Colegio de Postgraduados

Germination and morphology of *Sapindus saponaria* L. (Sapindaceae) seeds for their sustainable use

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ABSTRACT

Objective: To characterize morphological traits and to evaluate different pre-germinative treatments to break the physical dormancy of *Sapindus saponaria* L. seeds.

Design/methodology/approach: Seeds of *S. saponaria* were collected in the municipality of Cintalapa de Figueroa, Chiapas. Seed length, seed width and seed coat width were measured. The weight of 100 seeds, germination percentage, and purity were assessed. The following pre-germinative treatments were established: (T1) Hot water (80 °C) until room temperature; (T2) Hot water (80 °C) until room temperature followed by cold water shock (14 °C) until room temperature; (T3) Mechanical scarification; (T4) Mechanical scarification plus soaking in water for 24 hours (at room temperature); and (T5) Control. A completely randomized experimental design with four replications was used.

Results: This study found that *S. saponaria* seeds average 10.3 mm in length, 10.2 mm in width, and have a seed coat thickness of 1.2 mm. Mechanical scarification yielded the highest germination percentage (23.3%). This finding is significant given that the untreated germination percentage in this species is moderate to low. However, this result is lower than percentages documented in studies using chemical treatments.

Limitations on study/implications: Despite the low germination percentage, the advantages of the treatments implemented in this study is that they are cost-effective.

Findings/conclusions: It is necessary to expand assessments of pre-germinative treatments, both locally and regionally, to develop effective strategies for the sustainable management of *Sapindus saponaria* while harnessing its economic and medicinal potential.

Keywords: Conservation, dormancy, forest germplasm, impermeable seed coat, pre-germinative treatments.

Citation: Domínguez-Liévano, A., Hernández-Esquivel, K. B., Basave-Villalobos, E., Barrios-Calderón, R de J. (2025). Germination and morphology of *Sapindus saponaria* L. (Sapindaceae) seeds for their sustainable use. *Agro Productividad*. https://doi.org/10.32854/nw0ejg41

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: June 17, 2025.
Accepted: September 3, 2025.
Published on-line: September XX, 2025.

Agro Productividad, 18(9) supplement. September. 2025. pp: 157-164.

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INTRODUCTION

Sapindus saponaria L., commonly known as "jaboncillo" or "soapberry," is a tree species of great economic, ecological, and cultural importance in various regions of Latin America. It is distributed in Florida, the Greater Antilles, and from northern Mexico to southern Argentina. In Mexico, it occurs in tropical deciduous and sub-deciduous areas, in cloud forests, and occasionally in oak forests. The tannins and saponins derived from

S. saponaria are used as bioinsecticides, which exhibit low toxicity, degrade rapidly, and do not induce resistance (Souza *et al.*, 2023). Ecologically, this species has been used in landscaping projects and in the restoration of degraded areas (Albiero *et al.*, 2001; Sánchez and Silva, 2008). Additionally, it also has multiple traditional uses and potential industrial applications (Jozivan *et al.*, 2008; Rodríguez-Hernández *et al.*, 2016).

Studies at seed level are necessary to support the conservation of the species and its propagation, either inside or beyond its natural habitat (Lohbeck *et al.*, 2015). Seeds present several characteristics that strongly influence dispersal patterns, colonization, seedling establishment, and plant survival (Dalling, 2002). Traits such as size, shape, color, and texture can significantly influence processes like germination and seedling establishment (Luck *et al.*, 2012; Romero-Saritama and Castillo, 2021). Variation in these characteristics is commonly observed in many tropical tree species, both between and within populations; however, seed traits can also vary within a single genotype (Cohen *et al.*, 1991; Kröber *et al.*, 2012; Duncan *et al.*, 2019; Romero-Saritama and Granda, 2020; Basave-Villalobos *et al.*, 2024). Such variability, results from the combined influence of environmental and genetic factors during seed development (Upretee *et al.*, 2024). Therefore, it is essential to examine how seed traits vary in each seed source and how these variations affect germination, since the condition of the seeds themselves is one of the most critical factors governing the germination process (Shen and Cho, 2020).

