

Morphometric Characterization and Seed Dormancy Overcoming of *Sapindus saponaria* L.

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Received: March 28, 2018

Accepted: May 1, 2018

Online Published: June 15, 2018

doi:10.5539/jas.v10n7p329

URL: <https://doi.org/10.5539/jas.v10n7p329>

Abstract

S. saponaria L. is a Brazilian native arboreal species, with relevant ecological importance in the recovery of degraded and marginal areas. The objectives of the present study were to characterize morphometrically *Sapindus saponaria* L. seeds, to extract and to quantify oil content, establish the percent composition of fatty acids; to describe their different post-seminal stages; to describe the different post-seminal stages, as well as to evaluate the germinative performance of the seeds as a function of different treatments to overcome dormancy. Seeds were evaluated as to water content and biometric length and width. The extraction and quantification of the seed oil was obtained by the solvent extraction method using the Soxhlet extractor system. Regarding the post-seminal development, some processes triggered during growth and development of the seedlings were evaluated. The treatments for dormancy overcoming were: mechanical scarification, chemical scarification with sulfuric acid for 5, 10, 20, 30 and 40 minutes, immersion in hot water (80 °C) until reaching room temperature, immersion in distilled water at room temperature for 24 hours and intact seeds (control). The seeds were evaluated for germination, velocity, medium time, synchrony and germination uncertainty. The design was a completely randomized design with four replicates of 25 seeds per treatment. The seeds present on average 10.3 mm in length and 10.2 mm in width with hygiene-cryptocoleonar germination. The oil content found in the seeds was 7.25%, most of which was composed of unsaturated fatty acids (78.9%). The highest values of germination occur when the seeds are immersed in sulfuric acid for 30 minutes.

Keywords: forest species, germination, post-seminal, seedling.

1. Introduction

The search for forest species with potential to recovery degraded areas and for afforestation has increased in recent years. However, there is no information available on the management of most seeds of native species, which are basic inputs for ecosystem recovery and conservation projects. Therefore, there is a need for research aimed to determinate the basic information for the proper management of its seeds.

The forestry sector contributes significantly to the Brazilian economy by obtaining products for export or domestic consumption, generating employment for the population, as well as contributing to the conservation and preservation of renewable natural resources (Ladeira, 2002). Although there is a large number of native species traded in Brazil for forest recomposition, few are described in the Rules for Seed Analysis (Brasil, 2009). According to Sarmiento and Villela (2010), more research is needed with native tree seeds, especially regarding the physiological aspects involved in germination.

S. saponaria L., is a native, arboreal species and popularly known as soapwort, with relevant ecological importance in the recovery of degraded and marginal areas, that allow the reconstruction of the complex interactions existing within a community, as well as its local self-perpetuation. The demand for seeds and fruits of this species also has increased in recent years due to its use as an ornamental plant, its dense and globulous crown, medicinal properties, with roots and barks with calming, astringent, antispasmodic and antitussive substances. The fruits are rich in saponin, a natural surfactant, which can be used as soap (Ribeiro et al., 1999; Judd et al., 2009) and used for ulcers treatment, inflammations and skin lesions (Pelegrini et al., 2008). In

addition, they have several insecticidal and sarnicidal properties such as: tannin, fatty acids, triterpenes, anthocyanin, unsaturated steroids, rutin, luteolin, amirin and sitosterol (Previero et al., 2010; Souza & Lorenzi, 2012; Mena-Valdés et al., 2015). Its wood is heavy, hard and compact, used in construction and in the making of toys (Pio-Correa, 1984; Lorenzi, 1992).

S. saponaria L. species, has little information to analyze its seeds, being necessary to obtain information on germination aspects, allowing its use for a wide variety of purposes, as well as offering support to a wide variety of reforestation programs. In this context, the objective of the present work was to characterize the morphometrically of *S. saponaria* seeds, to describe the different post-seminal stages, as well as to evaluate their germinative performance as a function of different treatments of dormancy overcoming.

2. Material and Methods

2.1 Local and Execution Period

The tests were conducted at the Plant Propagation Laboratory of the Center of Agricultural Sciences (CECA) of the Federal University of Alagoas (UFAL), located in the municipality of Rio Largo, state of Alagoas, Brazil. *S. saponaria* seeds were harvested from trees located in the municipality of Maceió, state of Alagoas, at 9°37'40.90" latitude and 35°44'18.13" longitude, with an altitude of 59 m in December 2014.

