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Scientific article

Calidad de semilla y crecimiento de planta en poblaciones y altitudes de *Pinus hartwegii* Lindl.

Seed quality and plant growth in populations and altitudes of *Pinus hartwegii* Lindl.

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Abstract

Reproduction and growth of high mountain species adapted to low temperatures could be affected by global warming, particularly at marginal altitudes where stress is more intense. This study evaluated seed quality and initial growth in various altitudinal gradients of three *Pinus hartwegii* populations (two in the State of Mexico and one in *Veracruz* State); seeds were collected between 3 400 and 4 100 masl. The experimental design was completely randomized and data on seed germination, cespitose stage breakage, and plant growth were recorded. Seed germination capacity was 87 %, with a peak value of 3.2 and germination of 10.9, but with significant variation among populations and gradients. The lowest germination capacity (50 %) was obtained with seeds from the highest altitudinal gradient (4 100 m). The breakage of the cespitose stage varied according to population and altitude. Regarding the seedlings from *Veracruz*, 75 % of them broke the cespitose stage at five months of age, in contrast with those from the State of Mexico, where the percentage was below 35 %. Regarding altitude, the plants from the highest interval prolonged the break of the cespitose stage for more than two months, which influenced the low initial growth of the plants. This indicates that the 4 000 m population may be in decline, unlike the other altitudinal gradients, which exhibited excellent seed quality and plant growth.

Key words: High mountain, adaptive traits, cespitose stage, altitudinal gradient, germination parameters, reproduction.

Resumen

La reproducción y el crecimiento de especies de alta montaña adaptadas a bajas temperaturas, podrían afectarse por el calentamiento global, particularmente en altitudes marginales donde el estrés es más intenso. En este estudio se evaluó la calidad de semilla y el crecimiento inicial en varios gradientes altitudinales de tres poblaciones (dos del Estado de México y una de Veracruz) de *Pinus hartwegii*, donde las semillas se recolectaron entre 3 400 y 4 100 msnm. El diseño experimental fue completamente al azar y se registraron datos de germinación de semillas, rompimiento del estado cespitoso y crecimiento de plantas. La capacidad germinativa de las semillas fue de 87 %, con valor pico de 3.2 y germinativo de 10.9, pero con variación significativa entre poblaciones y gradientes. La menor capacidad germinativa (50 %) se obtuvo con semillas procedentes del mayor gradiente altitudinal (4 100 m). El rompimiento del estado cespitoso varió según la población y altitud. Respecto a las plántulas de Veracruz, 75 % de ellas rompieron el estado cespitoso a los cinco meses de edad, en contraste con las del Estado de México, cuyo registro fue menor a 35 %. Referente a la altitud, las plantas procedentes del mayor intervalo prolongaron el rompimiento del estado cespitoso por más de dos meses, lo cual influyó en el bajo crecimiento inicial de las plantas. Lo anterior indica que la población de 4 000 m podría estar en declinación, a diferencia de los otros gradientes altitudinales que mostraron excelente calidad de semillas y crecimiento de plantas.

Palabras clave: Alta montaña, caracteres adaptativos, estado cespitoso, gradiente altitudinal, parámetros germinativos, reproducción.

Introduction

Pinus hartwegii Lindl. is located at the altitudinal limit of arboreal vegetation (3 000–4 300 m), from northeastern Mexico to the northern region of *El Salvador* (Perry, 1991). Temperature is variable in this altitudinal range. Stands located at lower altitudes are exposed to warmer temperatures and lower humidity, while those at the upper limits are cold and with frequent frosts (López-Toledo *et al.*, 2017). In response to this environmental variation, species may develop certain adaptations or increase phenotypic plasticity in order to survive and grow (Di Pierro *et al*., 2017). *P. hartwegii* exhibits adaptive traits that allow it to coexist with natural fire and frost events (Robles-Gutiérrez *et al*., 2016).

As this taxon has a restricted distribution and is well adapted to high mountain conditions, increased temperature and low humidity in its niche would limit its natural regeneration and threaten its permanence (Ricker *et al*., 2007). A greater decrease in the recruitment of *P. hartwegii* plants is observed in the upper altitudinal limits of Mount *Tlaloc* as a consequence of the loss of soil moisture during the last decades (Astudillo-Sánchez *et al*., 2017). For other pines with lower altitude distribution, migration would be an alternative, but *P. hartwegii* would only be able to move up to sites with rocky, shallow soils and low moisture retention capacity (Gómez-Pineda *et al*., 2020).

