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Germination and initial growth of half-sibling families of *Pinus pseudostrobus* Lind. outstanding in resin production

A. Antonio-Bautista[1], H.J. Muñoz-Flores[2], D. Castillo-Quiroz[3]*, J.T. Sáenz-Reyes[2], R. Barrera-Ramírez[2], A. Rueda-Sánchez[4]

[1]Departamento de Fitomejoramiento, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro #1923, C.P. 25315, Buenavista, Saltillo, Coahuila, México.
[2]Campo Experimental Uruapan, Centro de Investigación Regional Pacífico Centro CIRPAC, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Av. Latinoamericana #1101, Col. Revolución, CP. 60150, Uruapan, Michoacán, México.
[3]Campo Experimental Saltillo. Centro de Investigación Regional Noreste CIRNE, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias INFAP. Carretera Saltillo-Zacatecas km 342+119 No. 9515, Col. Hacienda de Buenavista, C.P. 25315, Saltillo, Coah. Mexico.
[4]Campo Experimental Centro Altos de Jalisco. Centro de Investigación Regional Pacífico Centro CIRPAC, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias INIFAP. Km. 8, Carretera Libre Tepatitlán-Lagos de Moreno, Av. Biodiversidad # 2470 CP. 47600 Tepatitlán de Morelos, Jalisco, México.

***Corresponding author:** dacastilloq@gmail.com; castillo.david@inifap.gob.mx

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Abstract: The establishment of commercial forest plantations necessitates the use of high-quality genetic seed and the selection of superior individuals. This study aimed to evaluate the germination ability including germination percentage, germination velocity index, and germination rate as well as the initial growth characteristics (height and root collar diameter) of seedlings from 23 half-sibling families of *Pinus pseudostrobus* Lindl., selected for their exceptional resin production, across three altitudinal gradients. Half-sibling seeds of *P. pseudostrobus* were sown in 310 cm3 plastic-coated containers filled with a 1:1:1:1 substrate mixture of pine bark, Canadian sphagnum peat moss, vermiculite, and perlite, supplemented with Multicote® fertiliser at a concentration of 5 kg/m3. Each family was represented by four replicates, or experimental units, consisting of 25 completely randomised seeds. The evaluated variables were as follows: 1) germination percentage (PG), 2) germination vigour (VG) through the index of germination velocity (IVG), 3) germination rate (TG), 4) plant height (A), and 5) diameter of the root collar (DCR). Significant differences ($p < 0.05$) were found in the germination percentage and index of germination velocity between altitudinal gradients and between families in all measured variables. Our results suggest that germplasm collection should be performed within a gradient between 2,200 and 2,400 m, where seeds present the most intraspecific variation and the highest germination percentage, height, and root collar diameter.

Keywords: altitude; germination vigour; plantation; progeny; seed.
Abbreviations: A_ plant height; CINSJP_ Indigenous Community of Nuevo San Juan Parangaricutiro; DAS_ days after sowing; DCR_ diameter of the root collar; DDS_ days after sowing; F1….23_family; GA1, GA2 and G3_ altitudinal gradient; IVG_ index of germination velocity; PG_ Germination percentage; MDS_ months after sowing; TG_ germination rate; VG_ germination vigour.

Introduction

Conifer species that are widely distributed tend to differ genetically in quantitative characteristics, such as the pattern of buds, resistance to hydric stress, and low temperature, because of the effects of altitudinal gradients in response to different selection intensities imposed by the environment. Understanding genetic variation in traits relevant to species adaptation is essential for developing strategies that enhance the productivity of forest plantations and align the ecological conditions of plantation sites with the genetic characteristics of the saplings. This knowledge supports informed decision-making aimed at promoting the preservation of forest species and the restoration (Nienstaedt, 1990).
In this context, several studies have examined the influence of genetic variability on the growth of conifer species. For instance, García *et al.* (2001) evaluated different provenances of *Pinus teocote* Schltdl. & Cham. saplings grown in nursery

conditions. Farfán *et al*. (2002) determined genetic parameters and the efficiency of early selection in *Pinus ayacahuite* Ehren. var. *ayacahuite*. Moreover, Hernández *et al*. (2003) assessed seed variability among five provenances of *Pinus pseudostrobus* Lindl. Viveros *et al*. (2006) and Villegas *et al*. (2016) further investigated growth variability among *P. pseudostrobus* provenances, while Viveros-Viveros *et al*. (2005) analysed growth patterns of *Pinus oocarpa* Schiede ex Schltdl. saplings under nursery conditions. Similarly, Juárez *et al*. (2006) explored geographical variation in growth and initial development of *Pseudotsuga menziesii* (Mirb.) Franco saplings.
The establishment of commercial forest plantations requires seeds of high genetic quality and the selection of superior individuals with phenotypic characteristics of economic value (White *et al*., 2007), with the goal of identifying phenotypes with a genetic constitution. Therefore, it is necessary to determine the genetic quality of saplings obtained from selected trees. However, despite their relevance, the assessment of genetic quality in saplings of species of the genus *Pinus* is poorly developed (Farfán *et al*., 2002; Sáenz and Tapia, 2003).
Pinus pseudostrobus Lindl. is widely distributed across different climatic conditions (Sandoval-García *et al*., 2020) and is valued for its timber, which is suitable for sawmilling, plywood, veneer, and paper pulp production (Martínez, 1992). This species is notable for its high resin yield in the Mexican state of Michoacán (Muñoz-Flores *et al*., 2022) and is actively utilised in commercial forest plantation programs aimed at producing both timber and resin (Muñoz-Flores *et al*., 2022; Sáenz *et al*., 2011; Viveros *et al*., 2005). Additionally, it plays a key role in ecological restoration efforts, particularly in the rehabilitation of burned and degraded areas in Mexico (CONAFOR, 2009).
Given the commercial and ecological importance of *P. pseudostrobus* in the state of Michoacán, the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP, by its Spanish acronym) initiated a program focused on the selection and evaluation of early development in progenies derived from trees exhibiting high resin production. Accordingly, this study aimed to evaluate the germination ability and initial growth of saplings from 23 half-sibling families of *P. pseudostrobus* selected for their exceptional resin yield.