2.1.1 Assay 1: Morphometric Characterization of Seeds

For physical characterization, length (mm) and width (mm) were determined using a precision digital pachymeter of 0.1 mm using eight replicates of 100 seeds. The length was determined from the base to the apex (Figure 1A) and the width measured at the median line of the seeds (Figure 1B). For each variable, mean, modo, median, amplitude of variation, variance, standard deviation and coefficient of variation were calculated (Banzato & Kronka, 1992; Melo et al., 2015).

For the internal morphological observations (embryo and reserve tissue), the seeds were previously immersed in distilled water for 24 hours at 30 °C for softening and hydration. After this period, they were cut longitudinally with a blade and then observed in a magnifying glass.

Seed length and width data were grouped into class for better presentation on the frequency histogram. In order to indicate the degree of distortion of the distribution in relation to a symmetrical distribution, the asymmetry coefficient of Persons (1910) was calculated.

The weight of 1,000 seeds was determined using eight replicates of 100 seeds, and the mean, standard deviation and coefficient of variation were calculated (Brasil, 2009). The extraction and quantification of the oil was performed according to the methodology of Oliveira et al. (2009) using the Soxhlet extractor system, with hexane solvent.

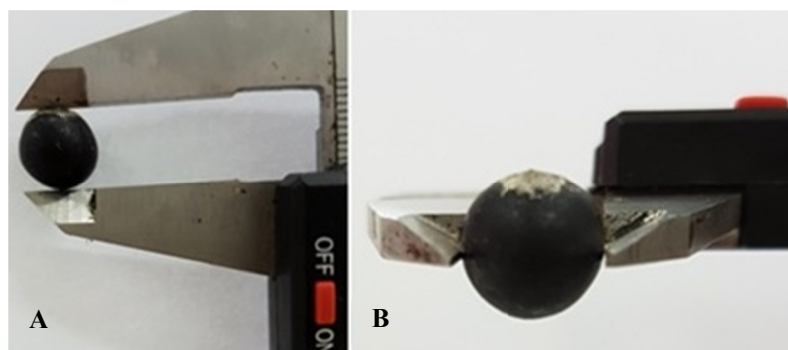


Figure 1. Length (A) and width (B) measurement of seeds of *Sapindus saponaria* L.

2.1.2 Assay 2: Post-seedling Development of Seedlings

The follow-up and registration of the different stages of post-seminal development were performed according to Oliveira (1993). In this study, the different stages of seedling development were observed daily. Abnormalities occurred in the seedlings, as well as the type of germination of the species in question, were also evaluated. For this, four replicates of 25 seeds were distributed over two sheets of paper towel, moistened with distilled water

equivalent to 2.5 times the dry weight of the paper, and covered by a third sheet. Afterwards, rolls were formed, and these were packed in a germinator regulated at a temperature of 30 °C under constant light.

2.1.1 Assay 3: Treatments for Overcoming Numbness

The seeds were previously submitted to asepsis, performed by immersion in 70% alcohol, for one minute, with subsequent washing in distilled water. The following treatments were performed:

- Mechanical scarification using sandpaper number 6, rubbing the integument on the side opposite the micropyle;
- Chemical scarification by immersing the seeds in concentrated sulfuric acid (98%) for different periods (5, 10, 20, 30 and 40 minutes), followed by washing under water;
- Immersion in hot water (80 °C) until water reaches room temperature;
- Immersion in distilled water (room temperature) for 24 hours;
- Control (intact seeds).

To evaluate the efficiency of the pre-germination treatments, the germination test was carried out with four replicates of 25 seeds. The seeds were distributed on two sheets of paper towel, previously moistened with distilled water in the proportion of 2.5 times the weight of the dry paper and kept in a germination chamber regulated at 30 °C (Camara et al., 2009).

The trials were conducted in a completely randomized design with four replicates of 25 seeds. The data were submitted to analysis of variance (ANOVA). For the biometric assays, a descriptive analysis of the data was performed. The averages were compared by the Tukey test at 5% probability, and for the germination percentage, the data were transformed into $\arcsin \sqrt{x/100}$, according to Banzatto and Kronka (1992).

