DOI: 10.5433/1679-0359.2019v40n6Supl3p3345

Dormancy breakage and germination in *Sapindus saponaria* L. seeds as a function of temperature and germination substrate

Superação de dormência e germinação em sementes de *Sapindus saponaria* L. em função do substrato da temperatura e germinação

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Abstract

The objective of this study was to evaluate different pre-germination treatments, histochemical characteristics and physiological performance of *Sapindus saponaria* L. seeds as a function of temperature and the germination substrate. The *S. saponaria* seeds were collected in the municipality of Rio Verde, GO, and analysed through the execution of 2 tests (dormancy breakage and germination tests). Test 1 consisted of 10 treatments for overcoming dormancy, whereas test 2 consisted of a 35-day evaluation of germination on several substrates (paper and sand) under the following 4 temperature conditions: 20, 25, 30°C and alternating 20-30°C (± 0.5°C). The results of the dormancy breakage and germination tests were submitted to analysis of variance (ANOVA), and the means were compared by the Tukey test. Scarifying *S. saponaria* seeds with concentrated sulfuric acid for 90 min provided a relatively high speed and percentage of emergence, without causing anatomical damage. Pre-germination treatments using high- (42 °C) or low-(10 °C) temperature stress caused cellular damage in the endosperm region of *S. saponaria* seeds. The greatest percentage of *S. saponaria* seeds germinated on a paper substrate at 30°C.

Key words: Western soapberry. Pre-germination treatments. Physiological quality.

Resumo

O objetivo deste estudo foi avaliar diferentes tratamentos pré-germinativos, características histoquímicas e o desempenho fisiológico de sementes de *Sapindus saponaria* L. em função da temperatura e de substrato para a germinação. As sementes de *Sapindus saponaria* foram coletas no município de Rio Verde, GO, e analisadas através da execução de 2 ensaios (superação de dormência e germinação). O ensaio 1 foi composto por 10 tratamentos de superação de dormência, enquanto o 2 foram avaliados a germinação em 2 substratos (papel e areia) e 4 temperaturas 20, 25, 30 e alternadas de 20-30°C (±0,5°C) por 35 dias. Os resultados do teste de superação de dormência na germinação foram submetidos à análise de variância (ANOVA) e as médias comparadas pelo teste de Tukey. A escarificação de sementes de *Sapindus saponaria* L. com ácido sulfúrico concentrado por 90 min, proporcionou maior velocidade e percentual de emergência, não acarretando danos anatômicos. Tratamentos pré-germinativos, utilizando de estresse por alta (42 °C) ou baixa (10 °C) temperatura, causaram danos celulares na região do

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Received: Sept. 19, 2018 - Approved: Sept. 29, 2019

endosperma de sementes de *Sapindus saponaria* L. A maior porcentagem de sementes de *S. saponaria* germinou em substrato de papel a 30 ° C.
Palavras-chave: Sabãozinho de macaco. Tratamentos pré germinativos. Qualidade fisiológica.

Introduction

The advancing agricultural frontiers and the uncontrolled extractivism of the Cerrado region have contributed to a gradual reduction in the occurrence areas of several potentially useful plant species (MORAES et al., 2017). Among these, *Sapindus saponaria* L., which belongs to the family Sapindaceae, stands out in the State of Goiás. This species, commonly known as western soapberry, wingleaf soapberry, jaboncillo, sulluku and mānele, occurs in rain forests and semi-deciduous forests (Amazon region to Goiás and Mato Grosso states) (LORENZI, 2002). Its seeds are exalbuminous, bitegumented, spherical and hard, with a dormancy imposed by integument impermeability.

The demand for seeds and seedlings of *S. saponaria* has increased over the years, particularly for its planting as an ornamental tree, due to its small size (up to 8 m), its dense and globose canopy and its use as a medicinal plant. The latter occurs because the roots, fruits and bark of *S. saponaria* present substances that are astringent, antispasmodic, calming and antitussive (LORENZI, 2002) as well as larvicidal and fungicidal (TSUZUKI et al., 2007).

Among the limiting factors for seedling production, temperature is critical because most seeds do not germinate below or above a certain temperature (FERREIRA; MORAIS, 2015). Thus, knowledge regarding the optimal temperature for the germination of *S. saponaria* seeds is determinant for obtaining maximum germination in a short period. In addition to the temperature, the substrate exerts a marked influence on the germination process because this process requires a specific relationship between water availability and aeration (PEREZ et al., 1999); this relationship should also be investigated to optimize the production of *S. saponaria* seedlings, given the absence of this information in the literature.

The production of *S. saponaria* seedlings is additionally limited by the occurrence of integument dormancy; this dormancy hinders the seed germination process, which naturally occurs slowly in a low percentage of seeds. Thus, to favour seed germination in this species, the use of dormancy overcoming treatments is necessary, together with knowledge regarding the best substrate and temperature.