Results and Discussion

Altitudinal gradients
The results of ANOVA revealed significant differences ($p \leq 0.05$) in the variables PG and IVG between GA (Figure 1) and between families in all variables.

Seed germination
Germination varied according to the evaluation date. First, 15 days after sowing (DDS), the families F1 and F18 at the "Pascuala" (GA1) and "Gómez" (GA2) sites presented the highest germination percentages (66.4 and 49.6%, respectively). Among the 15-17 DDS, nine families presented the highest germination percentages, corresponding to the "Gómez" site (F10, F11, F12, and F16), with 82.4-92.8% germination. However, family F22 at the "Pario" site (GA3) reached maximal germination, at 99.2%. Among the 17-19 DDS, only the families F22 of the "Pascuala" site and F13 of the "Gómez" site presented the highest germination percentages, with 51.2% and 47.2%, respectively. Between 19-21 DDS, the family F14 of the "Gómez" site registered the highest germination percentage, with 21.6%, one of the two families that recorded germination until the 25 DDS, together with F2 of the "Pascuala" site.
The ANOVA results obtained at 27 DDS indicated significant differences ($p > 0.05$) between families (Figure 2). The germination percentages of the F1, F5, and F8 families ("Pascuala" site in GA1); F11, F12, F16, and F18 ("Gómez" site in GA2); and F19 ("Tacarido" site in GA2) and F22 ("Pario" site in GA3) were the highest (100%), not significantly different ($p \leq 0.05$), but differed from those of the other families. The seeds of F20 at the "Tacarido" site (GA2) presented the lowest percentage of germination (65.6%).
The results of germination are greater than those reported in 11 natural populations of *P. menziesii* (7-84%) in the Mexican states of Chihuahua, Coahuila, Nuevo León, Querétaro, Hidalgo, Tlaxcala, Puebla, and Oaxaca from provenances of *P. teocote* (39.5%), *Pinus oaxacana* Mirov. (33.7% in the harvest of 2005 and 48.5% in 2007) in Veracruz, *Pinus engelmannii* Carr. (98.5%) in Durango, *P. oaxacana* (30%) in Puebla and Veracruz, *P. pseudostrobus* (forest-school 50%) in Nuevo León, *Pinus martinezii* Larsen (45.5%) in Michoacán, *Pinus greggii* (70.86%) in Veracruz and Querétaro, *Pinus cembroides* Zucc subsp. *orizabensis* D. K. Bailey (48%) in Tlaxcala, and *Pinus hartwegii* Lindl. (98% in the provenance from Ajusco, Mexico City) in the State of Mexico (Juárez *et al*., 2006; Ramírez *et al*., 2009; Quiroz *et al*., 2008; Bustamante *et al*. 2012; Alba *et al*., 2003; Domínguez *et al*., 2016; Morales *et al.*, 2017; Sánchez *et al*., 2005; Mendizábal *et al*., 2015; Ortega *et al*., 2003; Ramírez *et al*., 2001). The low germination percentage shown by some families of *P. pseudostrobus* in this study is not exclusive to this taxon but is also present in other *Pinus* and *Pseudotsuga* species.
However, other studies, such as that of Muñoz-Flores *et al*. (2023), have indicated that germination is similar to or greater than that reported in this study, with values ranging from 90 to 100%, if they work under controlled conditions in the laboratory, where germinated seeds already appear from the sixth day DDS. Therefore, it is important to note that moisture content at the time of sowing is a critical factor influencing the daily germination percentage, along with other variables such as seed size, weight, and health (Muñoz-Flores *et al*., 2023).
In another similar work, *P. montezumae* reported that more than 50% germination was reached from four DAS (Herrera-Hernández *et al*., 2024). In addition, there are reports where other species of the *Pseudostrobus-Montezumae* complex, such as *Pinus devoniana* Lindl., needed 5.5 DAS to reach half of the total germination (Romero-Rangel *et al*., 2017), or more than 12 DAS, as in the case of *Pinus hartwegii* Lindl. (Sánchez *et al*., 2023); therefore, the time and percentage of germination

Table 1. The altitudinal range of 23 half-sibling families of *Pinus pseudostrobus* Lindl. in the indigenous community of Nuevo San Juan Parangaricutiro (CINSJP), Michoacán, Mexico.

Families	Site	Altitudinal range (m)	Altitudinal gradient (GA)
F1, F2, F3, F4, F5, F6, F7, F8, F9	Pascuala	2200 - 2400	1
F10, F11, F12, F13, F14; F15, F16, F17, F18	Gómez	2401- 2600	2
F19, F20, F21	Tacarido	2401 – 2600	2
F22, F23	Pario	2601 – 2800	3

presented by the 23 families of *P. pseudostrobus* are good and allow us to demonstrate that there are indeed intraspecific differences.

