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Original Article

Silviculture

Dormancy Breaking in *Senna Pendula* (Willd.) H. S. Irwin & Barneby

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ABSTRACT

Senna pendula seeds dormancy is caused by impermeability of the seed coat to water. In order to determine the best methodology to overcome dormancy and to perform a morphological characterization, two batches of seeds were submitted to the following treatments: a) mechanical scarification of the seed coat; b) immersion in hot water at 70 °C for 5, 10, 15 and 20 minutes; c) immersion in hot water at 90 °C for 5, 10, 15 and 20 minutes; d) sulfuric acid concentrate for 5, 10, 15 and 20 minutes; e) no treatment. The experiment was conducted in laboratory using a completely randomized design, with four replications of 25 seeds each, with daily verification for 7 days. The results showed that the use of water does not overcome the dormancy, however mechanical and chemical scarifications were the most effective treatments, with germination percentage of up to 94%.

Keywords: germination, tegument, vigor.

1. INTRODUCTION

Seeds originated by the sexual reproduction of plants promote genetic variability of the population, and their dispersion provides the conquest of new environments, to the extent of the success of germination. The blocking of the germination process of intact and viable seeds, even in satisfying favorable environmental conditions, is called dormancy (Goudel et al., 2013). This is evolutionarily acquired mechanism is a promising adaptation for survival of the species to harsh environments and in long term (Hilhorst, 2007). The germination impediment is very common in the botanical family Fabaceae and in species of the pioneers' ecological group (Bewley & Black, 1994). Physical dormancy is the most common in this species (Perez, 2004; Pereira & Jacobi, 2014).

Many species of the Fabaceae family present dormancy because of the hardness of the integument, and thus are waterproof. In the genus *Senna* Mill., composed of 260 species (Rodrigues et al., 2005), many species have dormant seeds, as *Senna occidentalis* (L.) Linck. Handb.), *Senna siamea* (Lam.) H. S. Irwin and Barneby and *Senna spectabilis* (DC.) Irwin and Barneby (Floriano, 2004; Topanotti et al., 2014).

Dormancy, although important in ecological terms, is one of the great difficulties in the sexual propagation process, because it hinders the uniform production of seedlings (Dutra et al., 2015). Therefore, several studies have been developed aiming to improve techniques for overcoming seed dormancy in this family (Alves et al., 2004; Lopes et al., 2012; Pereira et al., 2014).

Physical dormancy is established when the seed coat, impermeable to water and gases, is the main modulator in the interaction between the internal tissues of the seed and the environment (Hilhorst, 2007). In some species, on the ripening stage, the seeds can be coated with suberin and/or lipids, making them waterproof. The impermeability is normally associated with the presence of one or more layers of palisade cells with lignified thick wall, mucilage in the testa and serous cuticle (Baskin & Baskin, 2004; Perez, 2004). This barrier formed by the palisade cells in the seeds of Fabaceae is a hereditary feature and causes a physical blocking, preventing both imbibition as the oxygenation of the embryo. In this sense, there are many methods to overcome dormancy in species of this family, among which mechanical scarification, chemical and immersion in hot water stand out, all of them aiming to overcome the integument impermeability (Agra et al., 2015).

The demand for studies of morphophysiological characteristics of native forest species is growing because of their economic and environmental potential use. Among the species of native Fabaceae is *Senna pendula* (Willd.) HS Irwin & Barneby, a pioneer species, found mainly in the Atlantic Forest (Souza & Bortoluzzi, 2016), widely used in reforestation and landscaping due to its yellow and showy flowers (Alves & Sartori, 2009).

In one of the few studies on the species, Alzugaray et al. (2007) recommends chemical treatment with sulfuric acid for 10 minutes to overcome dormancy. However, there is still little information on dormancy breaking, germination and storage processes that can enable production of more efficient and uniform seedlings.

Because of their environmental and landscaping potential and therefore, great interest in the nursery production of viable seedlings, it is important to better standardize their germination process. From this observation, this study aimed to morphologically characterize the *S. pendula* seeds, and evaluate the effectiveness of various treatments to overcome dormancy in two batches of seeds of different ages.