3. Results and Discussion

3.1 Physical Characterization of *Sapindus saponaria* L. Seed

The water content of *S. saponaria* seeds was 17%. The values of length and width presented an average of 10.3 mm and 10.2 mm, respectively. With a small amplitude of variation of approximately 1.4 mm for length and 1.5 mm for width (Table 1).

The biometric characterization of seeds allows to identify genetic variations within populations of the same species or differentiating species of the same genus from environmental factors (Gusmão et al., 2006; Virgens et al., 2016), besides being directly associated with as well as to determine seed dispersal and establishment of seedlings, thus making it possible to differentiate between pioneer species and climax in tropical forests (Cruz et al., 2001; Matheus & Lopes, 2007). Although biometric characteristics of seeds can be taxonomically questionable, due to the strong influence of latitudinal, seasonal and microclimatic variations, they have great biological significance, related to dispersing agents and dispersion syndromes (Rodrigues et al., 2006).

Table 1. Descriptive analysis of seed length and width of *Sapindus saponaria* L.

Statistical measures	Length (mm)	Width (mm)
Average	10.3	10.2
Modo	10.3	10.3
Medium	10.4	10.0
Minimum	9.4	9.2
Maximum	10.8	10.7
Standard deviation	0.2	0.3
C.V. (%)	2.7	3.0

The distribution of the relative frequency of *S. saponaria* seeds length and width is shown in Figure 2. The seeds have an average length of 10.3 mm (ranging from 9.3 to 10.9 mm) with a predominance of seeds with length between 10.2 and 10.4 mm. The average seed width was 10.2 mm (ranging from 9.1 to 10.8 mm), and 22% of these seeds predominate with a width of 10.2 to 10.4 mm. There was an asymmetric behavior for the length and width of the seeds being classified as positive high asymmetry.

According to Ferreira (2000), when the data are asymmetrical, both the right (positive asymmetry) and the left (negative asymmetry), the median is usually the best measure of central tendency. Since the average is sensitive to external observations, pushed towards the atypical values and may be excessively increased or reduced. In this study, the most important classes of seed length and seed width were used to evaluate the ecological aspects of the species (Macedo et al., 2009).

The *S. saponaria* (Figure 3A) is spherical, shiny black in color, with a rigid and smooth integument, in which the hilar region can be easily distinguished, surrounded by thin yellow whitish fur. This description is in accordance with that presented by Albiero et al. (2001), in his study on the anatomical characterization of the leaves, fruits and seeds of *S. saponaria*. The embryo is total, aclorophyllate, with crass cotyledons and hypocotyl-short radicle axis, yellow whitish color (Figure 3B).