Vigour and germination rate

In terms of vigour, the families that presented the highest IVG were F1 and F18, with values of 1.58 and 1.56, respectively, which were significantly different (p≤0.05) from those of the other families, where F20 presented the lowest value of 0.93 (Figure 3).

With respect to TG, the family that reached maximal germination in the shortest time was F14, with 19.89, whereas the family that required more time to reach maximal germination was F1, with 15.88 (Figure 4).

Families F1 and F18 reached 100% germination and the highest IVG (1.58 and 1.56, respectively) and TG (15.88 and 16.07, respectively), which were significantly different from those of the other families (p≤0.05), although F1 required more time to reach maximal germination. These results were lower than those reported by Mendizábal *et al*. (2015) in provenances of *P. greggii*, who reported that seeds from San Joaquín, Querétaro, Mexico, were more vigorous (1.92) but lower than those reported by Ortega *et al*. (2003) in provenances of *P. hartwegii* (6.80 in Ajusco, Mexico City) in the State of Mexico.

Hernández (2016) reported a germination capacity of 88% for *P. cembroides* and 84% for *P. orizabensis*, noting that *P. cembroides* exhibited greater germination vigour, requiring 12.7 days to reach 50% germination compared to 13.4 days for *P. orizabensis*. These findings indicate significant differences (p < 0.01) between the two species in terms of germination capacity, peak germination value, and the number of days required to reach peak and 50% germination. In this context, it is important to emphasise that germination capacity and vigour in *Pinus* species are key indicators of seed quality for nursery plant production. Furthermore, higher germination speed and uniformity are directly associated with greater seed vigour and more consistent plant development (Bonner *et al*., 1994; Trujillo, 1996).

The germination trend of the 23 evaluated families revealed that, between 15-21 DDS, 91% reached 90-100% germination, with F1 occurring at the "Pascuala" site with 100% PG and an IVG of 1.58, but it had the lowest TG of 15.88, followed by F18 at the "Gómez" site (GA2) with 100%, an IVG of 1, and a TG of 16.07. Moreover, the F5 individuals of the "Tacarido" site (GA2) and the F22 individuals of the "Pario" site (GA3) presented 100% PG. However, these individuals did not perform well in terms of IVG and TG since they required more time to reach the maximal percentage of PG. Therefore, these individuals should not be considered families with high vigour values. The F20 of the "Tacarido" site (GA2) presented the lowest values of vigour, with 65.6% PG and 0.93% IVG.

Height of the seedlings

The initial growth rate of seedlings in the nursery was closely linked to their ability to utilise environmental resources such as moisture, light, and nutrients. At 6.4 months after sowing (MDS), the families exhibiting the greatest height growth were F6, F5, and F9, with respective values of 26.6, 26.1, and 25.2 cm, all originating from the "Pascuala" site (GA1). In contrast, the progeny showing the least height growth was F21, with a value of 18.6 cm, from the "Tacarido" site, reflecting a difference of up to 8 cm compared to the F6 and F5 families.

At seven MDS, family F6 at the "Pascuala" site showed good growth, with a height of 26.9 cm, whereas families F5 and F10 also presented similar growth, with heights of 26.5 and 26.7 cm, respectively. In contrast, the families at the "Tacarido" site presented the lowest height growth (Table 2).

At 7.6 MDS, the results of the ANOVA indicated significant differences (p≤0.05) in height growth between families, as confirmed by Tukey's mean comparison test (p≤0.05), which revealed that family F6 presented the greatest growth at 28.4 cm, followed by F5 at 27.9 cm in GA1. In contrast, the families F3 from the "Pascuala" site (GA1), F20 from the "Tacarido" site (GA2), and F22 from the "Pario" site (GA3) presented the lowest heights (Table 2 and Figure 5). Therefore, this variable had no intraspecific variation in terms of growth between the families selected as abundant resin producers.

Although no significant differences (p ≤ 0.05) in A were observed between altitudinal gradients or among families, families F6 (28.4 cm) and F5 (27.9 cm) exhibited the highest values. These two families were statistically similar (p ≥ 0.05) but significantly different from the rest. The height growth achieved at 7.4 MDS surpasses that reported by Juárez *et al*. (2006) for the initial growth of progeny from 11 natural populations of *P. menziesii* (9.7-10.4 cm) at 9 MDS, as well as the values recorded by Ortega *et al*. (2003) for the initial growth of seven populations of *P. hartwegii* in the State of Mexico, where the Ajusco provenance from Mexico City reached heights between 1.8 and 3.2 cm at 7 MDS. Our values also exceed those obtained by Alba *et al*. (2003) for three populations of *P. oaxacana*, where the "Los Molinos" provenance exhibited the best growth at 23 cm after 11 MDS. Viveros-Viveros *et al*. (2005) found that the height of *P. oocarpa* saplings exhibited low heritability for the traits evaluated (0.27 < h2 < 0.40) from two to six months of age in nursery conditions; nevertheless, they highlighted the potential of early selection for identifying promising parent trees.

Table 2. Growth in height of saplings from 23 half-sibling families of *Pinus pseudostrobus* Lindl., outstanding in resin production.