2. MATERIAL AND METHODS

The work was carried out at the Seed Laboratory and Forest Ecophysiology of the Federal University of Espirito Santo (UFES) in Vitoria - ES.

Two batches of *Senna pendula* seeds were collected in 2011 and 2014 from five trees located in natural areas of the city of Piracicaba -SP. After collection, the seeds were taken to the Institute of Forestry Research and Studies (IPEF) also in Piracicaba (SP) and stored in a cold chamber at 10 °C and 50% humidity.

2.1. Morphological characterization

For the morphological analysis, the length and width of seeds were obtained with the aid of a digital caliper rule using four replications of 25 seeds each, and the results were expressed in mm.

To determine the weight of a thousand seeds, eight replications of 100 seeds each were used, following the established by Brasil (2009).

2.2. Seed moisture content

The moisture content of the seeds for the physical evaluation was determined. For this determination, we used three replications of 5 grams of seeds, which were weighed and submitted to an oven at 105 ° C ± 3 °C for 24 hours (Brasil, 2009).

2.3. Imbibition curve

For the study of imbibition, we used 3 sub-samples of 10 intact and 10 mechanically scarified seeds for 30 seconds in the opposite side of the embryo. They were placed in Petri dishes with two sheets of filter paper moistened with distilled water in the proportion of three times its dry weight. Each subsample was weighed each hour for 10 hours in a scale with accuracy of 0.001 g (Kern, KB 360-3N).

2.4. Dormancy breaking

Four replications of 25 seeds were submitted to the following treatments to overcome dormancy: a) mechanical scarification of the seed coat in the opposite part of the embryo, with sandpaper Nº. 40 for 30 seconds; b) immersion in hot water at 70 °C for 5, 10, 15 and 20 minutes; c) soaking in hot water at 90 °C for 5, 10, 15 and 20 minutes; d) immersion in concentrate sulfuric acid (H_2SO_4) for 5, 10, 15 and 20 minutes, followed by washing with running water; and e) seeds without treatment (control).

The seeds were then disinfected with sodium hypochlorite 2% for 2 minutes and placed in Petri dishes with two sheets of filter paper moistened with distilled water in the proportion of three times its dry weight and placed in BOD germination chamber, at constant temperature of 23 °C and constant light for a seven days period (Ferreira & Borghetti, 2004). The parameters analyzed were the germination percentage (G), relative frequency (RF) and the germination speed index (GSI).

The germination percentage (%G) was calculated according to the number of normal seedlings, following the established by Brasil (2009).

The relative frequency was calculated using the daily germination count as Labouriau & Valadares (1976) (Equation 1),

$$Fr = ni / \sum ni \quad (1)$$

where Fr = relative frequency; ni = number of germinated seeds per day; and Σni = number of germinated seeds.

The GSI was determined according to the methodology of Maguire (1962) (Equation 2),

$$GSI = \frac{G1}{T1} + \frac{G2}{T2} + \ldots + \frac{Gi}{Ti} \quad (2)$$

wherein GRI = germination rate index; G1 to Gi = number of germinated seeds occurred every day; and T1 to Ti = time of evaluation days.

2.5. Anatomical test

The seeds were fixed in acetic alcohol Formalin-acid (FAA) 70% and then stored in 70% alcohol. Later they were dehydrated using increasing alcoholic series, embedded in historesin (Leicester, Germany) and sectioned (10-12μm thick) using rotary microtome (Jung AG Heidelberg) (Johansen, 1940).

The statistical design was completely randomized with four replications of 25 seeds each. When necessary data were expressed in percentage were transformed into "arc sin ($\sqrt{x}$ / 100)", to meet the normality according Lilliefors and homogeneity of variances by Cochran (Banzatto & Kronka, 2006; Ribeiro, 2012); however, only the original data are shown. Data were submitted to analysis of variance (ANOVA), and the averages compared by Tukey test at 5% ($p < 0.05$) probability using the Assistat 7.7 software.

3. RESULTS AND DISCUSSION

Mature seeds of *Senna pendula* have flat and smooth shape and bright brown color. The morphometric analysis of seeds is found in Table 1.