In view of the above, the objective of the present research was to evaluate different pre-germination treatments, histochemical characteristics and the physiological performance of *S. saponaria* seeds as a function of the temperature and substrate used for germination.

Material and Methods

The fruit of *S. Saponaria*, fully ripe and yellowish, were collected from a rural area in the municipality of Rio Verde, GO, Brazil, and manually pulped to obtain the seeds. Subsequently, the batch was cleaned and homogenized in a homogenizer (boerner type, Motmco®). After homogenization, the seeds were stored at a laboratory room temperature of 25°C (± 2°C) in multilayered kraft paper bags for 6 months.

After the storage, 2 experiments were setup; these tests are described next.

Test 1 - Overcoming dormancy

This experiment consisted of 10 treatments, with 100 seeds, submitted to the following procedures: seeds *in natura* (without pre-germination treatment); immersion in concentrated sulfuric acid for 60, 75 and 90 min; manual scarification with sandpaper No. 80 in the region opposite the hilum and immersion in water for 24 h; immersion in

water at 70°C for 30 min; manual scarification with sandpaper No. 80 and immersion in gibberellic acid (1000 ppm) for 24 h at 25°C; storage at 41°C for 72 h in a germination incubator (TE-4013, Tecnal®, Brasil) ; and imbibition at 10°C for 72 h.

After exposure to the pre-germination treatments, 4 replicates of 25 seeds were sown in a sand bed, with a depth of approximately 2 cm, in a greenhouse using 3 daily irrigations.

To evaluate the treatment effects, several attributes were assessed. In the first test, emergence was determined from daily counts of emerged seedlings taken from the 14th to the 45th day after sowing, using eophyll emergence as the criterion for seedling emergence. At the end of the counts, the total number of emerged seedlings was recorded, and the percentage of seedlings emerged was calculated according to (BRASIL, 2009). In the second test, the emergence speed index (ESI) was determined concomitantly with the emergence test, by recording the number of seedlings emerged from the 14th to the 45th day, and calculated as proposed by Maguire (1962). In the third test, a measurement of the emergence speed (ES) was performed together with the emergence test by recording the number of emerged seedlings from the 14th to the 45th day, calculated according to Edmond and Drapala (1958). In the fourth test, the seedling length was recorded at the end of the emergence test; 10 normal seedlings of each replicate were measured with the aid of a ruler graduated in centimetres, with the results expressed in centimetres per seedling. Finally, the dry mass of the seedlings was established after the final count in the emergence test; the seedlings were dried in an oven regulated at 80°C for 24 h and then weighed with an analytical balance having an accuracy of 0.001 g, the test was performed after the emergence test, according to the Seed Analysis Rules (SAR), and normal plant and root and stem length evaluations were determined according to laboratory practices.

The results of the test on overcoming dormancy were submitted to an analysis of variance (ANOVA), and the means were compared by the Tukey test at the 5% probability level using the SISVAR software (FERREIRA, 2011).

For the anatomical and histochemical analyses, immediately after the tests on overcoming dormancy, the *S. saponaria* seeds were removed from the external protective pericarp and cut in the middle; then, the samples were fixed in solution for 24 h (KARNOVSKY, 1965). After this period, the samples were pre-washed in phosphate buffer and dehydrated in an ethyl alcohol series with increasing concentration (70% to 100%) as well as pre-embedded and embedded in historesin (Leica, Germany) according to the manufacturer's recommendations.

For structural evaluation, the samples were sectioned transversely at a 5 µm thickness on a rotary microtome (Model 1508R, Logen Scientific, China), and the sections were stained with toluidine blue for a polychromatic effect (0.05% 0.1 M phosphate buffer, pH 6.8) (O'BRIEN et al., 1964). For the histochemical analysis, the sections were stained with xylidine ponceau (XP) for total proteins (O'BRIEN; MCCULLY, 1981), periodic acid/ Schiff's reagent (PAS) for neutral polysaccharides (MCMANUS, 1948) and Sudan III for total lipids (PEARSE, 1972).

The observations were performed and the images were photographed under an Olympus microscope (BX61, Tokyo, Japan), coupled with a DP-72 camera Olympus®, Japan, using the clear-field option.

Test 2 - Germination

Before testing germination, the seeds were immersed in concentrated sulfuric acid for 90 min to overcome dormancy. The assay was installed with 8 treatments, arranged in a 4 x 2 factorial scheme (4 temperatures x 2 substrates) and having 5 replicates of 20 seeds.

To evaluate germination on a paper substrate, the number of germinated seeds was counted daily, considering the protrusion of the radical indicative of seed germination. For testing with a sand substrate, the seeds were considered germinated once the eophylls emerged. The counts of germinated seeds started at 12 and extended up to 35 days after sowing. At the end of the test, the numbers of germinated seeds, swollen seeds and hard seeds were evaluated. The results were calculated according to (BRASIL, 2009) and expressed as a percentage.