Family	Height (cm)		
	6.4 MDS	7.0 MDS	7.6 MDS
F1	23.4 ±4.84	24.4 ±3.20	24.7 ±5.40
F2	20.6 ±3.51	22.1 ±3.17	22.5 ± 3.86
F3	18.6 ±6.76	20.0 ±6.10	20.5 ±7.18
F4	22.0 ±5.00	23.6 ±4.02	23.8 ± 4.17
F5	26.1 ±3.74	26.5 ±3.22	27.9 ±4.24
F6	26.6 ±4.33	26.9 ±4.19	28.4 ±4.80
F7	25.1 ±5.25	25.8 ±5.19	27.2 ±5.69
F8	22.5 ±4.16	23.2 ±4.38	23.4 ±3.98
F9	25.2 ±5.42	25.8 ±4.77	27.5 ±6.06
F10	25.0 ±3.08	26.7 ±2.56	26.7 ±2.30
F11	20.6 ±5.47	21.7 ±5.61	22.7 ±4.64
F12	23.7 ±5.61	24.7 ±3.84	24.8 ±3.55
F13	24.6 ±6.00	24.8 ±5.50	25.9 ±6.50
F14	22.3 ±3.07	23.1 ±3.87	23.1 ±4.26
F15	24.7 ±2.86	25.5 ±3.05	24.7 ±2.33
F16	20.3 ±7.98	22.8 ±5.05	26.3 ±5.36
F17	19.6 ±5.89	21.5 ±6.58	24.3 ±6.44
F18	23.9 ±4.01	25.0 ±4.05	24.1 ±4.52
F19	19.8 ±4.90	19.8 ±4.04	26.5 ±4.16
F20	20.1 ±5.54	21.1 ±5.98	20.0 ±5.88
F21	18.6 ±3.42	19.6 ±3.09	22.8 ±3.85
F22	20.7 ±2.56	21.2 ±2.54	20.4 ±3.15
F23	21.1 ±4.77	21.2 ±5.07	21.3 ±6.07

MDS = Months after sowing.

These results indicate that when selecting an early-expression trait such as the height of *Pinus pseudostrobus* seedlings that is strongly correlated with a late-expression trait of economic importance, such as resin production, it is essential to understand the heritability of both early- and late-expression traits. Additionally, it is important to assess the degree of correlation between these traits (Lambeth *et al*., 1983; Vargas-Hernández & Adams, 1992; Zobel & Talbert, 1992; Wu, 1998; Adams *et al*., 2001).

Root collar diameter

In the first measurement at 6.4 MDS, family F7 from the "Pascuala" site (GA1) exhibited the highest root collar diameter growth, reaching 4.40 mm. In contrast, the lowest growth was recorded in family F21 from the "Tacarido" site (GA2), with a value of 3.34 mm. During the second measurement at 7 MDS, family F7 again showed the highest growth, reaching 4.64 mm, while the lowest growth was observed in family F17 from the "Gómez" site (GA2), with a value of 3.58 mm (Table 3).

At 7.6 MDS, the results of the ANOVA revealed significant differences ($p \leq 0.05$) among the families. Tukey's mean comparison test grouped the families into three distinct categories: (a) family F7 from the "Pascuala" site (GA1) with a DCR of 5.39 mm, (b) family F19 from the "Tacarido" site (GA2) with 4.83 mm, and (c) family F17 from the "Gómez" site with only 3.89 mm (Table 3 and Figure 6). Although there were no significant differences ($p \geq 0.05$) in DCR between GA, significant differences were observed among families. Tukey's test further confirmed that family F7 from the "Pascuala months after sowing" site (GA1) exhibited the highest growth at 5.39 mm. This value was higher than the maximum reported by Alba *et al*. (2003) for *P. oaxacana*, where the "Los Molinos" provenance achieved a DCR of 4.5 mm after 11 MDS.

In a study evaluating nine provenances of *P. pseudostrobus* and two provenances of *P. pseudostrobus* var. *apulcensis* (Lindl.) Martínez across two sites at different altitudes (2,200 and 2,800 m) within the Indigenous Community of San Juan Parangaricutiro (CINSJP) in Michoacán, Mexico, Viveros *et al*. (2006) found that, at 24 MDS, cumulative growth in height, basal diameter, and crown width was greater at the lower altitude. This trend is consistent with the present study, in which progenies from the lower-altitude site specifically families F5 and F6, identified as high resin producers and originating from elevations between 2,200 and 2,400 m in CINSJP forests exhibited superior growth performance.

Understanding the initial growth behaviour of seedlings is essential for guiding selection evaluations based on their growth potential, serving as an indicator of plant quality in nursery production and contributing to the successful establishment of

Table 3. Growth in diameter of the root collar of saplings from 23 half-sibling families of *Pinus pseudostrobus* Lindl. at different evaluation dates.

Family	**Diameter of the root collar (mm)**		
	6.4 MDS	7.0 MDS	7.6 MDS
F1	4.33 ±0.33	4.36 ±0.60	4.71 ±0.30
F2	3.92 ±0.42	3.99 ±1.09	4.24 ±0.61
F3	4.14 ±1.42	4.37 ±1.76	4.51 ±0.48
F4	4.03 ±0.63	4.49 ±0.79	4.49 ±0.45
F5	3.52 ±0.88	3.78 ±0.96	4.44 ±0.54
F6	4.08 ±0.54	4.23 ±0.64	4.45 ±0.37
F7	4.40 ±0.96	4.64 ±0.28	5.39 ±2.63
F8	4.00 ±0.64	4.35 ±1.46	4.44 ±0.48
F9	3.95 ±0.88	4.42 ±0.67	4.65 ±1.99
F10	3.75 ±0.71	4.28 ±1.86	4.41 ±0.34
F11	4.32 ±1.17	4.46 ±0.72	4.74 ±1.72
F12	3.91 ±0.60	4.07 ±0.99	4.24 ±0.63
F13	4.07 ±0.51	4.08 ±0.55	4.52 ±0.20
F14	3.81 ±0.81	3.96 ±0.82	4.24 ±0.38
F15	3.89 ±0.66	4.20 ±0.76	4.41 ±0.37
F16	4.19 ±0.93	4.41 ±0.46	4.68 ±0.36
F17	3.43 ±0.83	3.58 ±1.03	3.89 ±0.93
F18	4.07 ±0.52	4.29 ±0.40	5.08 ±2.28
F19	4.28 ±0.99	4.73 ±0.42	4.83 ±0.46
F20	4.18 ±0.72	4.46 ±0.57	4.47 ±1.75
F21	3.34 ±0.49	3.70 ±0.69	4.05 ±0.67
F22	4.10 ±0.71	4.17 ±0.52	4.43 ±0.63
F23	3.52 ±0.76	3.75 ±0.49	4.31 ±0.41

