

Germination of *Pinus maximartinezii* seeds from two relict populations: effects of morphological attributes, seed origin, and pre-germination treatments

José Leonardo García-Rodríguez, José A. Sigala, Homero Sarmiento-López, María Gabriela Ramírez-Valadez, Julio César Ríos-Saucedo, Erickson Basave-Villalobos*

Campo Experimental Valle del Guadiana, INIFAP. Carr. Durango-El Mezquital km 4.5, 34170 Durango, Durango, México

Abstract

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Pinus maximartinezii is an endemic and endangered Mexican pinyon pine highly restricted, only two relict populations are currently known. Effective conservation of this species requires the development of reliable seedling propagation protocols, but germination of *P. maximartinezii* seeds is problematic, likely due to water-impermeable seed coats. Methods to enhance germination are therefore necessary, yet this aspect remains poorly understood and understudied. This study aimed to evaluate the effects of mechanical and chemical scarification treatments on seed germination, linking treatment effectiveness with seed morphological traits and origin to identify, in turn, the influence of these factors. Provenances differed in seed size and seed coat thickness, thereby affecting water imbibition and germination dynamics. Seeds from Juchipila (Zacatecas) had thinner seed coats (1.4 mm) and imbibed water more rapidly ($0.10 \text{ g H}_2\text{O g}^{-1} \text{ h}^{-1}$), and germinated readily without any treatment (71.7%). In contrast, seeds from La Muralla (Durango) had slightly thicker coats (1.5 mm), imbibed more slowly ($0.07 \text{ g H}_2\text{O g}^{-1} \text{ h}^{-1}$) and required scarification to improve their relative low germination percentage (59.1%). Findings highlight the relevance of seed morphological traits and origin in defining effective pre-germination protocols for *P. maximartinezii*.

Keywords

conservation, endemism, forest germplasm, physical dormancy, scarification

Introduction

Among conifers recognized to date, the Pinaceae family stands out as one of the most important plant families worldwide. Within it, the genus *Pinus* is notable for its high species diversity and its ecological and economic significance across many regions. Mexico is renowned as a center of diversification for this genus, hosting 49 of the approximately 120 known species globally (GERNANDT and PÉREZ-DE LA ROSA, 2014), including several endemics such as *Pinus maximartinezii* Rzed., commonly known as blue pine. This species is not only endemic but also has

an extremely limited geographic distribution, confined to specific habitats and with small and isolated populations (LÓPEZ and GALVÁN, 2011). Only two relict populations are currently known: one in the municipality of Juchipila, in southern Zacatecas (RZEDOWSKI, 1964), and the other near La Muralla, in the indigenous territories of Santa María de Ocotán and Xoconoxtle, municipality of El Mezquital, Durango (GONZÁLEZ-ELIZONDO et al., 2011).

Early studies by PASSINI (1985) and more recently by GONZÁLEZ-ELIZONDO et al. (2011) reported that populations of *P. maximartinezii* comprise mainly long-lived individuals, aged between 220 and 250 years. This de-

*Corresponding author:

e-mail: basave.erickson@inifap.gob.mx

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mographic structure has negatively impacted the species' reproductive success. Like another pinyon pines such as *Pinus pinceana* Gordon (QUIROZ, 2015), *P. maximartinezii* exhibits low seed production in terms of quantity and viability, likely due to inbreeding. Furthermore, the species is under pressure from unregulated seed gathering for ornamental propagation and commercial use as pine nuts in national and international markets (GONZÁLEZ-ELIZONDO et al., 2011). As a result, *P. maximartinezii* is classified as endangered under the Mexican legislation (NOM-059-SEMARNAT-2010; SEMARNAT, 2010) and listed on the IUCN Red List of threatened plants (FARJON, 2013).

These challenges underscore the urgent need for conservation efforts of *P. maximartinezii*. One promising strategy is in situ conservation, which involves nursery propagation for reforestation or enrichment plantations. However, this is hindered by the species' low germination rates, attributed to the hard and thick seed coat, as well as the presence of water-repellent organic compounds, traits common in pinyon pines (LÓPEZ-MATA, 2001; RAMÍREZ-HERRERA et al., 2008; VALERO-GALVÁN et al., 2019). This germination barrier is a type of physical dormancy (BEWLEY et al., 2013), where seed coat structures prevent water uptake, gas exchange, or radicle emergence (BEWLEY, 1997; BEWLEY et al., 2013). Similar dormancy mechanisms have been observed in other pines, such as *Pinus bungeana* Zucc. ex Endl. (GUO et al., 2018) and *Pinus lambertiana* Dougl. (SHEN and CHO, 2021).