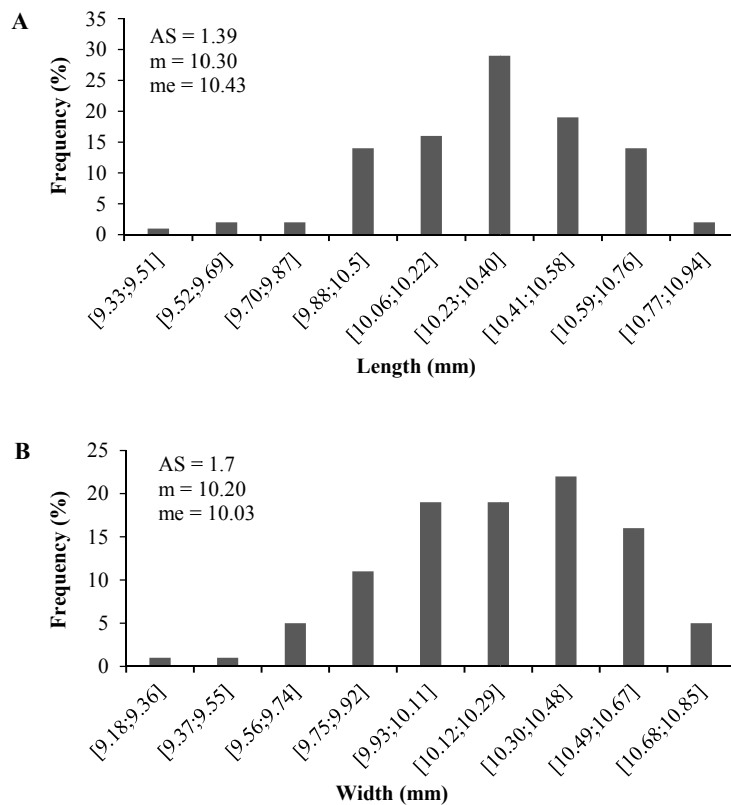


Figure 2. Distribution of the relative frequencies of length (A) and width (B) of *Sapindus saponaria* L. seeds (AS = asymmetry; m = average; me = median)

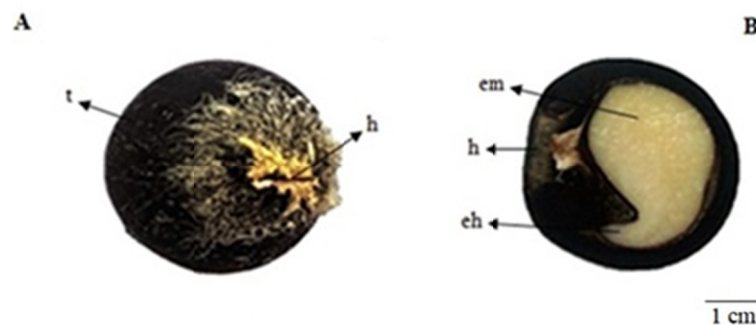


Figure 3. Morphological characterization of the *Sapindus saponaria* L. seed, external view of the seed (A), seed after longitudinal cut (B). (h = thread, t = integument, in = embryo, and h = axis-hypocotyl-radicle)

The weight of 1,000 seeds presented an average of 672.4 g, corresponding to the approximate number of 1,487 seeds per kilogram (Table 2). These data are lower than those found by Lorenzi (1992) and Frigieri et al. (2016), where they mentioned a total of 1,870 and 1,545 units, respectively. Possibly, the explanation for the variation between the results can be related to the difference in the water content of the seeds, which is variable according to the season and harvest environment. According to Vaughton and Ramsey (1998), and Leishman et al. (2000), this change in the weight of the seeds within the plant itself may be related to the effects caused by changes in the environment during its formation.

According to Brazil (2009), the seeds are considered small when they present values above 5,000 units kg^{-1} , and large, when lower. Thus, following this classification the seeds of *S. saponaria* can be considered large, since in one kilo it contains 1,487 units.

Table 2. Descriptive statistics of the weighing obtained to calculate the one thousand seeds of *Sapindus saponaria* L. weight

Statistical measures	Thousand seeds weight
Average of repetitions (g)	67.2
Variance (s^2)	0.8
Standard deviation (s)	0.9
C.V. (%)	1.35

The profiles of methyl esters of fatty acids obtained from the seeds of *S. saponaria* are present in Table 3. Oleic acid (C18:1) was predominant in the oil extracted from the seeds of *S. saponaria*, with 57.6% of the following gadoleic acid (C20:1), linoleic (C18:2), eicosanoic (C20:0) palmitic (C16:0), lignoceric (C24:0), stearic (C18:0), linolenic (C18:3), behenic (C22:0), erucic (C22:1), palmitoleic (C16:1) and caprylic (C8:0), total quantification twelve fatty acids. The oil content was found to 7.25%, most being composed of unsaturated fatty acids (78.9%) and 21.1% saturated. Lovato et al. (2014) obtained similar results when quantifying the oil *S. saponaria*, where the oleic acid was the most abundant, with about 52.45%. Also according to these authors, it was possible to quantify a total of seven fatty acids, being 57.60% of unsaturated and saturated 42.40%.

The quality of oil is given by its fatty acid composition and its various stability according to the percentage of the predominant fatty acid in this, which indicates whether or not the use of antioxidants against seed storage.

Table 3. Fatty acid profiles obtained from oil seeds of *Sapindus saponaria* L.