Recognizing the factors that affect seed germination is particularly important for species with dormancy, such as *S. saponaria*, because the seed's morphological and anatomical characteristics regulate the development and expression of mechanisms that inhibit germination (Upretee *et al.*, 2024). *Sapindus saponaria* seeds exhibit physical dormancy, which hinders germination (Nascimento *et al.* 2009; Cabral *et al.*, 2019). In this type of dormancy, the tissues surrounding the embryo (seed coats) can interfere with water absorption or gas exchange, preventing the release of inhibitors from the embryo, or act as a mechanical barrier to radicle emergence (Bewley *et al.*, 2013). To overcome this dormancy and promote germination, scarification treatments, usually referred as "pre-germinative" treatments, are required to soften, remove, break or abrade the seed coats by applying methods of mechanical or chemical scarification (Baskin and Baskin, 2014). However, the effectiveness of each treatment depends on the specific characteristics of the seeds (above mentioned), making it essential to carefully apply and evaluate these methods, as proposed by Ramírez-Herrera *et al.* (2008).

In summary, understanding and assessing the morphological traits of seeds and germination protocols is essential for sustainable *ex situ* propagation efforts and for developing specific strategies in the management of native tropical forest species, while also preserving their genetic diversity —an important factor in conserving local populations (Jozivan *et al.*, 2008; Rodríguez-Hernández *et al.*, 2016). The aim of the present research was to describe the morphological traits and to test pre-germinative treatments used to break seed dormancy in *Sapindus saponaria*. Additionally, this study seeks to examine how knowledge of seed morphological traits can serve as a valuable tool to optimize the *ex-situ* propagation process for this tropical species and thereby contribute to its sustainable use.

MATERIALS AND METHODS

Collection site and plant material

Seeds were collected in the municipality of Cintalapa de Figueroa, Chiapas, Mexico (421538.60 UTM X, 1847315.60 UTM Y), at an elevation of 534 m. The seed lot evaluated was obtained directly from the tree canopy in June 2023, when seeds were observed on the ground beneath the dripline area. After collection, laboratory tests were carried out using randomly selected samples in each case. A backup herbarium specimen (ADL01) was collected, pressed, and deposited at the Herbario ECOSUR Tapachula (ECO-TA-H).

Seed morphological characterization and yield variables

Twenty-five seeds in four replicates were used to measure seed coat thickness (mm), seed length (mm), and seed width (mm) with a digital caliper (Figure 1). Following the rules of the International Seed Testing Association (ISTA, 2010), a homogeneous sample size was used. One hundred seeds were weighed on a digital scale (0.1 g precision) in four replicates. The following weights were recorded: (a) weight of 100 uncleaned fruits, (b) weight of 100 cleaned fruits, and (c) weight of 100 clean seeds without exocarp. Inert material was then removed from the seeds with forceps to obtain pure seeds. The percentage of pure seeds was calculated with the following formula:

$$\%Purity = \left(Weight\ of\ pure\ seeds \,/\, Weight\ of\ the\ sample\right) \times 100$$

Germination

After collection, seeds were disinfected with 2% sodium hypochlorite for five minutes and then rinsed with tap water. Germination percentage was evaluated under five different pre-germinative conditions: (T1) seeds were immersed in hot water at 80 °C until they reached room temperature; (T2) the same process was followed, but a subsequent cold-water shock at 14 °C was applied until room temperature; (T3) the seeds were cracked (mechanical scarification); (T4) seeds were also cracked and then soaked in water for 24 hours at room temperature. (T5) control, in which no treatment was applied. The germination test was carried out on paper according to Agustín-Sandoval *et al*. (2017), with four replicates of 30 seeds each. Seeds were placed on paper towels, labeled, and then kept in plastic bags at room temperature, ensuring the paper remained adequately moistened. Evaluations were made three times per week until germination.