Seed coat impermeability, particularly of the testa, is often due to layers of palisade cells, including sclereid cells with lignified walls (BEWLEY et al., 2013; UPRETEE et al., 2024). For tree species, seed scarification is a common practice used to break physical dormancy, using mechanical or chemical methods such as abrasion, testa removal, or the application of substances like sulfuric acid or hydrogen peroxide to erode seed coat (BASKIN and BASKIN, 2014). While pre-germination treatments are well-established for many forest species, especially tree legumes, their effectiveness in seeds of *P. maximartinezii* is little known. Moreover, effective germination strategies must not only consider pre-germination treatments (as suggested by RAMÍREZ-HERRERA et al., 2008), but also ponder the influence of seed functional traits, such as size, weight, and coat thickness, which are known to vary by provenance and can affect germination capacity (HERNÁNDEZ-ANGUIANO et al., 2018; LUNA-CAVAZOS et al., 2024).

This approach is justified by the fact that seed morphological traits related to dormancy are shaped by both genetic and environmental factors acting on the maternal plant during seed development (UPRETEE et al., 2024). In the case of *P. maximartinezii*, it is therefore essential to assess how responses to pre-germination treatments can be affected by seed morphology traits, which in turn could be associated with seed origin. Understanding these relationships is key for developing more effective propagation protocols, crucial for future conservation efforts. In this context, we aimed to examine the morphological variation in seeds from the two relict populations of *P. maximartinezii* and to evaluate how these traits influence the ef-

fectiveness of mechanical and chemical pre-germination treatments.

Materials and methods

Germplasm characterization

Seeds of *P. maximartinezii* were obtained from the two relict populations: Juchipila (Zacatecas) and La Muralla (Durango). Germplasm collection was done in 2023 and seeds were storage at 5°C inside glass containers. For simplicity, the provenances were referred to as Juchipila and La Muralla, respectively, throughout the study. A sample of 500 seeds from each provenance was used for morphological characterization. Seed length (mm) and diameter (mm) were measured using a digital caliper (Truper® CALDI-6MP, Mexico). Diameter was measured along two perpendicular axes: 1) the maximum width at the seed's natural resting position (Fig. 1a), and 2) the lateral profile of the seed (Fig. 1b). Additionally, a subsample of 41 seeds per provenance was selected for seed coat measurements. Each seed was split longitudinally (Fig. 1c) using a mini-cutting disc (Dremel® 3001, Illinois, USA). Seed coat thickness (mm) was measured at four points on each half: two at the ends (tip and base) and two at the mid-points of the longitudinal walls (Fig. 1d). This resulted in a total of eight measurements per seed.

Additionally, the number of seeds per kilogram was estimated for each provenance. Five groups of 100 seeds were made per provenance and weighed using an analytical balance with a precision of 0.001 g. The average weight of 100 seeds per origin was extrapolated to the weight of 1,000 seeds. Based on this value, the average number of seeds per kilogram was determined (FAO, 1991). A seed purity analysis was also conducted. Two seed samples of 1,000 g each were separated by provenance. From each sample, the following components were isolated and weighted (g): clean seeds, empty seeds, and impurities. Seed purity was computed as follow: Purity (%) = [Weight of clean seeds / (Weight of clean seeds + Weight of impurities)] × 100.

Seed imbibition test

An imbibition test was conducted using five groups of 20 seeds from each provenance. The initial average dry weight of each group was recorded at time zero. Each group was then placed in a 100 mL beaker and fully submerged in distilled water. Wet weight measurements were taken at 6-hour intervals over a 24-hour period, resulting in four measurements per group. The amount of water absorbed per group ($\text{g H}_2\text{O g}^{-1}$) at each time point was calculated by subtracting the dry weight from the corresponding wet weight.