Fatty Acid	Representation	Percentage%
Caprylic	C8:0	0.2
Palmitic	C16:0	6.5
Palmitoleic	C16:1	0.2
Stearic	C18:0	2.4
Oleic	C18:1	57.6
Linoleic	C18:2	8.1
Linolenic	C18:3	1.6
Eicosanoic	C20:0	8.1
Gadoleic	C20:1	10.9
Behenic	C22:0	0.9
Erucic	C22:1	0.5
Lignoceric	C24:0	3.0

The oxidative stability of oils tends to be lower with the increase of polyunsaturated compounds such as linoleic and linolenic acids, being susceptible to auto-oxidation. Thus, high levels of linoleic and linolenic acid resulted in a higher oxidation rate of 64 to 100 times higher, respectively, than the oxidation of oleic acid (Oetterer et al., 2006; Ramos et al., 2009). It is noteworthy that changes in oil quality can be realized by changing the proportion of the fatty acids, these in turn are strongly influenced by ambient conditions, particularly temperature during seed development. Oliveira and Vieira (2004) reported that higher temperatures provide increased levels of oleic

acid and reduced levels of linoleic acid. In species *Sapindus mukorossi* represented oleic acid 62.8% (Sengupta et al., 1975).

According to Santos et al. (2010) the Sapindaceae family stands out for presenting seeds with potential for oil production, as well as differences in their fatty acid composition. In this study, the oil content found in *S. saponaria* seeds was 7.25%, which was low compared to other species of the same family as was the case of the species *Magonia pubescens*, *Paullinea* sp. and *Dilodendron bipinnatum* studied by Nunes (2012), showed that high levels of oil above 25% were considered potentially promising for biofuel production due to a higher proportion of monounsaturated fatty acids in the composition.

3.2 Post-seminal Seedlings Development

The first visible signs of germination began four days after sowing, with tegument rupture, evidencing the hood (Figure 4A). At fifth days (Figure 4B), the protrusion of the primary root, which was 0.6 cm long and whitish in color, occurred mainly in the region of the hood. Seven days after sowing (Figure 4C), the elongation of the primary root was observed with a slight change in the color of the epidermis, becoming less whitish, measuring around 1.2 cm, with the slow and gradual appearance of the eophylls (first distinct leaves of adult leaves), light green in color. Structural variations in the post-seminal phase, among them, heterofilia is a common factor in the seedling phase in forest species, where the first leaves (eophylls) may present distinct morphology of the adult leaves (Montoro, 2008).

With the elongation of the primary root (Figure 4D), nine days after sowing, it was possible to observe the beginning of the appearance of the first secondary root and the change in coloration of the primary root, from whitish to cream; the eophylls appeared totally free of the integument, green, delicate and opposite. Seeds with eophylls totally free from the integument were considered germinated.

On the thirteenth day after sowing (Figure 4E), the rapid development of epicotyl and root system was observed. The primary root was initially thick, becoming thinner with development. At this stage, seedlings had few secondary roots and a root system classified as pivoting, measuring approximately 4.6 cm in length. The epicotyl was, however, elongated with an average of 5.3 cm. At the end of the trial, thirty days after sowing (Figure 4F), the seedlings presented with all their essential structures formed and developed, measuring on average 11.3 cm in length in total. The seed germination presented the cryptocotyledonar-hypogean type.

In the classification of seedlings of different angiosperms species, the seed attributes (size, function, position of the cotyledons and reserve material) are directly related to the initial morphology of the seedlings, characterizing the morpho-functional types (Garwood, 1996; Pereira et al. al., 2008). For Hladik and Miquel (1990) post-seminal development is characterized by five types of seedlings: phanero-colony-epigeal with foliaceous cotyledons (PEF); (PHR), cryptocotyledonar/hygegea with reserve cotyledons (CHR) and cryptocotyledonar-epigeal with reserve cotyledons (CER), which result from the functional adaptation of the phytocotyledonar-epigea with reserve cotyledons (PER) to the environment, associated to ecological factors, such as seed dispersal capacity and regeneration strategy (Garwood, 1996; Ibarra-Manríquez et al., 2001).

Some types of *S. saponaria* plantlet abnormalities (Figure 5) were observed, such as congenital malformation on early onset, stunted eophytes, atrophied roots and epicotyls, and abnormality at the main root. There was also the occurrence of fungus-infested seedlings throughout the trials (Figure 6). However, infested seedlings continued their development without apparent damage throughout the germination test (30 days).