On the other hand, natural regeneration depends on the quality of the seeds (Cheng *et al*., 2009). A decrease in seed production and quality was determined in *Abies georgei* Orr var. *smithii* (Viguié & Gaussen) C. Y. Cheng, W. C. Cheng & L. K. Fu as the altitudinal gradient increases. This is reflected in the low regeneration and density of trees at these altitudes. Therefore, their migration to higher altitudes is limited (Wang *et al*., 2016).

The reproductive physiology of species subjected to prolonged water stress conditions is affected. Small, empty seeds, aborted or poorly formed embryos, and deficient structures are observed when reproductive trees are often exposed to low-humidity conditions (Lauder *et al*., 2019).

Various anthropogenic activities have negative effects on the natural regeneration of *P. hartwegii* forests and reduce the density of its populations through the frequent elimination of seed-producing trees (Iglesias and Tivo, 2006; Espinoza-Martínez *et al.,* 2008). Moreover, the drought condition is expected to increase mortality and modify the reproductive phenology of the species (Gómez-Pineda *et al.*, 2020). This could result in the production of low-quality seeds and consequently poor seedlings for establishment (Aguilar *et al.*, 2019). Therefore, determining the quality of seed produced in different altitudinal gradients is essential for the management of this taxon.

In conifers, seed quality is an indicator of reproductive success that allows to know the status of the population in order to propose conservation strategies (Castilleja *et al.*, 2016). Seed quality is defined by both germination capacity (percentage of seeds germinated in a period) and vigor (germination speed) (Navarro *et al.*, 2015). Therefore, the faster and more homogeneous the germination, the greater the vigor and uniformity of the plants produced (Hernández-Anguiano *et al*., 2018).

The cespitose growth of *P. hartwegii* seedlings can be prolonged for several years as a result of disturbances and competition with invasive shrub species, which prevent their establishment (Jin *et al*., 2019). Invasive taxa are favored under conditions of disturbance and climate change; therefore, rapid breaking of the grass stage of plants is desirable. However, this is variable and depends on habitat conditions; in certain populations, plants from higher altitudinal limits remain in a cespitose state for longer than those from lower altitudes (Viveros-Viveros *et al.*, 2009).

The objective of this study was to analyze the effect of the altitudinal condition on the seed quality and initial growth of *P. hartwegii* seedlings from two populations in the State of Mexico and one in the state of *Veracruz*. It is considered that the seeds produced by trees located in marginal, low and high altitude, zones will exhibit lower quality and, therefore, plants of lower growth, unlike seeds from trees located in intermediate altitudinal sites.

Materials and Methods

Plant material

P. hartwegii seed collection was carried out between 2017 and 2018, in two natural populations in the State of Mexico (Mount *Tlaloc* and *Nevado de Toluca*) and *Veracruz* (*Cofre de Perote*). In each population, several sites with different altitudinal levels were considered for the collection (Table 1). Cones (mature strobili) from 5 to 15 trees were collected at the sites, depending on the density, spatial distribution, and distance between them (100 m when possible). The selection of individuals was based on the presence of cones and the absence of pests.

Table 1. Geographic location of *Pinus hartwegii* Lindl. populations in the State of Mexico and *Veracruz* and number of trees sampled per site.

Population	Number of trees	Altitude (m)	Latitude	Longitude
Mount *Tlaloc*, State of Mexico	5	3 600	19°24’04.48’’	98°44’11.55’’
	5	3 700	19°24’52.11’’	98°44’29.66’’
Nevado de Toluca, State of Mexico	5	3 900	19°07’47.79’’	99°46’50.31’’
	5	4 000	19°07’25.66’’	99°46’43.77’’
	5	4 100	19°07’18.93’’	99°45’11.85’’
Cofre de Perote, *Veracruz*	15	3 400	19°31’13.33’’	97°09’49.65’’
	15	3 600	19°31’13.33’’	97°09’51.31’’
	15	3 800	19°30’19.42’’	97°09’31.37’’
	15	4 000	19°29’44.47’’	97°09’09.27’’

The cones collected from each tree were taken to the laboratory for seed extraction, where they were then placed in an air column to separate them by weight. The empty seeds (without megagametophyte or embryo) floated and were removed, whereas the filled seeds remained at the base of the column and were refrigerated (at 4 to 6 °C).