The cross-section reveals a thick seed cutaneous layer separating the embryo from the external environment. It can be noted a double epidermal layer with the presence of macrosclereids in the underlying band, in contact with the mesophyll (Figure 1).

We observed, by seeds imbibition curves of both batches of *S. pendula*, a rapid imbibition and consequent increase in weight throughout the experiment (Figure 2). The same was not observed in intact seeds, which confirms the existence of a physical dormancy, thus preventing the absorption of water.

Table 1. Physical characteristics of S. pendula seeds.

Parameters	Batch 2014	Batch 2011
Weight of 1000 seeds (g)	27.7	25.6
Length (mm)	5.5	5.3
Width (mm)	3.2	3.3
Moisture Percentage	13.8%	10.1%

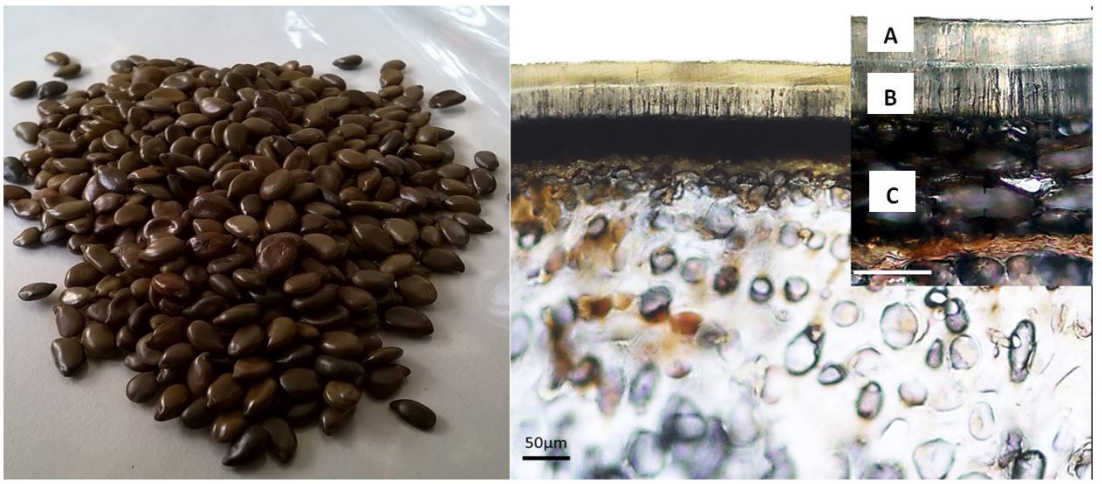

Figure 1. Characterization of Senna pendula seed: Seed overview and Histological cross section. (A) Epidermis; (B) Epidermis with the presence of macrosclereids; (C) Mesophyll.

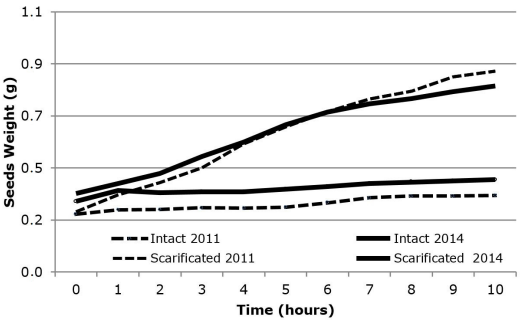

Figure 2. S. pendula seed imbibition curve intact and mechanically scarified with sandpaper.

In general, the untreated seeds, used as a control, did not show satisfactory germination percentage (Table 2), with maximum values of 10 and 12%, for batches 2011 and 2014, respectively.

Dormancy established in the seed tends to be attenuated with time, either in the environment or storage (Fowler & Bianchetti, 2000). Studies with sunflower show dormancy weakening with 80% germination percentage after 2 years of storage (Maeda et al., 1987). The same was observed with seeds of *Ilex paraguariensis* (Yerba mate) and the genus *Magnolia* (Floriano, 2004).