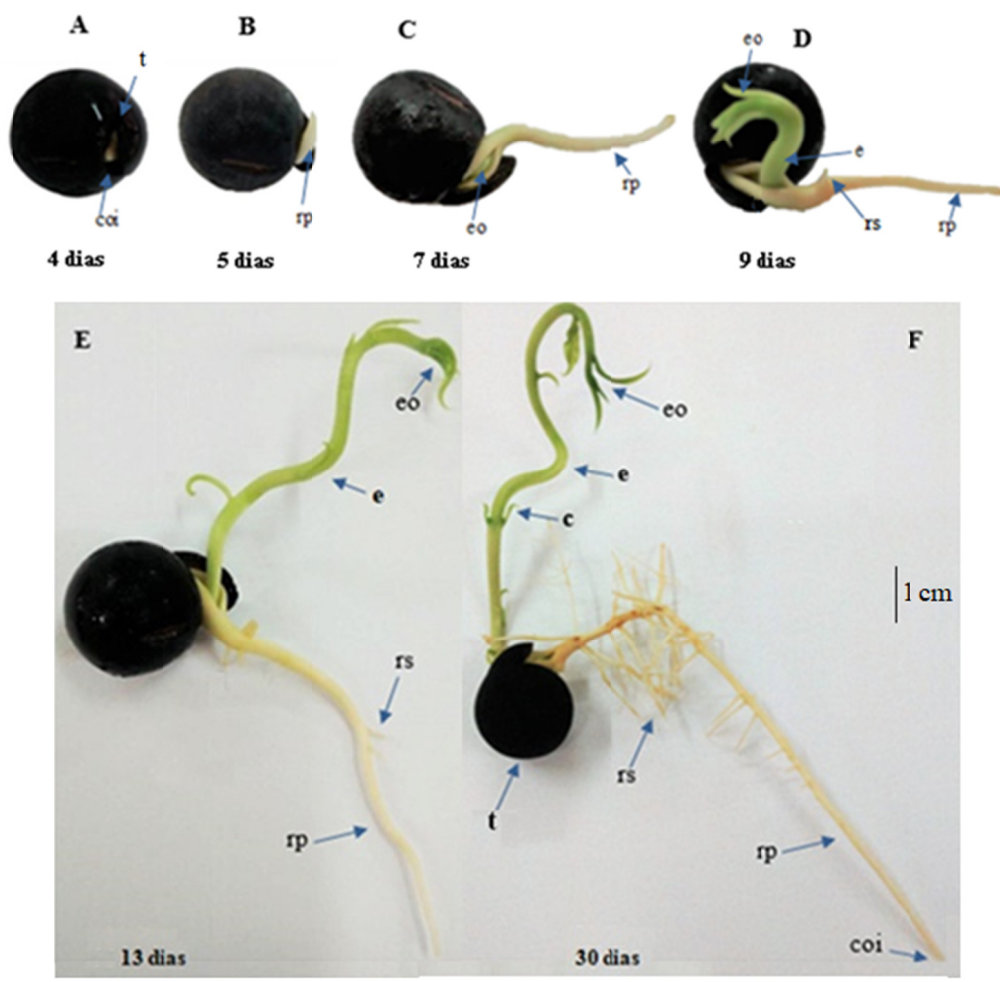


Figure 4. Successive stages of *Sapindus saponaria* L., with four (A), five (B), seven (C), nine (D), thirteen (E) and thirty (F) days after sowing. (c = cataphyll, co = cotyledons, coi = coif, e = epicotyl, eo = eophile, rp = primary root, rs = secondary root, t = tegument)