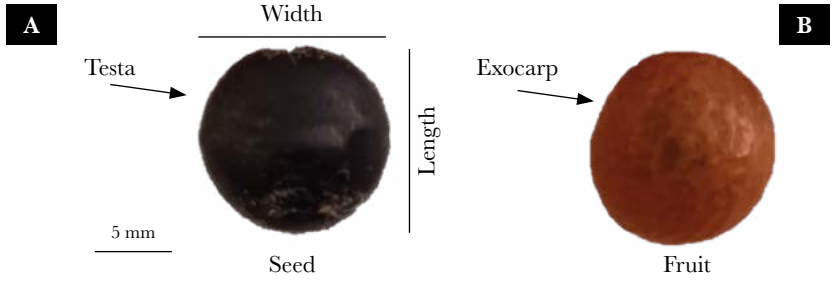

Figure 1. Representation of three measured traits to determine the size of *Sapindus saponaria* L. seeds. A) Seed without protective coating: length, width, and thickness of the seeds; and B) Fruit before removing exocarp.

Statistical analysis

Germination percentage data (p) were transformed using the arcsine square-root function to approximate a normal distribution (Sokal and Rohlf, 1981). A completely randomized design with four replicates per treatment was used. Tests of normality (Shapiro-Wilk) and homogeneity of variances (Bartlett) were applied, followed by analysis of variance (ANOVA) and Tukey's multiple comparison tests at a significance level of $p<0.05$. Data were analyzed using R statistical software for Windows v3.6.3 (R Core Team, 2020).

RESULTS AND DISCUSSION

Study species

Trees from 3 up to 25 m tall; to 18 cm diameter to breast height, bark gray. Leaves pinnate from 10 up to 57 cm. Leaflets 5 to 10, lanceolate to oblong, unequal; petioles winged or wingless. Inflorescence a terminal panicle, from 15 to 25 cm long, the branches and pedicels densely puberulent to tomentose. Flowers white; sepals elliptic, puberulent; petals ciliolate, glabrous; disc fleshy spreading; stamens exserted, filaments filiform; ovary ovate, 3-lobed, glabrous. Indehiscent fruits of 1 to 3 cocci; cocci brown to yellow, globose, ca. 1.5 cm of diameter, shiny, often lenticellate at maturity, the surface smooth; seeds globose, 1.2 cm of diameter, set at the base of the carpel in a cottony mat pubescence It grows from 0 to 2000 m.a.s.l., flowering and fruiting all year round (WFO, 2025).

Morphology and yield

According to the measurements, the seeds are as long as they are wide, and the seed coat is relatively thick (Table 1). These findings are consistent with those reported by das Neves *et al.* (2018), who also found similar values for length and width. Bonilla *et al.* (2007) reported an average seed length of 10 mm and a width of 8 mm, the latter being lower than the value obtained in the present study.

The average weight of 100 seeds was 144±0.96 grams for uncleaned fruits; 140±1.29 grams for cleaned fruits; and 76±1.26 grams for cleaned seeds without exocarp (Table 1; Figure 1). The weight of 100 pure seeds (cleaned and without exocarp) is slightly higher in this study than that reported by das Neves *et al.* (2012) (67.2 g/100); these authors mention that variation in seed weight may be because environmental changes during seed development. It was found that *S. saponaria* seeds exhibit a purity level of 97.2%,

Table 1. Average weight and size of morphological and yield-related variables of *Sapindus saponaria* L. seeds.

Variables	Mean±SD
Seed coat thickness (mm)	1.2±0.10
Seed length (mm)	10.3±0.08
Seed width (mm)	10.2±0.06
Weight of 100 uncleaned fruits (g)	144±0.96
Weight of 100 cleaned fruits (g)	140±1.29
Weight of 100 cleaned seeds without exocarp (g)	76±1.26

SD=Standard error.

with the remaining 2.8% consisting of residual fruit material. Although *S. saponaria* seeds are relatively easy to clean, the fruit's exocarp has an oily and flexible consistency, which hinders its removal (Figure 1).