MDS = Months after sowing.

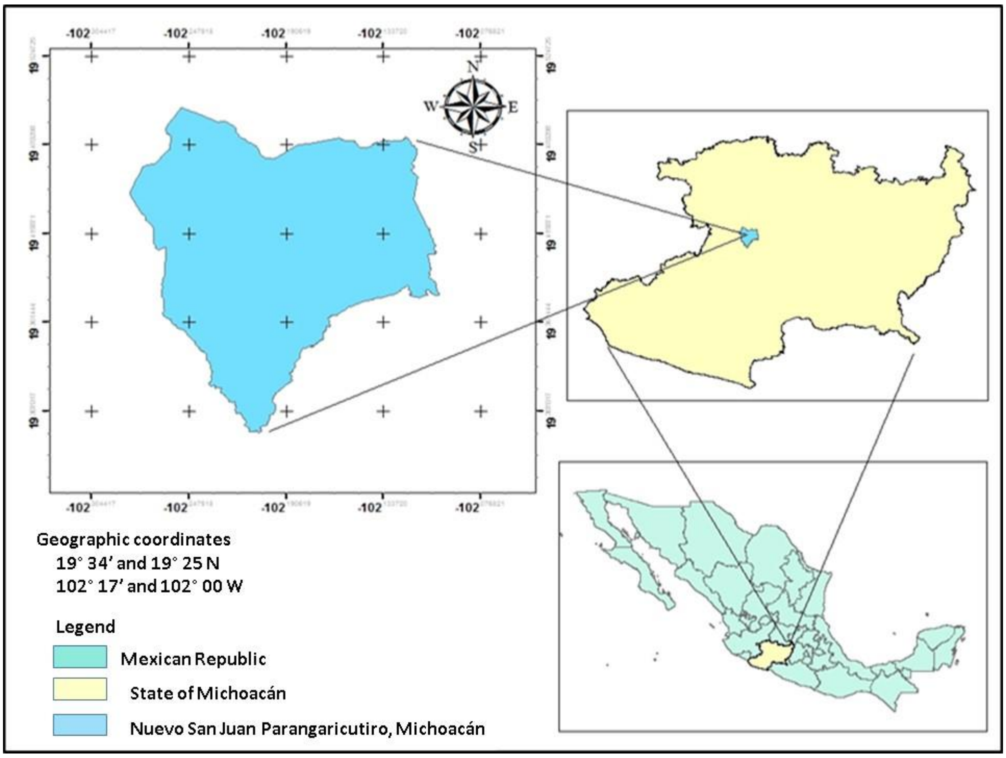

Fig 1. Location of the study area in the indigenous community of Nuevo San Juan Parangaricutiro (CINSJP), Michoacán, Mexico.