To evaluate germination in sand, the substrate was washed in concentrated hydrochloric acid for 7 days, followed by oven sterilization at 100°C. To wet the substrate, the field capacity of the sand was first determined as recommended by (BRASIL, 2009), and the substrate was moistened with distilled water to 60% of its maximum retention capacity. Subsequently, 1 kg of sand was deposited in a plastic bottle with a volume of 1.5 L, and the seeds were sown in the sand by distributing them in a circular manner at a 2 cm depth. After sowing, the bottles of sand were stored in a transparent plastic bag to maintain moisture.

For the test of germination between paper layers, sowing was performed by distributing the seeds in a linear and alternating manner on 2 germitest paper sheets moistened with 2.5 times their dry weight using distilled water; then, the 2 sheets were covered with another sheet, moistened in the same way. Finally, the sheets were formed into rolls, which were packed in a transparent plastic bag. For both substrates, the seeds were maintained for 35 days in a Mangelsdorf-type germinator at constant temperatures of 20, 25 and 30°C as well as alternating 20-30°C (± 0.5°C).

For the rate of emergence, the daily count data recorded in the germination test were used, and the calculations were performed according to the formula proposed by Maguire (1962). For germination and the speed of emergence, the daily count data recorded in the germination test were used, which were calculated according to Edmond and Drapala (1958).

The data were submitted to analysis of variance (ANOVA), and the means were compared by the Tukey test at the 5% probability level, using the SISVAR software (FERREIRA, 2011).

Results and Discussion

Overcoming dormancy

The chemical scarification in concentrated sulfuric acid for 90 min and 60 min resulted in a greater emergence of *S. saponaria* seedlings (Figure 1) as well as a higher emergence speed index in relation to the other treatments tested in this study (Figure 2). Together, these results demonstrate the efficiency of the chemical scarification for 90 min and 60 min with concentrated sulfuric acid in overcoming the dormancy imposed by the integument. These treatments contributed in maximizing water absorption and increase the speed of the emergence process. Oliveira et al. (2012) corroborated this information, reporting that scarification for 60 min promotes an increase in the emergence of *S. saponaria* seeds. Albuquerque et al. (2007) reported similar results for the seeds of *Bowdichia virgilioides* Kunth (sucupira-preta).

Figure 1. Percentage emergence of *Sapindus saponaria* L. seeds submitted to different pre-germination treatments (means topped by the same lowercase letter above the bar do not differ by the Tukey test at the 5% probability level).

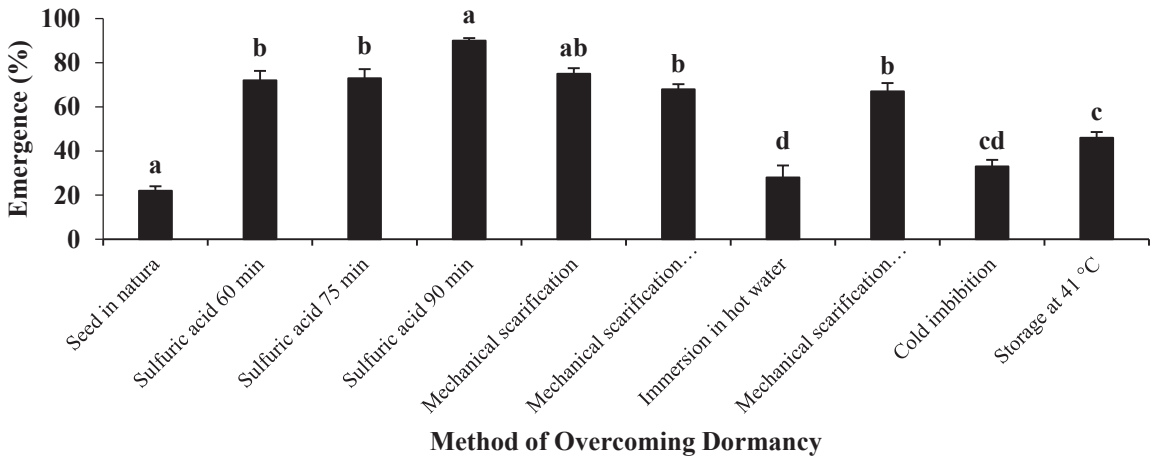
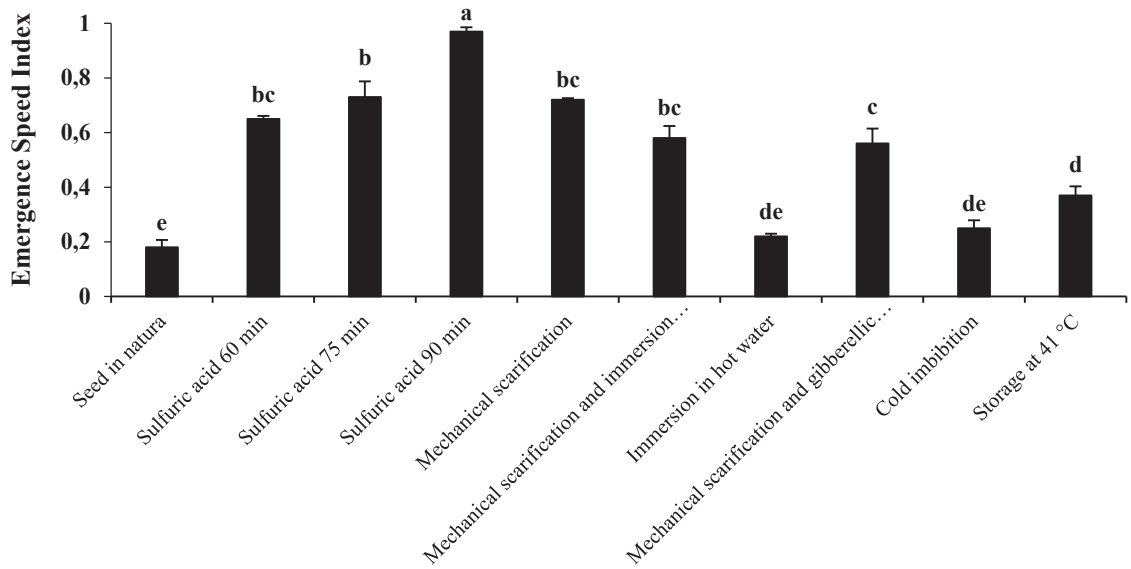