Germination

The experiment revealed that *S. saponaria* seeds began to germinate 13 days after the start of the trial. Germination ceased after seven weeks. Hernández-Jaramillo *et al.* (2012) reported a similar germination onset of approximately 12 days, whereas Bonilla *et al.* (2007) documented a broader range, from as early as 5 days to up to 2 months. The results of this study show significant differences in seed germination among the pre-germinative treatments ($p<0.001$) (Figure 2). Manual scarification by cracking the seed coat (T3) proved to be the most effective treatment for enhancing germination (23.3%), followed by T4, which consisted of cracking followed by a 24-hour water soak (13.3%). These findings are consistent with those of Cuitláhuac (2017), who reported a 25% emergence rate after soaking seeds in water for 10 minutes. In contrast, Lima-Diniz *et al.* (2018) found a 74% germination rate using manual scarification with sandpaper (No. 100), while Oliveira *et al.* (2012) reported a 65% emergence rate using sulfuric acid as a pre-germinative treatment. The control treatment (T5) showed a 0.0% germination rate. This may be due to the presence of a narrow micropyle and micropyle canal in the seeds, which potentially hinders the imbibition of internal tissues or storage reserves (Bonilla *et al.*, 2007). Some studies have shown that sulfuric acid treatments can enhance germination rates in seeds with impermeable coats, although they may also cause damage (Santarém and Áquila, 1995; Albiero *et al.*, 2001; Mendes, 2012; Oliveira *et al.*, 2012). Nascimento *et al.* (2009) and Cabral *et al.* (2019) reported that *S. saponaria* seeds possess a very thick and sclerenchymatous seed coat, and that prolonged exposure to sulfuric acid (60 to 90 minutes) may increase germination percentage, although it may also lead to seed damage.

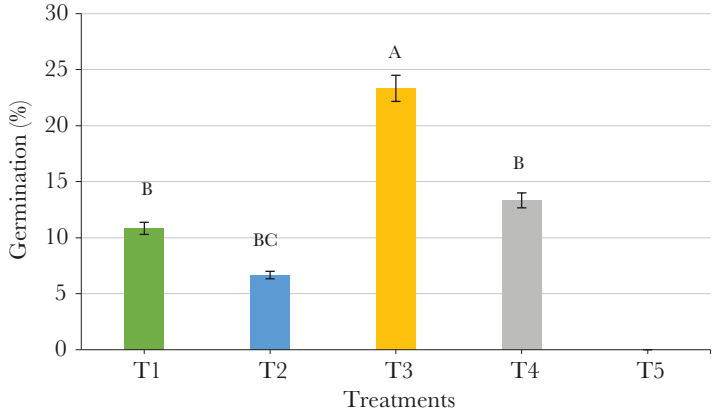

Figure 2. Germination percentage of *Sapindus saponaria* L. seeds under the established treatments: (T1) Hot water until room temperature (80 °C); (T2) Hot water until room temperature (80 °C) followed by thermal shock with cold water (14 °C) until room temperature; (T3) Mechanical cracking; (T4) Mechanical cracking followed by soaking in water for 24 h (room temperature); (T5) Control. Different uppercase letters indicate significant differences ($P<0.05$) among treatments.

CONCLUSIONS

This study found that *S. saponaria* seeds are spherical and possess a thick seed coat. Mechanical scarification through seed cracking was observed to be effective in overcoming seed coat hardness. In spite that this treatment yielded a germination rate of less than 50%, it is an inexpensive and simple technique to accelerate germination and can be implemented by any person who wishes to reproduce the seeds, only caution is needed when performing cracks to do not damage the embryo. Understanding the dynamics of natural plant community regeneration largely depends on knowledge of seed germination processes and the application of pre-germinative treatments to break seed dormancy. Nevertheless, in the southern region of Chiapas State, as well as in many other tropical areas, little is still known about the biology of numerous native species, including *Sapindus saponaria*. This knowledge gap is closely linked to the limited availability of species proposed for ecological restoration programs in disturbed areas, particularly those with economic potential. Furthermore, collecting significant quantities of seeds should be conducted under protocols aimed at minimizing the impact on the species' natural progeny, a process that requires time and careful implementation. Therefore, it is crucial to continue investigating and deepening our understanding on the reproduction of *S. saponaria* and other native tropical species. This will aid in developing effective strategies for the conservation and sustainable management of these species both in their natural habitats and in ex situ conservation settings, while also harnessing their economic and medicinal potential.

ACKNOLEDGEMENTS

ADL thanks to the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI), from Mexico, for the Doctoral scholarship received while performing this study (CVU 953615). KBHE thanks to SECIHTI for the Posdoctoral scholarship received while doing this study (CVU 435594).

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