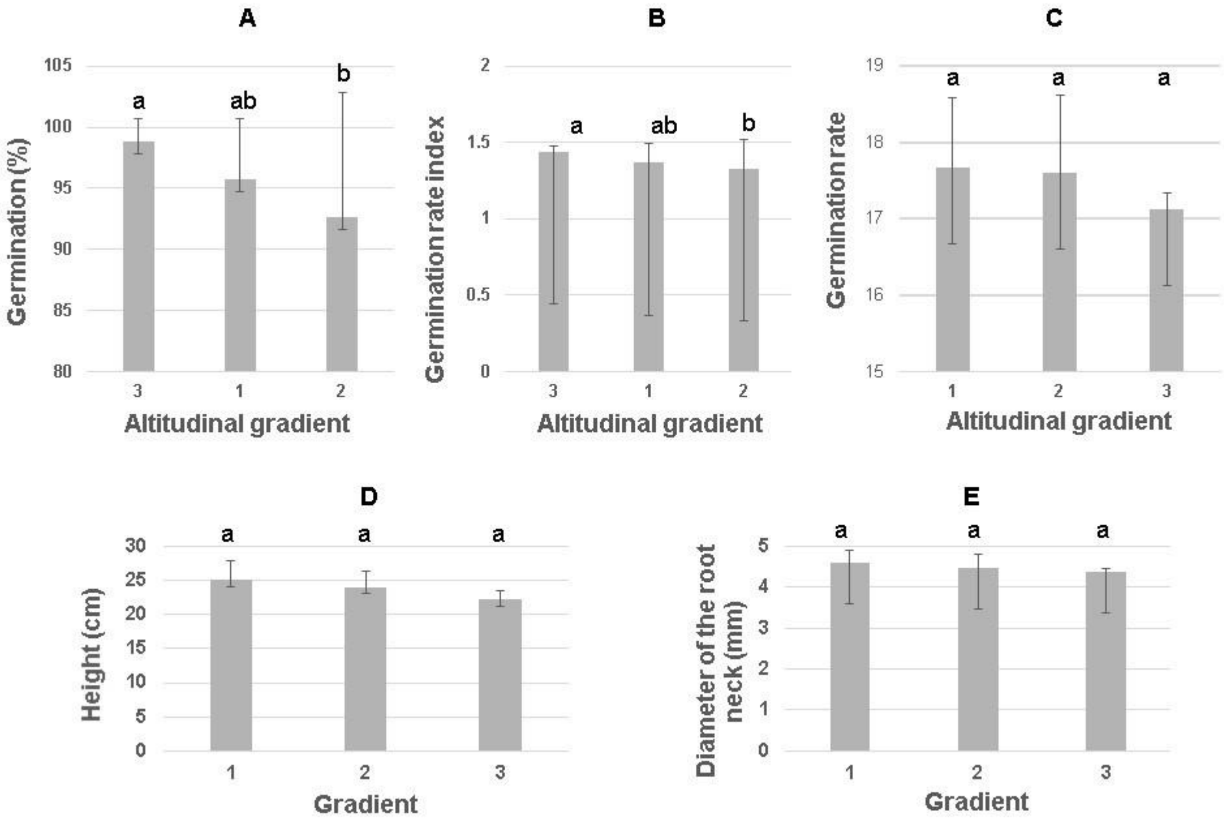

Fig 2. Tukey's mean comparison test (p≤0.05) for (A) germination, (B) index of germination velocity, (C) germination rate, (D) height, and (E) diameter of the root collar for 23 half-sibling families of *Pinus pseudostrobus* Lindl. at three altitudinal gradients. Means with different letters are significantly different (p≤0.05). The lines over the bars indicate the standard deviation.

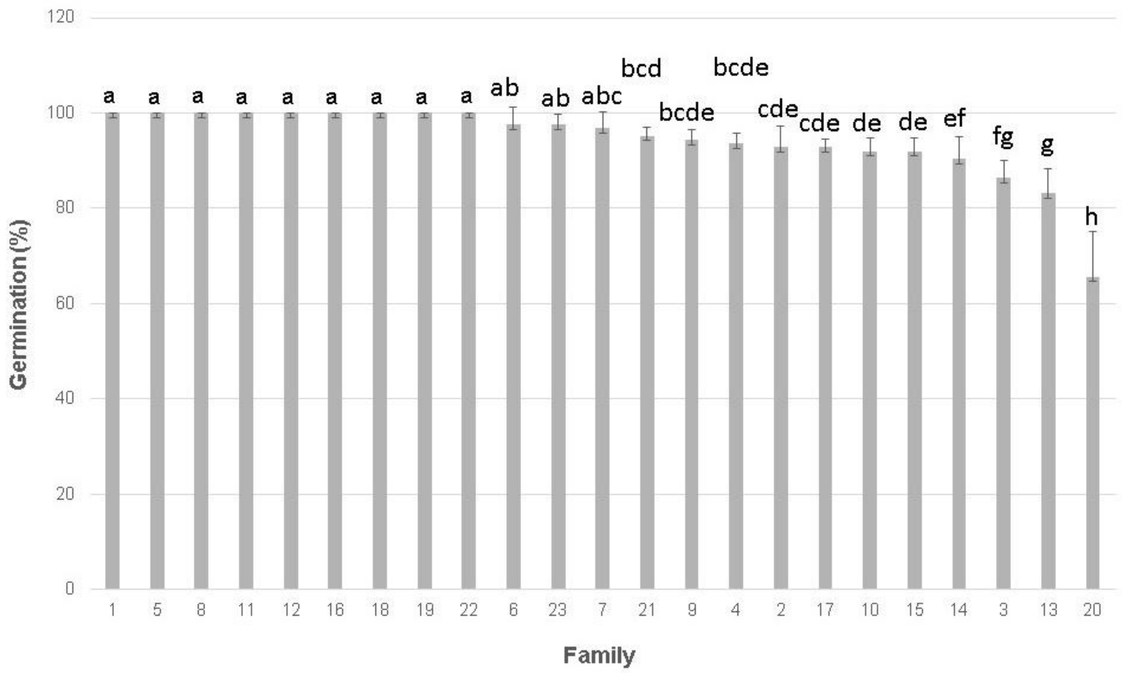

Fig 3. Tukey's mean comparison test (p≤0.05) for the percentage of 23 half-sibling families of *Pinus pseudostrobus* Lindl. Means with different letters indicate a significant difference (p≤0.05). The lines over the bars indicate the standard deviation.