Figure 2. Emergence speed index for *Sapindus saponaria* L. seeds submitted to different pre-germination treatments (means topped by the same lowercase letter above the bar do not differ by the Tukey test at the 5% probability level).



Among the other pre-germination treatment strategies investigated in this study, the scarifications with concentrated sulfuric acid for 90 minutes resulted in higher emergence rates (Figure 2). However, the use of mechanical scarification, coupled with immersion in 1000 ppm gibberellic acid for 24 h, did not provide an increase in emergence or in the speed of emergence compared with the mechanical scarification treatment followed by imbibition in water for 24 h. This result may indicate that *S. saponaria* seeds have no physiological dormancy.

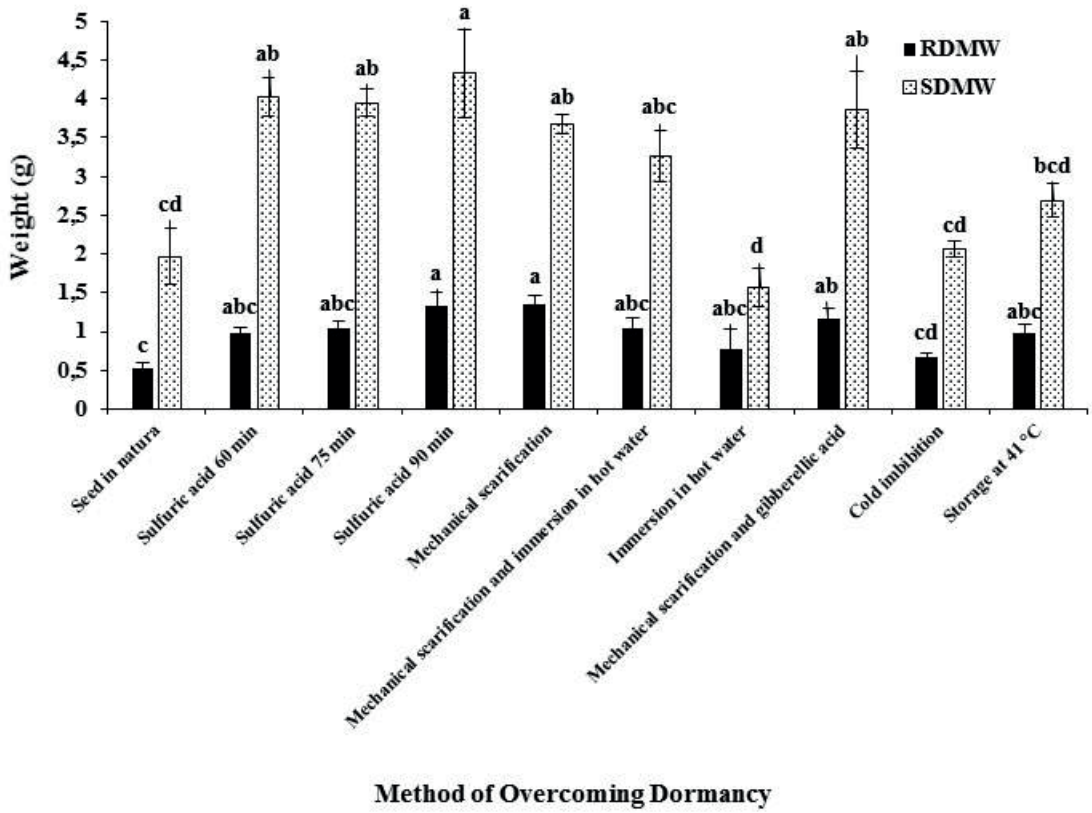
In studies with seeds of *Stylosanthes viscosa* (melosa or meladinha*)*, Araújo et al. (2000) reported that sulfuric acid scarification can efficiently guarantee a high emergence percentage and ESI because this treatment was determinant in avoiding

the impermeability to water imposed by the integument during the seed dormancy of this species. In turn, Sousa et al. (2009) found that scarification with sulfuric acid reduced the emergence and the ESI of *Ricinus communis* (castor bean) seeds.