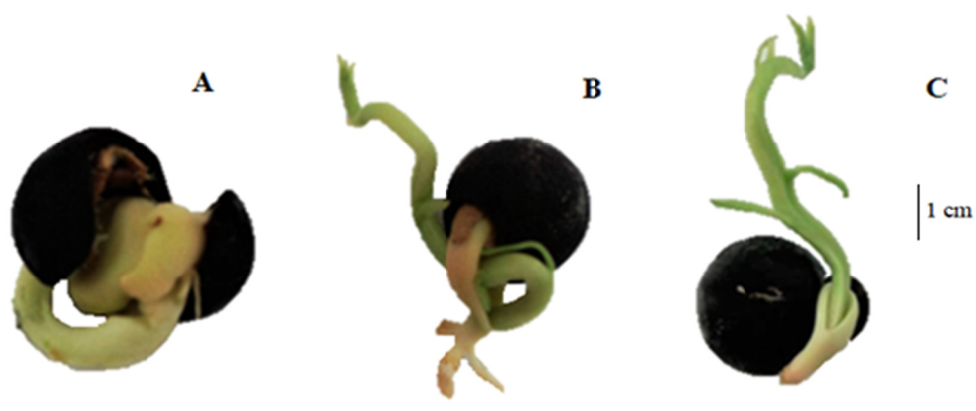


Figure 5. *Sapindus saponaria* L. abnormal seedlings. A = poor seedling formation, with atrophy; B = stunted eophiles, atrophied roots and epicotyl; C = seedling with abnormality at the main root



Figure 6. *Sapindus saponaria* L. seedling, infested with fungus

3.3 Pre-germination Treatments for Seed Dormancy Overcoming

The highest values of germination and GRI were obtained when seeds were immersed in sulfuric acid for 30 minutes, with values of 72% and 1.7, respectively (Table 4). This increase was probably caused by the action of sulfuric acid in the softening of the seed coat, acting on the removal of the cuticle, with consequent exposure of the macroscleride layers, providing more homogeneous degrees of permeability (Santarém & Áquila, 1995).

Table 4. Germination (G) and germination rate index (GRI) of *Sapindus saponaria* L. seeds, submitted to different treatments of dormancy overcoming

Treatments	G (%)	GRI
Control	40 BC	0.5 D
Mechanical scarification (sandpaper nº 6)	52 ABC	0.9 BCD
Chemical scarification (sulfuric acid for 5 min)	38 C	0.6 D
Chemical scarification (sulfuric acid for 10 min)	52 ABC	1.0 BCD
Chemical scarification (sulfuric acid for 20 min)	62 AB	1.2 ABC
Chemical scarification (sulfuric acid for 30 min)	72 A	1.7 A
Chemical scarification (sulfuric acid for 40 min)	62 AB	1.3 AB
Immersion in distilled water for 24 hours	46 BC	0.7 CD
Immersion in hot water (80 °C) to ambient temperature	40 BC	0.5 D
“F” value for dormancy treatment	5.9 **	11.9 **
C.V. (%)	18.2	23.4

Note. ns: not significant; * significant at 5 % by the F-test.

Albiero et al. (2001) observed in an anatomical study of the tegument of the saponaria seeds, that these have double tegument, with many layers of cells in the forehead, being the exotesta formed by macrosclerids, the sclerotic mesotesta and the apparently fibrous endotesta, with parenchymic tegmem constituted by approximately 13 layers of wall cells, characterizing the dormancy imposed by the impermeability of the integument, which constitutes a barrier to the water absorption by the seeds. Thus, as the seed coat of *S. saponaria* is thick and fairly scleridified, it is justified the need for a prolonged period of exposition of the same in sulfuric acid to overcome dormancy. It is also worth noting that the efficacy of this method depends on some factors such as the exposure time to sulfuric acid and the age of the seeds (Albuquerque et al., 2009).

Seeds of *S. saponaria* when immersed in sulfuric acid for 40 minutes showed a decrease in percentage values and germination rate index (Table 3). For Santos et al. (2014) the corrosive effect of this compound can promote irreversible injuries to the embryo, which may explain the decrease in the germination of the seeds submitted to this treatment. Contradictory results were found by Oliveira et al. (2012), who stated that the immersion of *S. saponaria* seeds in sulfuric acid for 60 minutes was able to promote the overcoming of seed dormancy, however in this study the concentration of sulfuric acid used was not specified, which may justify the favorable germinative performance over the long period of immersion (60 minutes).

When mechanically scarified, the seeds of *S. saponaria* showed little more than 50% germination (Table 3). A relatively expressive result, but not indicated for the species, due to the difficulty of manual scarification caused by stiffness of the tegument, as observed by Albiero et al. (2001).