Pre-germination treatments

Pre-germination treatments were designed to break physi-

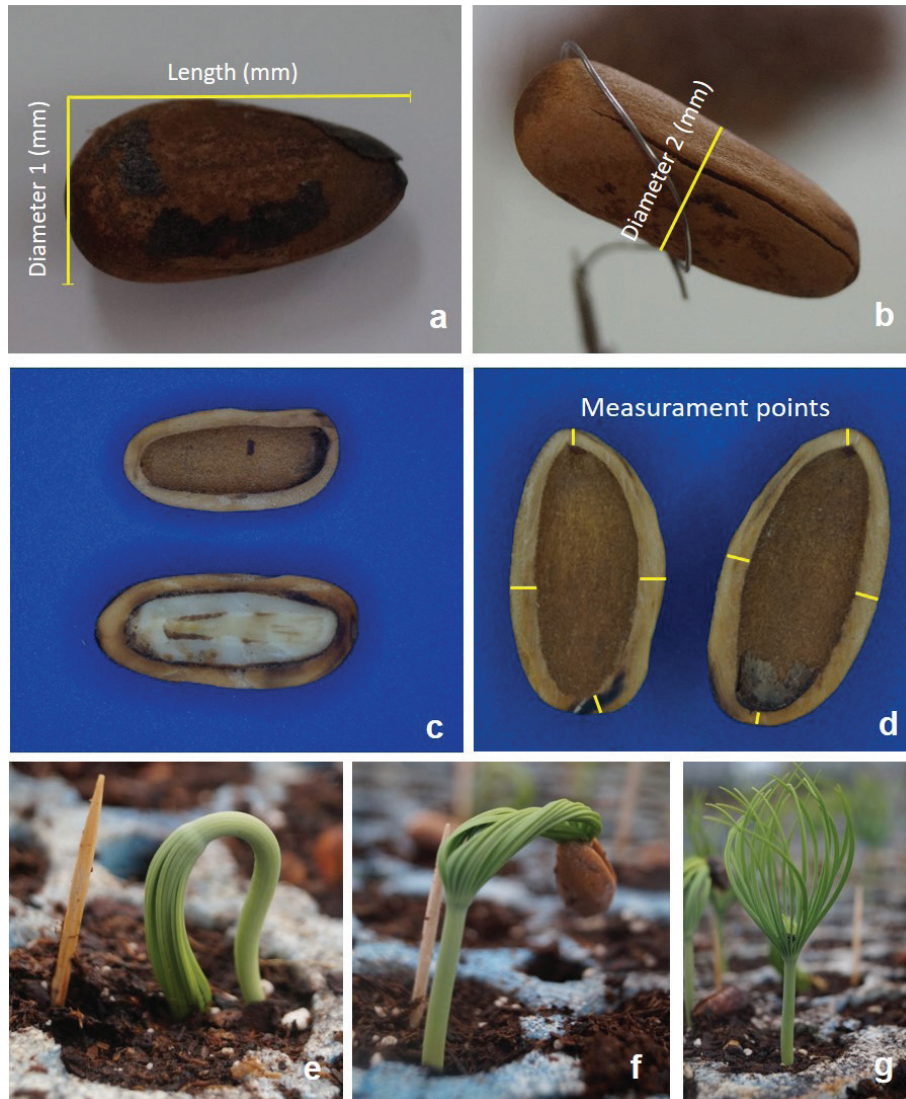


Fig. 1. *Pinus maximartinezii* seeds: (1) dissected seed with and without nut; (2) measurement points for seed coat thickness (mm); (3) seed length (mm) and diameter 1 (mm); (4) diameter 2; (5) seed considered germinated; (6) matchstick stage; and (7) seedling with cotyledonary leaves released from the seed coat and developing the epicotyl (shoot or apical bud).

cal dormancy through both mechanical and chemical seed scarification. A factorial experimental design was implemented, combining two levels of mechanical scarification: 1) sanding of the seed coat and 2) no sanding (control), with four chemical treatments: 1) immersion in distilled water for 24 hours, 2) immersion in 30% hydrogen peroxide (H_2O_2) with periodic agitation for 1 hour, 3) immersion in 97% sulfuric acid (H_2SO_4) with periodic agitation for 15 minutes, and 4) no immersion (control) (Table 1). All chemical treatments were applied at room temperature between 20–25°C. For the chemical scarification with acid, seeds from each provenance were placed in 2 L beakers and fully submerged in sulfuric acid, at which point the 15-minute treatment began. After the allotted time, the seeds were poured into a sieve positioned over another beaker to recover the acid. Once drained, they were immediately and thoroughly rinsed with potable water. Remaining surface moisture was removed with absorbent

paper, and the seeds were then sown directly into the containers. Mechanical scarification was done using a metal container lined with 80-grit metal sandpaper on the inner walls. Seeds were placed inside, the container was sealed and manually shaken with oscillating movements for 35 minutes to abrade the seed coat. After sanding, four groups from each provenance were separated and subjected to the chemical treatments individually. For each combination of mechanical and chemical scarification, four groups of 77 seeds were assigned. Following treatment, seeds were manually coated with 2 g of captan (Captan 50 Plus, ADAMA, Mexico) to prevent potential fungal infections during germination.