The sulfuric acid treatment of seeds for 90 min resulted in an increase of 0.82 and 2.36 g in the root dry matter weight (RDMW) and shoot dry matter weight (SDMW), respectively, of *S saponaria* seedlings compared with seedlings grown from the seeds *in natura* (Figure 3). Similarly, Silva et al. (2011), used *Tamarindus indica* L. (tamarind) seeds and obtained a higher dry matter value when the seeds were soaked in sulfuric acid for 15 min.

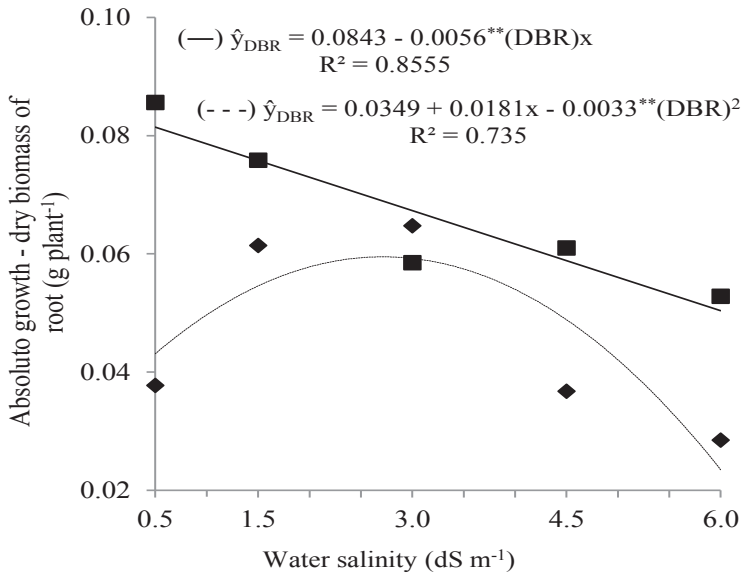
The SDMW (Figure 3) of the plants from the seeds submitted to imbibition at 10°C and immersion in hot water was similar to that of the plants from seeds without treatment to overcome dormancy. This result indicates that these treatments do not benefit the post-seminal development of *S. saponaria*.

Figure 3. Root dry matter weight (RDMW) and shoot dry matter weight (SDMW) for seedlings of *Sapindus saponaria* L. submitted to different pre-germination treatments (means topped by the same lower case letter above the bar do not differ by the Tukey test at the 5% probability level).



Fresh seeds presented mean values equal to scarified seeds, increased root length (RL) and other attributes of physiological quality (Figure 4). In contrast, immersion in water at 70°C for 30 min, water-immersion scarification, mechanical and gibberellic acid immersion scarification, cold soaking and storage at 41 ° C resulted in lower RLs in relation to the other treatments. Alexandre et al. (2009) found higher growth, both root and aerial, in *Enterolobium contortisiliquum* (Vell.) Morong (tamboril) using seed immersion in sulfuric acid for 10 min compared with mechanical scarification and seed cracking.

Figure 4. Root length (RL), shoot length (SL) for *Sapindus saponaria* L. seedlings submitted to different pre-germination treatments (means topped by the same lower case letter above the bar do not differ by the Tukey test at the 5% probability level).