Although mechanical scarification is also widely used as an efficient treatment to overcome the integumentary dormancy of many seeds, it requires manpower and availability of time and is indicated for small seed lots. For larger plots, this scarification could be mechanized, but it is necessary to pay scarification uniformity level, which can result in significant losses, especially for species with production irregular, as well as those that rapidly lose their viability (Aguiar, 1995; Bortolini et al., 2016).

Faria and Davide (1991) reported that the integumentary dormancy presented by *S. saponaria* seeds can be overcome by scarification with sandpaper No. 60 for 30 seconds, although handling with sulfuric acid is dangerous, this treatment is considered more practical when for cases with large seed lots, where their use may be a viable alternative in terms of research with this species (Oliveira et al., 2003).

For germination time average (GTA) (Table 5), there was no statistical difference between the control and pre-germinated seeds treated. Higher values of germination velocity average (GVA) were observed in the treatments: immersion in sulfuric acid for 30 and 40 minutes, with 0.073 and 0.070 days⁻¹. Treatments with lower GVA values indicate that germination was slow and at longer time intervals.

Table 5. Germination time average (GTA) and germination velocity average (GVA) of *Sapindus saponaria* L. seeds, at different treatments of dormancy overcoming

Treatments	GTA	GVA
Control	19.6 A	0.050 AB
Mechanical scarification (sandpaper nº 6)	16.9 A	0.060 AB
Chemical scarification (sulfuric acid for 5 min)	14.9 A	0.045 B
Chemical scarification (sulfuric acid for 10 min)	16.6 A	0.060 AB
Chemical scarification (sulfuric acid for 20 min)	16.4 A	0.061 AB
Chemical scarification (sulfuric acid for 30 min)	13.6 A	0.073 A
Chemical scarification (sulfuric acid for 40 min)	14.4 A	0.070 A
Immersion in distilled water for 24 hours	17.3 A	0.058 AB
Immersion in hot water (80 °C) to ambient temperature	18.9 A	0.052 AB
“F” value for dormancy treatment	1.5 ^{NS}	3.0*
C.V. (%)	14.5	18.3

Note. ns: not significant; * significant at 5 % by the F-test.

Regarding the uncertainty and synchrony results (Table 6), a high degree of uncertainty and low synchrony were observed, with the germination distributed in the medium time. According to Santana et al. (2010), when the uncertainty values are above zero ($U > 1.80$) and Z close to zero ($Z < 0.32$) means that the germination and seed emergence processes are spread in relation to the medium time, with a high degree of uncertainty and low synchrony. These authors obtained similar results, when they studied the germination of Pau-santo (*Kielmeyera coriacea*), with high degree of uncertainty, low synchrony and germination distribution in relation to the mean time.

Table 6. Synchrony of germination (Z) and uncertainty of germination (U) of *Sapindus saponaria* L. seeds, to different dormancy overcoming treatments

Treatments	Z	U
Control	0.077 AB	2.652 A
Mechanical scarification (sandpaper nº 6)	0.130 AB	2.585 A
Chemical scarification (sulfuric acid for 5 min)	0.054 B	2.262 A
Chemical scarification (sulfuric acid for 10 min)	0.068 AB	3.099 A
Chemical scarification (sulfuric acid for 20 min)	0.073 AB	3.142 A
Chemical scarification (sulfuric acid for 30 min)	0.105 AB	2.920 A
Chemical scarification (sulfuric acid for 40 min)	0.144 A	2.553 A
Immersion in distilled water for 24 hours	0.083 AB	2.812 A
Immersion in hot water (80 °C) to ambient temperature	0.084 AB	2.614 A
“F” value for dormancy treatment	3.2*	0.9 ^{ns}
C.V. (%)	20.0	21.5

Note. ns: not significant; * significant at 5 % by the F-test.

The synchrony, uncertainty, time and germination speed average are aspects that can be calculated, and which demonstrate the dynamics of the germination process. Considered important characteristics not only for physiologists and seed technologists, but also for ecologists, precisely for the ability to predict the degree of success of the species, based on the ability of the seed crop to distribute germination over time (Ranal & Santana, 2006)

4. Conclusions

The seeds of *S. saponaria* present on average 10.3 mm in length and 10.2 mm in width, germination of the cryptocyledonar-hypogean type, with higher values of percentage of germination when the seeds are immersed in sulfuric acid for 30 minutes.

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