Sowing and germination monitoring

Seeds were sown in 77-cell expanded polystyrene trays (170 mL per cell) filled with a moistened substrate

Table 1. Pre-germination treatments involving mechanical and chemical seed scarification evaluated on *Pinus maximartinezii* seeds

Mechanical scarification	Chemical scarification treatments
No sanding (control)	No immersion (control); H ₂ O, H ₂ O ₂ ; H ₂ SO ₄
Sanding	No immersion (control); H ₂ O, H ₂ O ₂ ; H ₂ SO ₄

composed of peat moss (55%), composted pine bark (24%), and perlite (21%). One seed was placed vertically in each cell. Four trays were used per treatment. After sowing, the seeds were covered with a thin layer of the same substrate mixture and lightly watered. Trays were placed on benches inside a greenhouse covered with 720-gauge milky-white polyethylene film with UV protection. The average internal temperature was 22 °C and the relative humidity was 63%, with a natural photoperiod. The average photosynthetically active radiation (PAR) registered during the study was 835 $\mu\text{mol m}^{-2} \text{s}^{-1}$ inside the greenhouse, corresponding to a PAR transmittance of 44% relative to external PAR conditions.

Germination was monitored daily for 30 days after sowing. For this study, we considered a germinated seed to be one in which the hypocotyl had emerged from the substrate in a hook-shaped form (Fig. 1e). This criterion was based on the epigeal-emergence pattern of pine seeds, in which the cotyledons initially remain below the substrate surface, still attached to the seed coat. As the hypocotyl elongates, it gradually lifts the seed coat above the surface (Fig. 1f). In the following days, the seed coat eventually detaches, releasing the cotyledons and allowing the epicotyl or apical bud to begin development (Fig. 1g).

Throughout the evaluation period, manual watering was performed every other morning, ensuring the substrate remained at 60–70% moisture content. During germination observations, seed repositioning was also carried out as needed. This involved adjusting seeds whose radicles had protruded excessively from the substrate, a common characteristic in pinyon pines. Repositioning consisted of placing the seed with the micropyle facing downward to ensure the radicle remained properly embedded in the substrate.

Statistical analysis

Seed morphological variables were analyzed using descriptive statistics, and t-tests were performed to assess differences between the two provenances. Imbibition data were fitted to a monomolecular function: $y = A(1 - e^{-kt})$, where y is the amount of water absorbed per unit of initial seed weight, t is the imbibition time, A is the asymptote representing the maximum water uptake, and k is the rate constant. The latter indicates how quickly water absorption approaches equilibrium. The model was fitted for each replicate using the *nls* function from the ‘stats’ package in R. The estimated k values were then extracted and compared between the two provenances using a t -test.

The effects of pre-germination treatments on cumula-

tive germination were analyzed using a two-way ANOVA. For this analysis, the data were fitted to a generalized linear model (GLM) with a quasi-binomial distribution and a logit link function, using the *glm* function from the ‘stats’ package in R. In addition, the following germination parameters were calculated from the daily germination data using the ‘germinationmetrics’ package in R: time to reach 50% of total germination (T50), germination rate, peak value, and germination value. To assess the effects of scarification treatments on these germination parameters, a two-way ANOVA was also performed using general linear models with the *lm* function from the ‘stats’ package. When significant effects of factors or their interactions were detected, pairwise comparisons were conducted using Tukey’s test ($\alpha = 0.05$), implemented with the ‘emmeans’ package in R. For all models, residuals were evaluated graphically to check assumptions of normality and homoscedasticity.

Results

Morphological traits of seeds

The *P. maximartinezii* seed lot from Juchipila contained a total of 854 seeds per kilogram, with a purity of 99.54%; the remaining 0.46% comprised empty seeds and other impurities. In contrast, the seed lot from La Muralla had an estimated 773 seeds per kilogram and a higher purity of 99.89%, with only 0.11% containing empty seeds and impurities. Statistical differences in morphological seed traits were observed between the two provenances. Seeds from La Muralla exhibited greater diameter, length, and seed coat thickness (Fig. 2).

Seed imbibition

After 24 hours of imbibition, seeds from La Muralla exhibited a slightly higher overall water uptake (16.3%) compared to those from Juchipila (15.4%). However, during the initial 15 hours, seeds from Juchipila absorbed more water, showing a constant imbibition rate statistically higher than those from La Muralla (Fig. 3).