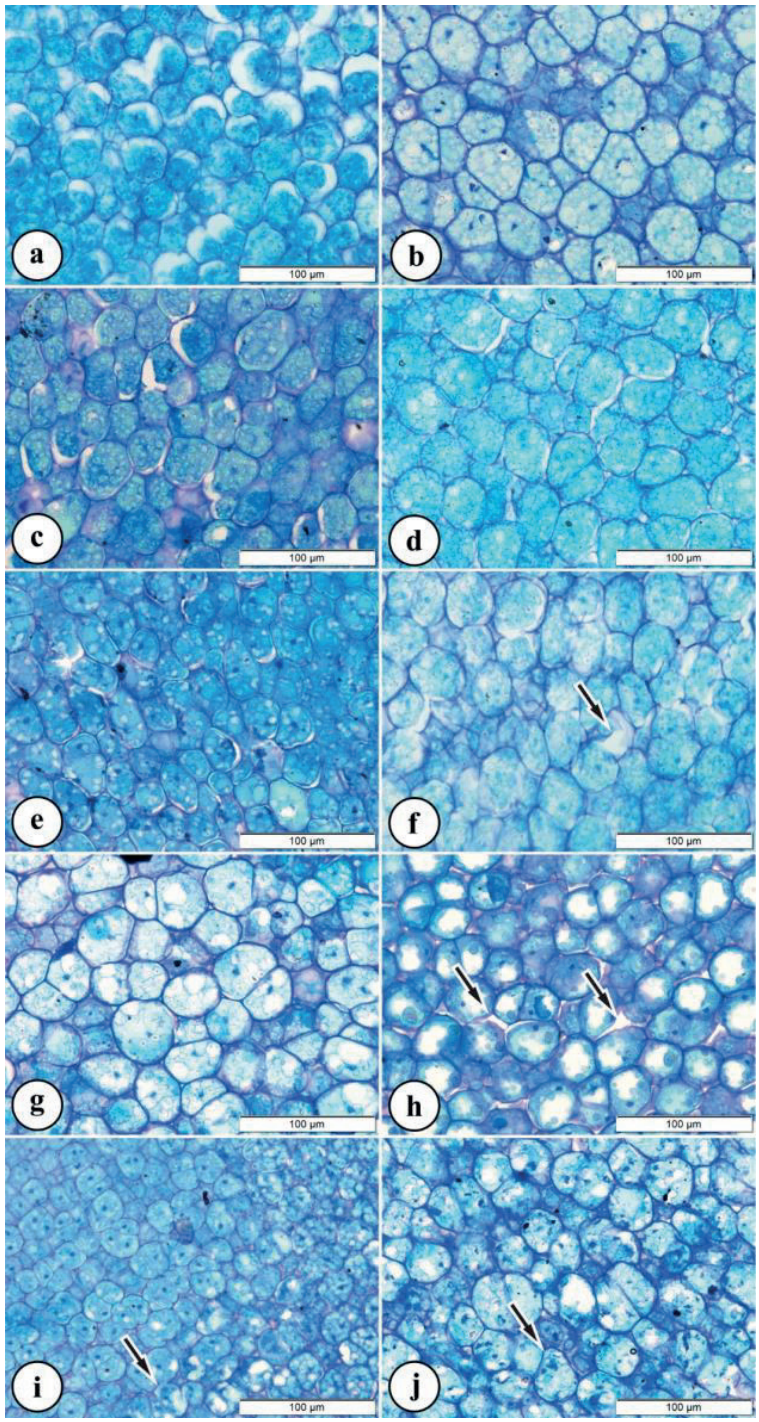


The shoot length (SL) responded best to the scarification with sulfuric acid for 90 and 60 min (Figure 4). The treatment with sulfuric acid for 75 min and the control treatment resulted in similar SLs, which were also observed between the following treatments: mechanical scarification and imbibition with water and with 1000 ppm gibberellic acid. The worst result occurred for the immersion in water at 70°C for 75 min, indicating that this treatment does not overcome the dormancy of western soapberry seeds. Similar results were observed for the seeds of *Hymenaea courbaril* L. (jatobá) (SAMPAIO et al., 2015).

In all the physiological quality evaluations conducted for this study, the dormancy of the western soapberry seeds was observed to be best overcome using the method of scarification in sulfuric acid for 90 min. Similar results were also observed with *Hymenaea courbaril* L. (jatobá), for which scarification in sulfuric acid for 24 min was found to be the best treatment to overcome jatobá seed dormancy (SOUZA; SEGATO, 2016).

The anatomic and histochemical evaluations demonstrated well-defined *S. saponaria* endosperm cells, presenting a circular shape with thick cell walls (Figure 5A, B, C, D, E and G). The treatments using manual scarification with sandpaper No. 80, immersion in gibberellic acid (1000 ppm) for 24 h at 25°C, storage at 41°C for 72 h and imbibition at 10°C for 72 h promoted a collapse of the endosperm cells and an increase in the number of intracellular spaces (Figure 5F, H, I and J). The cellular collapse results from physical damage to the seed; this damage disrupts the endosperm cells, altering the imbibition process and promoting the loss of leachates (SILVA et al., 2008).

Figure 5. Anatomy of *Sapindus saponaria* L. seed endosperm after tests to overcome dormancy. (A) without treatment to overcome dormancy; (B), (C) and (D) immersion in concentrated sulfuric acid for 60, 75 and 90 min, respectively; (E) manual scarification with sandpaper No. 80 in the region opposite the hilum; (F) manual scarification with sandpaper No. 80 in the region opposite the hilum and immersion in water for 24 h; (G) immersion in water at 70°C for 30 min; (H) manual scarification with sandpaper No. 80 and immersion in gibberellic acid (1000 ppm) for 24 h at 25°C; (I) storage at 41°C for 72 h; and (J) imbibition at 10°C for 72 h. Arrows indicate intracellular spaces and cell collapse.