Storage may have been responsible for a decline in the viability of the *S. pendula* seeds, as observed in Table 2. Even with the best treatments for breaking dormancy, in which the germination rates were above 80% in batch 2014, the germination rate remained below 50% in the 2011 batch. The storage conditions can accelerate the deterioration processes in seeds (Marcos, 2005; Silva et al., 2010) and this may have been one of the factors for the decrease in the viability of seeds of 2011 batch. The deterioration is evidenced by genetic damage, loss of integrity of cell membranes, changes in respiratory activity and enzyme activity of seeds, which may cause delay in germination, uneven emergence and finally death of seeds (Santos et al., 2004).

Garcia (2013) worked with two batches of *Senna rugosa,* one new and other artificially aged, and also observed a decrease in the percentage of germination, with a significant difference between samples. In another study analyzing the deterioration suffered by the seeds over time, Ferreira et al. (2004) observed sharp decline of viability in *S. macranthera* seeds.

Table 2. Germination percentage of different batches of S. pendula seeds under different dormancy breaking treatments.

Treatments	Batch 2011	Batch 2014
Control	10 A e	12 A g
Sandpaper n. 40 30”	49 A a	94 Ba
Water 70 °C 5’	17 A d	22 A f
Water 70 °C 10’	20 A d	49 B e
Water 70 °C 15’	19 A d	52 B e
Water 70 °C 20’	20 A d	52 B e
Water 90 °C 5’	11 A e	49 B e
Water 90 °C 10’	0.0 A f	19 B f
Water 90 °C 15’	0.0 A f	3 A h
Water 90 °C 20’	0.0 A f	4 A h
H_2SO_4 5’	44 A ab	80 Bd
H_2SO_4 10’	39 Abc	91 Bab
H_2SO_4 15’	37 Ac	88 B bc
H_2SO_4 20’	39 A bc	85 Bcd

Means followed by the same capital letter in line and lowercase letters in the column do not differ (5% Tukey test). ” = Seconds; ’ = Minutes.

For *S. pendula* seeds, the treatment with most promising result was scarification using sandpaper # 40 for 0.5 min, at which it was obtained 94% germination (seed batch of 2014), followed by immersion in sulfuric acid for 10 minutes, at which 91% of germinated seeds was obtained in the 2014 batch (Table 2). Treatment of scarification with sandpaper for 0.5 minute was the most promising for both 2011 and 2014 batches. The second best batchesresult for both batches was chemical scarification with sulfuric acid. However, the seeds of 2011 batch did not support as well the 10 minutes of exposure as the 2014 batch and the best results for the older batch occurred when subjected to acid for 5 minutes.

Santarém & Aquila (1995) and Lopes et al. (2012) obtained similar results with *Senna macranthera* seeds, scarificated in the opposite area of the embryonic axis, resulting in the highest percentage of germination in relation to other treatments to overcome dormancy. However, Lopes et al. (2012) recommends the use of Nº 100 sandpaper, but without establishing for how long.

Despite the good results obtained to overcome seed dormancy of *S. pendula* with treatments by mechanical scarification with sandpaper and chemical with acid, the impact of the seed loss of viability due to deterioration over time is outstanding. This fact is evidenced when comparing the percentage of seed germination between the two batches studied (stored for 1 year and 4 years), subjected to the same treatments to overcome dormancy. The results obtained with chemical and mechanical scarification demonstrate the success of these methods in seeds with physical dormancy (integument). However, when subjected to hot water, we observed that the increase in temperature to 90 °C caused severe damage to the embryo, resulting in the death of seeds from 10 minutes exposure to 2011 batch and 15 minutes to the 2014 batch. *Bowdichia virgilioides* seeds also showed impaired germination when subjected to hot water at 80 °C (Albuquerque et al., 2007). These results show that, although the boiling water softens the outside of the seed and increase fluid absorption capacity, it can also denature both tegument as embryo proteins, affecting the viability and integrity of this proteins of seed tissues (Guedes et al., 2013).