Germination parameters

Without pre-germination treatment, *P. maximartinezii* seeds from Juchipila (71.7%) showed significantly higher germination than those from La Muralla (59.1%; $\chi^2 = 36.3$, $p < 0.001$). However, germination performance varied by provenance in face to pre-germination treatments. Mechanical scarification alone significantly increased total germination in seeds from La Muralla ($\chi^2 = 23.1$, $p < 0.001$). Conversely, in seeds from Juchipila it had no significant effect on ($\chi^2 = 0.15$, $p = 0.703$). On the other hand, chemical scarification also significantly influenced germination in both provenances, but its effect depended on whether mechanical scarification was applied (mechanical \times chemical treatment interaction, $p < 0.001$ for both

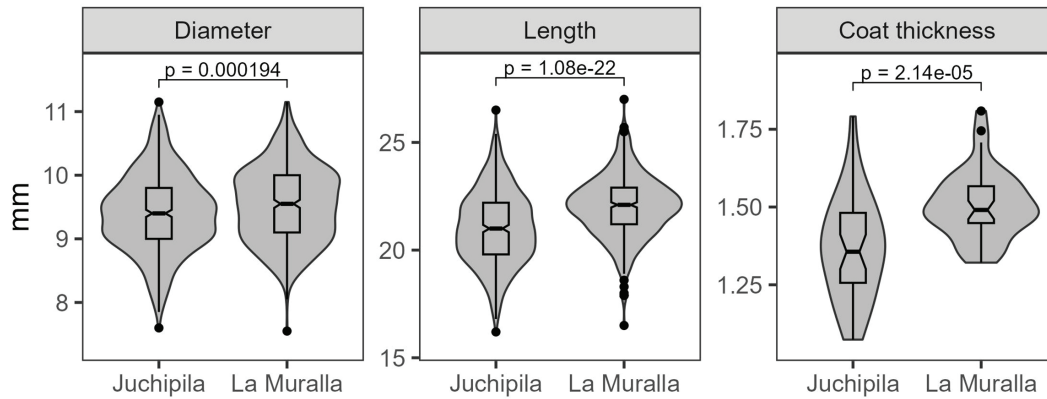


Fig. 2. Dimensional characteristics of *Pinus maximartinezii* seeds from two relict populations in Juchipila, Zacatecas, and La Muralla, Durango, Mexico.

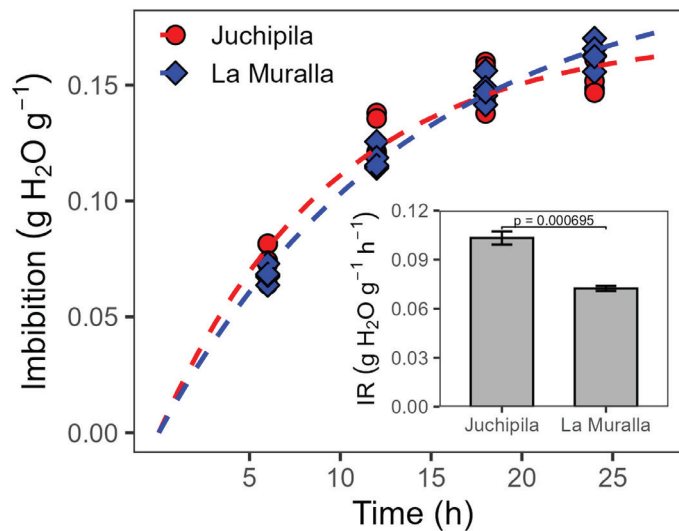


Fig. 3. Imbibition curves of *Pinus maximartinezii* seeds from Juchipila, Zacatecas (red) and La Muralla, Durango (blue). Observed (points) and predicted data (lines) from monomolecular model. Subfigure represents the constant imbibition rate.

cases). For example, in seeds from La Muralla, the highest germination percentages were observed in the control (no immersion) and H₂O₂ treatments, regardless of mechanical scarification. Water immersion alone resulted in low germination, although it improved when combined with mechanical scarification. In contrast, seeds from Zacatecas achieved the highest germination in the no-immersion control. All other chemical treatments reduced germination, with the reduction being more pronounced when combined with mechanical scarification. In both provenances, H₂SO₄ immersion produced the lowest germination percentages and completely inhibited germination when combined with mechanical scarification (Fig. 4).

In general, germination in both provenances began around day 12 and reached maximum cumulative values between days 28 and 30 (Fig. 4). The time to reach 50% of total germination (T_{50}) was approximately 20 days, with no significant difference between provenances. However, in the absence of scarification treatments, seeds from Juchipila exhibited a significantly higher germination rate (F

= 10.1, $p = 0.019$), peak value ($F = 60.6$, $p < 0.001$), and germination value ($F = 45.4$, $p < 0.001$), compared to those from La Muralla (Table 2).