The histochemical test performed in the endosperm region showed the formation of globular corpuscles, characterized as lipid droplets inside the cells throughout this tissue (Table 1). The presence of lipids in the cells may be related to the characteristic odour of *S. saponaria* fruit at maturity (ALBIERO, 2001). Lipids are generally consumed during seed germination and have important roles in this process (HAN et al., 2017). However, the high lipid intensity in the endosperm cells of *S. saponaria* seeds observed during the present study indicates that lipids are not being modified and consumed during germination.

The total carbohydrate concentration in the *S. saponaria* seeds identified by the PAS test was of low and medium intensity (Table 1). This result shows that the carbohydrates present in the endosperm are consumed for energy during seed germination and during the construction of plant tissues that will constitute the seedling (CARVALHO; NAKAGAWA, 2000).

Table 1. Histochemistry of endosperm in *Sapindus saponaria* L. seeds after tests to overcome dormancy.

Treatment	Metabolite Group Test		
	Sudan IV Endosperm	**PAS Endosperm**	**Ponceau Xylidine Endosperm**
1 S/Overcoming	+	++	+++
2 Sulfuric acid, 60 min	++	+	+++
3 Sulfuric acid, 75 min	+	+	+++
4 Sulfuric acid, 90 min	+	+	+++
5 Sandpaper	++	++	+
6 Sandpaper and imbibition	++	++	+++
7 Immersion in hot water	+	+	+
8 Sandpaper and gibberellic acid	+++	+	+
9 Cold imbibition	+++	++	+
10 Ageing	+	+	+

Low-, medium- and high-intensity positive results are indicated by +, ++ and +++, respectively.

In *S. saponaria* seeds, proteins were the most abundant compounds in the reserve tissues (Table 1). This conclusion was evidenced by the increased protein intensity detected throughout the endosperm cells (Table 1). For the XP tests, the treatments without dormancy breakage and those overcoming dormancy via sulfuric acid exposure for 60, 70 or 90 min presented a higher protein intensity compared with the other treatments (Table 1). Proteins are degraded and used during germination, so the large protein accumulation in *S. saponaria* tissues may be directly related to the germination potential of their seeds (PRITCHARD et al., 2002).

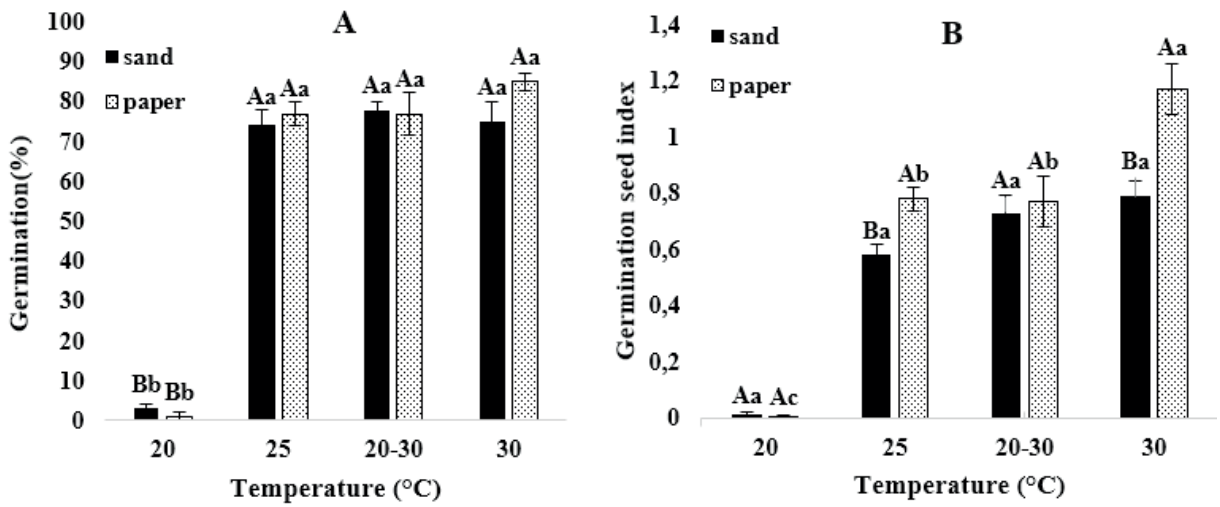
Germination test

The percentage of germination on the sand and paper substrates, in association with the temperatures of 25, 20-30 and 30°C, was higher compared with that of the other treatments (Figure 6A). In turn, the temperature of 20°C, regardless of the substrate, resulted in lower germination percentages for the *S. saponaria* seeds (Figure 6A). The seeds of *Eugenia involucrata* (cherry of the Rio Grande) and *Eugenia pyriformis* (uvaia) present a similar response, as Gomes et al. (2016) verified that the germination tests of these species should be conducted at 25°C.