The exposition to water 70 °C did not cause damage to *S. pendula* seeds, but there was no significant increase in germination (Table 2). Although soaking in hot water is considered a practical method to overcome dormancy (Santarém & Aquila, 1995), including seeds of *Senna obtusifolia* (Topanotti et al., 2014), its effectiveness could not be confirmed for *Senna pendula*.

The results observed for the two batches showed the decrease in the germination percentage and seed vigor from the 2011 batch (four years in cold chamber). Although the vigor is determined primarily by genetic component, thus species-specific, post-harvest handling and environmental conditions are relevant (Flávio, 2014). For the species *Calliandra foliolosa* Benth., another

Fabaceae, stored in cold chamber, at 5 °C, the germination percentage was maintained, as well as the vigor, even after 4 years of storage (Calil et al., 2008). For *Senna* seeds, stored in the same temperature conditions in cold chamber, we observed that in only 240 days there was loss in the vigor and the GSI values were reduced (Rodrigues, 2013). However, in *S. macranthera* seeds also kept at 5 °C, but coated with polymeric blanket, it was not observed a sharp decrease in germination rate and viability after 380 days (Pozitano, 2011).

The conditions and the storage period influenced the seed vigor of *S. pendula,* despite being considered an orthodox species. This can also be seen in the treatment with hot water at 90 °C for both batches, which, with vigor decrease, did not support the thermal stress, not reaching satisfactory germination percentages(<50%).

The temperature and relative humidity have major role to ensure the longevity and viability of seeds during storage (Pinho et al., 2009), and a rise in the values of these two factors may increase the seeds deterioration (Garcia et al., 2014).

Thus, it is estimated that, even being a pioneer species with physical dormancy, which would ensure a long period of longevity (Carvalho et al., 2006), the *S. pendula* seeds have demonstrated its viability affected over four years of storage, under the conditions in which they were stored (10 °C/50% RH). It is suggested, for this species, storage at a lower humidity and temperatures around 5 °C, as established by several authors, including Fabaceae species (Borges et al., 2009; Aguiar et al., 2010).

The treatments with higher germination percentage (mechanical scarification with sandpaper and H_2SO_4 for 10 minutes) (Table 2) showed GSI equal to 24.85 and 28.15 respectively for the 2014 batch (Table 3), demonstrating that these methods, by causing the weakening of the seed coat, enable immersion and subsequent germination. These values show a significant difference when compared with the Batch of 2011. This decrease in GSI supports the Smaniotto et al. (2014) who reported decrease in the viability and GSI of soybeans after 6 months of storage, as a result of natural deterioration process of seeds.

Souza et al. (2005), in their work with seed *Tabebuia serratifolia* also observed along the storage for six months the loss of force and decrease of the GSI. The same could be observed in *Dalbergia nigra* seeds stored for 2 years in cold chamber (Aguiar et al., 2010).

The seeds show a growing trend of GSI when subjected to hot water (70 °C) for up to 15 minutes. Similar results were presented by Lopes et al. (2012) with *S. macranthera* seeds submitted to the same temperature for up to 10 minutes. When exposed to water at 90 °C, the results of this study were similar to that of Lopes, noting reduction in GSI values at all time intervals.

Germination started on the third day, ending at the sixth. We observed differences in the germination peaks

Table 3. Germination Speed Index (GSI) of different batches of seeds of S. pendula under different dormancy breaking treatments.

Treatments	Batch 2011	Batch 2014
Control	2.02A a	2.31B b
Sandpaper n. 40 for 0.5'	9.90A d	24.85B c
Water 70 °C for 5'	4.26A b	4.89B d
Water 70 °C for 10'	4.57A c	12.43B e
Water 70 °C for 1'	4.53Ac	15.36B f
Water 70 °C for 20'	4.29A b	14.2B g
Water 90 °C for 5'	2.35A	12.76B
Water 90 °C for 10'	-	4.75 d
Water 90 °C for 15'	-	0.9 j
Water 90 °C for 20'	-	0.86 j
H_2SO_4 for 5'	10.12A d	23.7B h
H_2SO_4 for 10'	9.39A e	28.15B i
H_2SO_4 for 15'	10.46A f	28.56B a
H_2SO_4 for 20'	10.96A g	28.73B a

Means followed by the same capital letter in line and lowercase in the column do not differ (5% Tukey test). ' = Minutes.