Mechanical scarification alone had a positive effect on germination parameters in both provenances. Nevertheless, in seeds from La Muralla, this effect was not statistically significant compared to the no-sanded seeds (Table 2). In contrast, chemical scarification did not enhance germination performance and, in some cases (particularly with H₂SO₄), had a detrimental effect. Water immersion also reduced germination parameters relative to the control. However, in seeds from La Muralla, the combination of mechanical scarification with water immersion increased the germination rate, peak value, and overall germination to levels comparable to those of the control. The H₂O₂ immersion yielded intermediate germination values. When applied alone to La Muralla seeds, its effect was similar to that of mechanical scarification. However, when combined with mechanical treatment, H₂O₂ had a negative effect on germination parameters (Table 2). In summary, the results

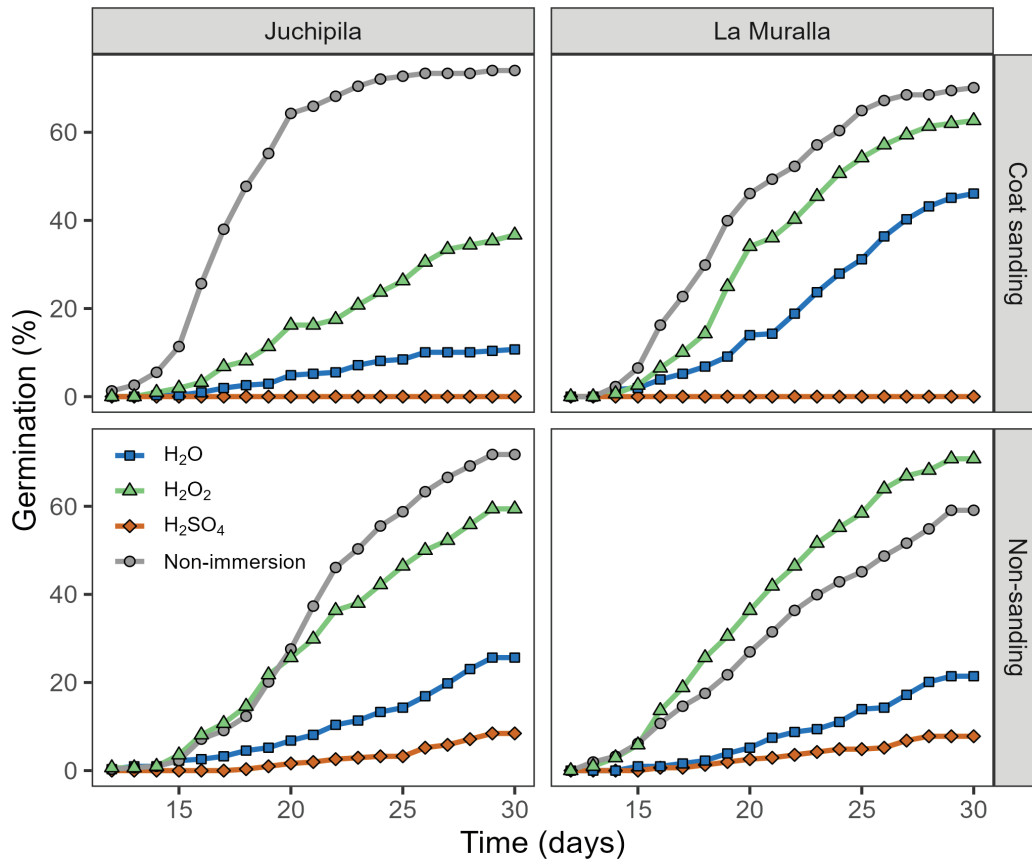


Fig. 4. Germination dynamics of *Pinus maximartinezii* seeds from Juchipila, Zacatecas and La Muralla, Durango, after the application of treatments involving mechanical and chemical seed scarification. The points represent the data observed on each day of evaluation.

indicate that *P. maximartinezii* seeds are non-dormant or mildly physiologically dormant, and that germination can be improved through simple treatments without the need for complex dormancy-breaking procedures.