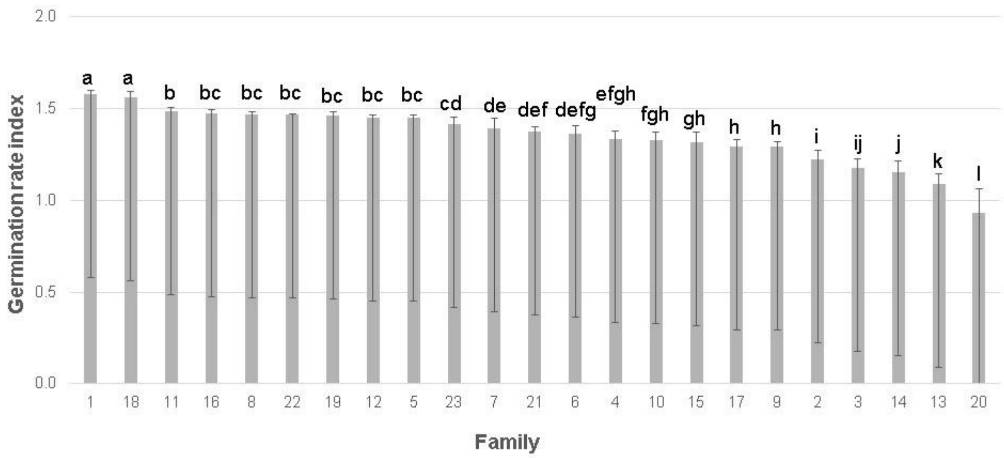

Fig 4. Tukey's mean comparison test (p≤0.05) for the index of germination velocity of 23 half-sibling families of *Pinus pseudostrobus* Lindl. at 7.6 months after sowing. Means with different letters are significantly different (p≤0.05). The lines over the bars indicate the standard deviation.

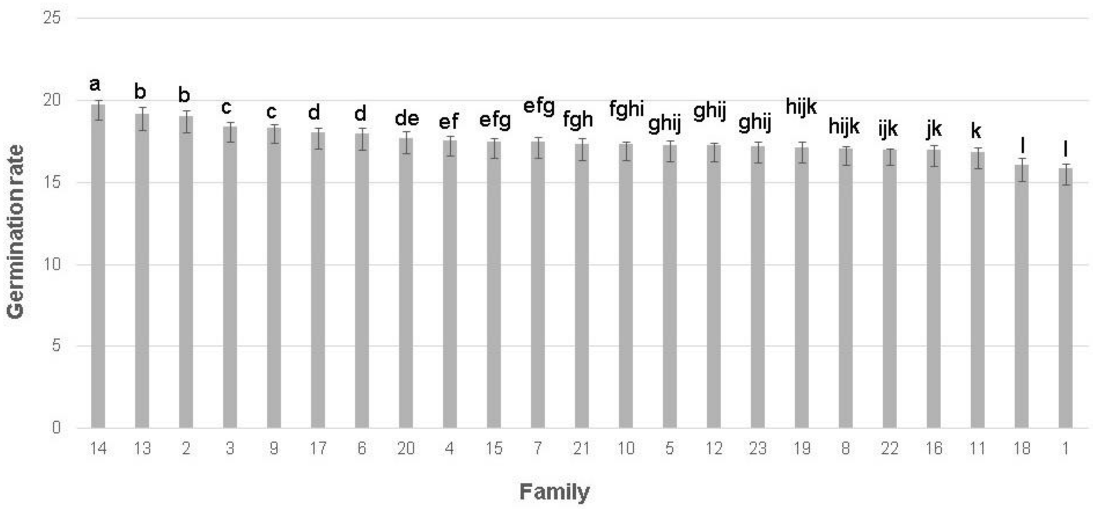

Fig 5. Tukey's mean comparison test (p≤0.05) was used to compare the germination rates of 23 half-sibling families of *Pinus pseudostrobus* Lindl. Means with different letters are significantly different (p≤0.05). The lines over the bars indicate the standard deviation.

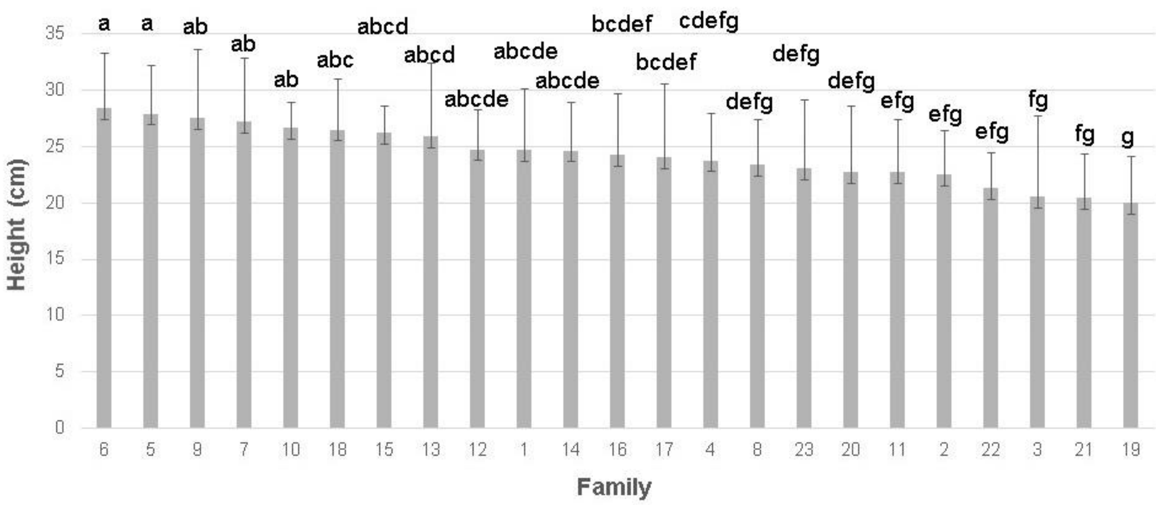

Fig 6. Tukey's mean comparison test (p≤0.05) for the heights of 23 half-sibling families of *Pinus pseudostrobus* Lindl. at 7.6 months after sowing. Means with different letters are significantly different (p≤0.05). The lines over the bars indicate the standard deviation.