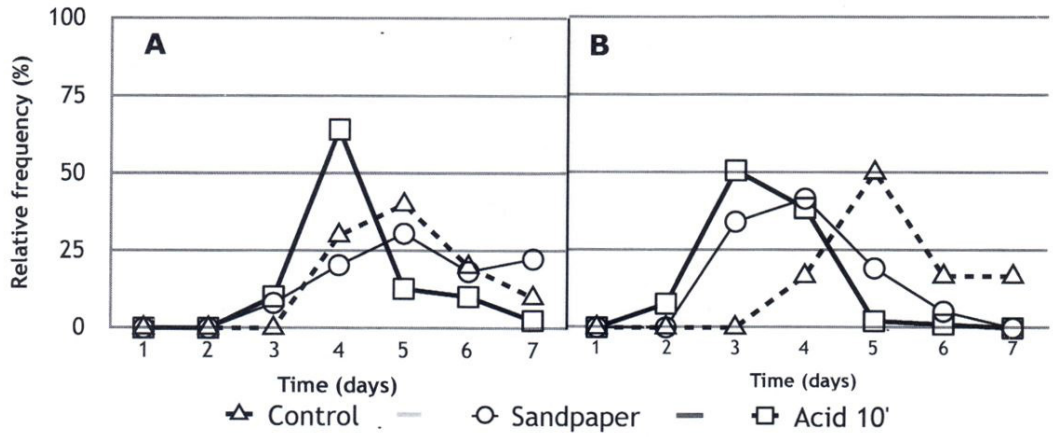

Figure 3. Relative frequency of S. pendula seed germination after a few treatments: (A) Batch 2011; (B) Batch 2014.

in the relative frequencies of the treatments, which had better germination percentage (acid 10' and sandpaper) when compared to control. Thus, the highest number of germinated seeds was observed at the fifth day. In the others treatments this value was verified between the third and fourth days, having reached in the latter about 60% in the treatment with H_2SO_4 for 10 minutes (Figure 3). Both in the treatment with acid as in the mechanical scarification of the seed, the germination was faster and uniform. These results are in agreement with Menezes Silva et al. (2011) who submitted seeds of *S. virgata*, other species from Fabaceae family, which is pioneer and dormant, to the same chemical and mechanical treatments, and verified similar germination rate, but the chemical scarification has noticed slight non-uniformity after the first germination peak.

The comparison of the two batches showed again a higher speed in the germination of seeds treated with acid and sandpaper. To the 2014 batch it was established a germination peak 1 day prior to the 2011 batch. This can be explained by the higher vigor of the newer batch of seeds, thereby ensuring a faster metabolic reactivation as well as more efficient development of the plants (Oliveira et al., 2015).

In the natural environment, mainly due to dormancy, it is expected a higher heterogeneity of germination with distribution spread over time, presenting an adaptive advantage. However, determining what plant constitutes an ecological advantage is a disadvantage for growers who seek greater uniformity of germination because the logistics of marketing, transportation and planting (Bortolini et al., 2011).

Although the use of acid has been indicated by Alzugaray et al. (2007) and is considered more practical than the sandpaper to large scale (Oliveira et al., 2003), such method can be dangerous and unfeasible financially and ecologically because of its high commercial value and residues effect to the water (Lin, 1999). Thus, the scarification with sandpaper is the safest and most efficient method (environmentally, sustainable and cheap) for breaking dormancy in *S. pendula* seeds.

4. CONCLUSIONS

The *S. pendula* seeds present a physical barrier that prevents imbibition during its return to growth, so characterized as dormant.

The hot water treatment, both as 70 °C to 90 °C was not feasible to overcome the dormancy. Although the treatment with sulfuric acid was an effective and practical way to overcome the dormancy, it can be dangerous and unfeasible financially and ecologically. Therefore, the mechanical scarification for 0.5 min is the suggested treatment as efficient in terms of cost benefit to overcome dormancy of *S. pendula.*

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