Discussion

This study examined the effects of pre-germination treatments, specifically mechanical and chemical scarification, on *Pinus maximartinezii* seeds from the only two known populations in Mexico. It also considered the influence of seed origin and key morphological traits that can affect the germination capacity. Morphological differences were observed between both provenances, particularly in seed size and seed coat thickness. The seeds from La Muralla were the larger and had thicker seed coats. In both provenances, these values exceeded those reported for other pinyon pines, such as *Pinus cembroides* and *P. orizabensis*, which have average seed coat thicknesses of 0.87 mm and 0.78 mm, respectively (HERNÁNDEZ-ANGUIANO et al., 2018).

Variation in morphological traits of seeds is common among tree species and occurs at both the population and individual levels (ROMERO-SARITAMA, 2016; CALIXTO-VALENCIA et al., 2022; BASAVE-VILLALOBOS et al., 2024). As reported by HERNÁNDEZ-ANGUIANO et al. (2018) for the

pinyon species *P. cembroides* and *P. orizabensis*, seed traits in *P. maximartinezii* also varied by origin and, consequently, influenced imbibition dynamics. For example, seeds from La Muralla, which were larger and had thicker seed coats, absorbed more water overall but at a slower rate, i.e., over a longer period. In this context, water uptake is a critical physiological process in mature seeds, as it triggers metabolic activity and cell expansion events necessary for root emergence (BEWLEY et al., 2013).

The seed coat in many species contains specialized structures that regulate water uptake, with the micropyle, hilum, or chalazal region often playing a key role depending on the species (BEWLEY et al., 2013; UPRETEE et al., 2024). In conifers, the micropyle is one of the primary pathways for water entering (GUO et al., 2018). For instance, in *Pinus monticola* Douglas ex D. Don, both the seed coat and the megagametophyte (embryo sac) have been identified as the main structures through which water is absorbed. This helps explain why germination is often restricted in hard-coated pine species such as *P. maximartinezii*, *P. cembroides*, and *P. pincea*. In these species, the limited permeability of the seed coat hinders water movement to the embryo, thereby delaying or inhibiting germination (BEWLEY et al., 2013). Nevertheless, it is important to note that in pine seeds, despite their hardness and thickness, the seed coat is not completely imperme-

Table 2. Average germination of *Pinus maximartinezii* seeds from two relict populations (La Muralla, Durango, and Juchipila, Zacatecas) after mechanical (coat sanding vs. control) and chemical scarification (immersion in H₂O, H₂O₂, H₂SO₄, or control)

Mechanical scarification	Chemical scarification	G [‡]	T ₅₀ [¶]	Germination speed	Peak value	Germinative value
<i>La Muralla, Durango</i>						
Control	Control	59.1 a	20.6 ab	2.91 a	2.04 a	4.02 a
	H ₂ O	21.4 c	22.9 b	0.94 b	0.74 b	0.55 b
	H ₂ O ₂	70.8 a	20.0 ab	3.56 a	2.53 a	6.11 a
	H ₂ SO ₄	7.8 c	22.0 b	0.35 c	0.28 c	0.09 b
Sanding	Control	70.1 a	18.7 a	3.68 a	2.63 a	6.15 a
	H ₂ O	46.1 b	23.0 b	2.08 a	1.56 a	2.42 a
	H ₂ O ₂	62.6 ab	20.4 ab	3.06 a	2.26 a	5.15 a
	H ₂ SO ₄	—	—	—	—	—
<i>Juchipila, Zacatecas</i>						
Control	Control	71.7 a	20.7 b	3.41 b	2.50 b	5.99 a
	H ₂ O	25.6 c	23.0 b	1.15 cd	0.89 c	0.76 b
	H ₂ O ₂	59.4 b	21.0 b	2.86 b	2.07 b	4.14 a
	H ₂ SO ₄	8.4 d	23.4 b	0.35 e	0.30 d	0.10 c
Sandig	Control	74.0 a	16.9 a	4.23 a	3.23 a	8.04 a
	H ₂ O	10.7 d	20.6 b	0.51 de	0.40 d	0.15 c
	H ₂ O ₂	36.7 c	21.9 b	1.72 c	1.27 c	1.59 b
	H ₂ SO ₄	—	—	—	—	—

[‡] Total germination; [¶] Time to reach 50% of total germination.

able. This assumption is supported by our results of the imbibition test. Seed coat permeability largely depends on its lignin content, a phenolic compound that forms a protective matrix with cellulose and hemicellulose microfibrils, imparting hydrophobic properties (GUO et al., 2018; UPRETEE et al., 2024). Similar blocking mechanisms have been observed in seeds of agriculturally important families such as Fabaceae, Malvaceae, Cannaceae, Chenopodiaceae, and Convolvulaceae (ROLSTON, 1978; BEWLEY et al., 2013).