For *S. saponaria* seeds, Oliveira et al. (2012) improved conditions for performing the germination test using a constant temperature of 30°C combined with the sand substrate. This result corroborates that found in the present study, during which the above-mentioned conditions similarly provided good germination.

In the sand, the germination speed index (GSI) of the *S. saponaria* seeds was higher at temperatures of 25, 30 and 20-30°C in relation to other temperatures (Figure 6B). By contrast, on the paper, the GSI was superior at 30°C. However, the use of a paper substrate interfered with the seed germination performance of this species.

Figure 6. (A) Percent germination (% G) and (B) germination speed index (GSI) for *Sapindus saponaria* L. seeds submitted to different pre-germination treatments (means followed by the same capital letter for temperature and lowercase letter for substrate do not differ by the Tukey test at the 5% probability level).



Analysing only the temperature factor indicated that 20-30, 25, 25-30 and 30°C were the temperatures that provided the highest rate of germination, a fact that was also verified by Oliveira et al. (2005) in seeds of *Annona montana* (araticum).

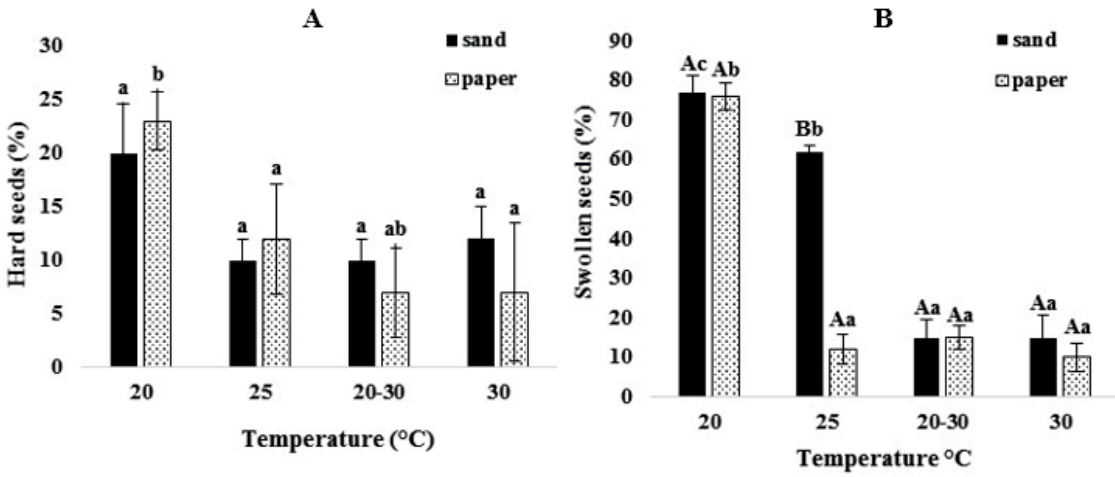
A relatively low percentage of *S. saponaria* hard seeds occurred at temperatures of 30 and 25°C (Figure 7A). In this sense, the percentage of hard seeds on the paper substrate was higher at 20°C in relation to the other temperatures, a pattern that was not observed for the sand. Thus, the temperatures favouring good germination presented lower percentages of hard seeds. In contrast, temperature does not affect the percentages of hard seeds in *Chloroleucon foliolosum* (Benth.) G.P. Lewis (tatarena) (SILVA et al., 2014).

The low percentage of hard seeds (13%) in the *S. saponaria* seed germination test may be an indication that at the population level, the seeds of this species require a period of more than 35 days to complete germination. Time was found to be more critical than temperature in germination evaluations conducted for 22 days at different temperatures using the seeds of *Bowdichia virgilioides* (sucupira-do-cerrado) (SMIDERLE, 2011). This result can be explained by the imbibition process, which is the first stage of the germination process, and with an earlier start to imbibition, the trend is for the next germination stages to be anticipated (MARCOS FILHO, 2009).

The percentage of western soapberry seeds that were swollen at 20°C was higher than that at

the other temperatures (Figure 7B). This finding demonstrates that even with seed soaking, no activation of their metabolism occurs, thus inhibiting germination. Similarly, Machado et al. (2002) found that temperatures below 25°C are detrimental to seed germination in *Tabebuia serratifolia* (Vahl) Nicholson (yellow ipê).

Figure 7. (A) Hard seeds (%) and (B) swollen seeds (%) for *Sapindus saponaria* L. submitted to different pre-germination treatments (means followed by the same capital letter for temperature and lowercase letter for substrate do not differ by the Tukey test at the 5% probability level).



Conclusion

The scarification of *S. saponaria* seeds with concentrated sulfuric acid for 90 min provided higher germination speed and percentage in relation to the other treatments, without causing anatomical damage. Pre-germination treatments that apply high-temperature or low-temperature stress can cause cellular damage in the endosperm region of *S. saponaria* seeds.

The seed germination test for Sapindus saponaria L. indicated no differences between temperatures and substrates.

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