses to provenances and sowing sites (Tables 3 and 4).

In a controlled or uncontrolled environment, seed germination after 18 hours' water soaking was significantly lower than other dormancy-breaking methods; however, this effect may be related to different responses obtained in relation to Sinop provenance, as already pointed out (Tables 3 and 4). Sinop stock may present a higher proportion of hard seeds than the other packages, thus requiring more abrasive techniques for its germination, a result supported by mechanical scarification. In this case, thermal shock, with 4 to 18-hour water soaking, was more efficient than just imbibitions, in order to reduce integument impermeability and stimulate germinative processes (Tables 3 and 4).

Although heat shock treatments differed in germination, emergence and germination speed (GSR), especially in uncontrolled environment, were not efficient in providing germinability similar to obtained in control treatment, as verified by Silva et al. (2012). Mechanical scarification was more effective, with germination/emergence higher than 77% and GSR above 2.0. The hard structure that surrounds the embryo exerts physical impediment, which restricts water entrance and primary root protrusion. The rupture of the tegument through scarification allows water soaking and initiation of the embryonic axis elongation (Aref et al., 2011; Botsheleng et al., 2014).

The increase in the germination percentage and the GSR (Tables 3 and 4) and the decrease in seeds mean germination time (Figures 1 and 2) when submitted to mechanical scarification are an indication that integument hardness is responsible for dormancy in *E. schomburgkii*. This can be influenced by the environmental factors, generating more or less degree of dormancy in seeds (Alexandre et al., 2009). Scalon et al. (2005) detected dormancy due to integument impermeability in *E. contortisiliquum* seeds, while evaluating germination by chemical scarification with sulfuric acid, which may indicate the need to use more pre-germinative treatments in order to obtain better percentages of germination.

Other abrasive methods, such as mechanical scarification, can be used by farmers, especially if carried out at radicles emission extreme opposite side (Borges et al., 1980; Souza & Varela 1989; Matos et al., 2010; Linhares et al., 2020). As for the heat shock application to break dormancy, results of two treatments tested here were similar to those found by Silva et al. (2014) in *E. contortisiliquum*, although less efficient than mechanical treatments, which does not exclude its application. The use of each method may depend mainly on the labor required by each method, seed availability and execution costs.

It is important to highlight that, despite notable advances and adaptations to an immense variety of scenarios related to collection, processing and commercialization of native seeds practices in Brazil, many procedures have been based on consolidated routines for agricultural seeds sector, of breeding and establishment of stocks with standardized seed quality (Guimaraes et al., 2021). The direct application of these norms to the native seeds sector, where hundreds or even thousands of species still lack greater taxonomic, ecological and physiological knowledge (Ribeiro-Oliveira & Ranal, 2014; Figliolia, 2015; Pedrini & Dixon, 2020; Marques et al., 2022), can make it difficult to apply expected standards described in IN nº 17/2017 and, consequently, the commercialization.

Analyzing standardized methods official list for the analysis of forest seeds (Brazil, 2013), there are only one or two citations for dozens of species, as a study reference for standards suggestion. Considering landscapes richness and genetic diversity present in Brazilian flora species, especially those with a broad floristic distribution, more research is needed to give greater robustness to official recommendations. In addition, many techniques suggested in literature indicate use of restricted circulation substances to break integumentary dormancy (such as H_2SO_4), a situation that is far from the reality (and legality) of many seed collectors and traders, who need to present, according to IN nº 17/2017, bulletins or reports of germination.

4. Conclusion

E. schomburgkii is a species that allows the use of simple dormancy breaking methods, such as mechanical scarification, for the production of seedlings. The method proved to be more efficient, independent of environmental control, without requiring large investments for the production process. Future researches shall focus on enhance robustness to official information, since Brazilian biodiversity is great and requires basic research on seed germination and emergency for restoration and commercialization.

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