Consistent with the findings of HERNÁNDEZ-ANGUIANO et al. (2018), our results show that *P. maximartinezii* seeds possess a hard seed coat regardless of their provenance. However, we found that seed coat thickness varies between provenances. Thicker seed coats, often associated with higher lignin content, have been shown to slow water absorption, thereby affecting the imbibition process (UPRETEE et al., 2024). Consequently, variations in seed coat thickness may influence the effectiveness of pre-germination treatments. In this regard, seeds from Juchipila had thinner seed coats and germinated readily without any treatment, as they absorbed water at a higher rate, suggesting less restriction to water uptake. In contrast, seeds from La Muralla, which had thicker seed coats, required either chemical or mechanical scarification to promote germination. This aligns with the intended function of scarification, which is to stimulate germination in seeds that exhibit dormancy due to barriers imposed by the seed coat or external structures (BASKIN and BASKIN, 2014).

Although scarification enhanced germination in *P. maximartinezii*, its effectiveness varied depending on the method used. In the same seed lot from La Muralla, treatment with reagent-grade hydrogen peroxide (30%) significantly promoted and accelerated germination compared to the control. Hydrogen peroxide is a strong oxidizing agent

widely used as a pre-germination treatment in various tree species, particularly those exhibiting physical dormancy, such as members of the Fabaceae family (RUIZ-CARRANZA et al., 2024). There are also precedents in pine species where hydrogen peroxide has improved germination. For instance, MERÁZ and BONILLA (2000) reported positive results in *Pinus arizonica* Engelm. and *Pinus durangensis* Martínez.

The effectiveness of hydrogen peroxide treatment likely stems from an adequate exposure time that allowed for the removal of epicuticular waxes and partial degradation or softening of the seed coat, without compromising embryo viability. In contrast, sulfuric acid treatment had a detrimental effect on germination. The application of concentrated sulfuric acid as a pre-germination treatment is typically based on its ability to emulate the chemical scarification that occurs when seeds pass through the digestive tracts of animals (TRAVESSET, 1998; RAMOS-FONT et al., 2015). However, in our study, the immersion time (15 minutes) or the concentration may have been excessive, despite following the protocol recommended by PRIETO et al. (2012) for hard coated pine seeds. These findings diverge from those of RUIZ-CARRANZA et al. (2024), who reported improved germination responses in several tree and shrub species subjected to sulfuric acid treatments with several immersion periods.

In their study, RUIZ-CARRANZA et al. (2024) reported that sulfuric acid treatment can enhance germination primarily in tree legumes, although responses varied among species depending on immersion time. These divergent results may be explained by the observations of BASKIN and BASKIN (2014), who emphasized that the effectiveness of scarification treatments, whether mechanical or chemical, differs across species due to inherent biological traits,

seed coat properties, and the intensity and duration of the applied treatments. Overall, these findings highlight the importance of defining pre-germination protocols not only through empirical testing of their effectiveness, as suggested by RAMÍREZ-HERRERA et al. (2008), but also by considering seed traits specific to each species and provenance.

While seeds from Juchipila germinated successfully without the need for pre-germination treatment, sanding was found to further enhance germination speed. Sanding, a form of mechanical scarification, has been shown to be an effective and practical method for promoting germination in neotropical species such as *Enterolobium cyclocarpum* (Jacq.) Griseb. (VIVEROS VIVEROS et al., 2015; SALAZAR and RAMÍREZ, 2018), as well as in pines, as demonstrated by NAWROT-CHORABIK et al. (2021) in *Pinus sylvestris* L. Importantly, the practicality of germination-enhancing techniques is a key consideration when managing large quantities of seeds (RUIZ-CARRANZA et al., 2024). However, when working with limited germplasm, as is the case with *P. maximartinezii*, practicality must be balanced with treatment efficacy. In this context, the findings of the present study provide valuable insights for developing more effective germplasm management strategies for *P. maximartinezii*, a species of high conservation priority.

Conclusions

The morphological characteristics of *Pinus maximartinezii* seeds differ between the two relict populations in Mexico. Seed coat thickness influences water uptake capacity, which is greater in seeds from Juchipila (Zacatecas), whereas seeds from La Muralla (Durango) exhibit a more gradual imbibition rate. The data support the use of population-specific germination protocols. Variation in seed coat thickness and imbibition dynamics necessitates distinct pre-germination treatments, with scarification being necessary solely for the thick-coated La Muralla seeds.

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