forest plantations (Alba *et al*., 2003). Therefore, it is recommended to prioritise seed collection from the "Pascuala" site (GA1), followed by the GA2 region, as *P. pseudostrobus* seeds from these sites exhibited the greatest intraspecific variability, yielding the highest germination percentages, along with A and DCR values.
Similar studies have demonstrated that, in *Pinus* species, both the origin or provenance and the parent tree (family) are key factors influencing germination percentage as well as plant development and growth in nursery conditions (Pérez-Luna *et al*., 2024). Rodríguez (2013) reported greater height and diameter growth in *Pinus patula* Seem. families grown in a nursery in Puebla, Mexico, originating from Ahuazotepec (2350 m), Huayacocotla (2100 m), and Zacatlán (2360 m). These findings are consistent with those of the present study, which involved families from altitudinal gradients ranging from 2200 to 2600 m. This confirms that greater growth is often observed in progenies originating from sites with lower elevations and higher precipitation particularly in species with a wide geographic distribution, such as *P. pseudostrobus* (Sáenz-Romero *et al*., 2020). This relationship helps explain the observed differences in height and diameter growth among families of the same species.

Materials and methods

Tree selection and germplasm collection

We selected 23 half-sibling families (trees) of *P. pseudostrobus* from natural populations located across three altitudinal gradients (Table 1) on lands belonging to the CINSJP in Michoacán, Mexico (Figure 1). Selection was based on resin production, as reported for the 2016-2017 period (Muñoz-Flores *et al*., 2022). For each tree (family), 50 cones were collected from the middle section of the crown using a telescopic cutter during the fall-winter of 2017. The selected trees exhibited appropriate physiological maturity. Collected cones were placed in jute bags and labelled with family and collection site information. The cones were then transported to the nursery of the Experimental Field Uruapan of INIFAP, located in Uruapan, Michoacán, Mexico (19° 24' 26" N, 102° 03' 07" W; elevation 1614 m), for seed extraction and germination testing. Germplasm was extracted by air-drying the cones outdoors to induce opening and facilitate seed release. The seeds were subsequently cleaned manually using cribs. Each lot of seeds was stored until sowing in brown paper bags and labelled by family.

Sowing

Sowing was carried out in March 2018, following a completely randomised design involving 23 families, each represented by four replicates of 25 seeds per experimental unit. Plastic containers with 25 cavities, each with a capacity of 310 cm3 (one seed per cavity), were used. The substrate was pre-moistened with running water and consisted of a mixture of pine bark compost (25%), *Sphagnum canadensis* (peat moss) (25%), vermiculite (25%), and perlite (25%), supplemented with Multicote®, a controlled-release fertiliser, at a dose of 5 kg/m3. Each container was labelled with the corresponding seed lot to ensure identification during the germination phase. Throughout the evaluation period, irrigation was applied every 72 h using running water to maintain adequate moisture levels for seed germination.

Data collection

The evaluated variables were as follows: 1) germination percentage (PG), 2) germination vigour (VG) through the index of germination velocity (IVG), 3) germination rate (TG), 4) plant height (A), and 5) root collar diameter (DCR).
For the variable PG, a germinated seed was considered when it had a height of 2 cm above the substrate surface. PG was calculated with the following formula:

$$PG = \left[\frac{Number\ of\ germinated\ seeds}{Number\ of\ sowed\ seeds}\right] 100 \qquad (1)$$

VG was determined through the index of germination velocity (IVG) as follows:

$$IVG = \sum[ni/(\sum ti)] \qquad (2)$$

Where: *ni* is the number of germinated seeds within the time interval *to*
Σti is the time in days between sowing and the last day of the experiment (Ranal and García, 2006).

TG was determined using the following formula:

$$TG = (\ N_1T_1 +\ N_2T_2 + \cdots N_nT_n)/(N_1 +\ N_2 + \cdots N_n) \qquad (3)$$

Where: N is the number of non-accumulated germinated seeds.
T is the time in days (Hartmann and Kester, 2001).

The recording of A was performed from the plant base to the apical bud with a Stanley® tape measure of 3.0 mm, graduated in centimetres, and precision in millimetres. The DCR was measured at the root collar with an electronic Truper® Vernier with precision in millimetres.

Data analysis

The variable measurements were recorded using Microsoft Excel® 2016, where the mean values for each variable were calculated. Subsequently, a one-way analysis of variance (ANOVA) was performed, with the percentage of germination (PG), germination velocity index (IVG), germination rate (TG), plant height (A), and root collar diameter (DCR) as the dependent variables. Family (F) was taken as an independent variable, while the altitudinal gradient (GA) was included as a covariate. Statistical analyses were conducted in Rstudio. We conducted a Tukey mean comparison test (p≤0.05) when there were using RStudio. When significant differences were detected, Tukey's mean comparison test was applied (p ≤ 0.05).

Conclusion

There were significant differences (p≤0.05) between the altitudinal gradients in terms of the germination variables and the index of germination velocity (Gradient 3 = 2,600-2,800 m) but not in TG, A, or DCR or between families in all the variables, independent of the altitudinal gradient. Families F1, F5, F8, F11, F12, F16, F18, F19, and F22 presented the highest PG; F1 and F18 presented the highest PG in IVG and TG, whereas F5 and F6 presented the highest PG in A; and F7 presented the highest DCR. The collection of seeds should be conducted along a 2,200-2,400 m altitudinal gradient, where seeds presented the highest intraspecific variability and the best PG, A, and DCR.

Based on the results obtained, it is possible to initiate a tree selection program aimed at identifying superior individuals of *P. pseudostrobus* that can ensure improved outcomes in terms of reduced yield time and increased resin production within the forests of CINSJP, Michoacán, Mexico.

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