Germination

The study was conducted during the first week of February 2019, in the greenhouse of the Graduate Program in Forest Sciences of the *Colegio de Postgraduados*. The experiment was established in a completely randomized design, in which for each altitudinal level (nine in total) four replications of 100 seeds of *P. hartwegii* were used, for a total of 3 600 seeds.

First, the seed was germinated in 310 mL forest tubes (one seed per tube), previously filled with a mixture of peat moss, agrolite, and vermiculite (3:1:1, respectively). The seeds were kept on the surface of the substrate, inside a small hole, without covering them completely. Then, the tubers were placed in 54-cavity trays and maintained in greenhouse conditions, with an average temperature of 24±12 °C. The humidity of the substrate was kept constant by irrigation. Germination was recorded daily for a period of 30 days. Once the seed germinated, it was buried in the substrate.

The germination data were used to calculate: 1) The germination percentage (germination capacity (*GC*)=(germinated seeds/total seeds) 100), 2) The peak value (*PV*), i.e., the maximum value of the sum of the germination percentage divided by the number of days in the evaluation and the number of days to reach 50 % of the total germination achieved, and 3) the germination value (*GV*) as the peak value multiplied by the average germination (Capilla-Dinorin *et al.*, 2021).

The seedlings produced remained in greenhouse conditions, and irrigation was provided every four days or earlier when required.

Assessment of plant growth and development

For this phase of the experiment, in the first week of March 2019, a sample of 60 plants per altitudinal level was taken and distributed in four replications of 15 plants under a completely randomized design. The diameter at the base (*DB*) and the total height (*TH*) of each plant were measured. Subsequently, during the last week of October 2019, the number of cotyledonary leaves (*NCL*) was recorded; the breakage of the grass stage, that is, the emission and elongation of epicotyl, which was evaluated as the percentage of plants with (*PWCS*) and without (*PWOCS*) grass stage, and the diameter of the apical bud (*ABD*). The *DB* and *TH* were obtained with a Mitutoyo® digital vernier model 500-702 (±0.01mm) and Abacus® graduated ruler (±0.1 cm), respectively. The growth in *DB* and *TH* was calculated from the differences between the initial and final measurements.

Statistical analysis

The variable data were subjected to Shapiro-Wilk and Bartlett's tests in order to verify the assumptions of normality and homogeneity of variances, respectively, using the UNIVARIATE and GLM procedures, with the SAS/PC for Windows version 9.4 statistical package (SAS Institute, 2003). Since the *GC* data did not meet the assumptions of normality and homogeneity of variances, we chose to use the nonparametric Kruskal-Wallis test.

The other germination parameters met normality ($p \geq 0.0714$) and homogeneity of variances ($p \leq 0.3356$); therefore, they were analyzed using the GLM procedure of the SAS® statistical package (SAS Institute, 2003) with the model:

$$Y_{ijk} = \mu + R_i + P_j + A_{k(i)} + E_{ijk} \qquad (1)$$

Where:

Y_{ijk} = Feature value of the k^{th} altitude nested in the j^{th} population in the i^{th} repetition

μ = Effect of the overall mean

R_i = Random effect of the i^{th} repetition

P_j = Effect of the j^{th} population

A_k = Effect of k^{th} altitude within j^{th} population

e_{ijk} = Experimental error

The data for *DB*, *TH*, *NCL*, *ABD*, and *PWOCS* after checking the assumptions of normality ($p \geq 0.772$) and the homogeneity of variances ($p \geq 0.0994$) were analyzed with the MIXED procedure of the SAS® statistical package (SAS Institute, 2003) using the model:

$$Y_{ijk} = \mu + P_i + A_{k(i)} + E_{ijk} \qquad (2)$$

Where:

Y_i = Value of the characteristic of the k^{th} altitude nested in the i^{th} population

μ = Effect of the overall mean, P_i is the effect of the i^{th} population

A_k = Fixed effect of the K^{th} altitude within the i^{th} population

e_{ijk} = Experimental error

Finally, the separation of means was performed with the Tukey test (p=0.05).

Results and Discussion

Germination

The analysis of variance showed no significant differences between the three populations for the germination parameters evaluated, except for the peak value, while there were differences between altitudes in all parameters (Table 2).

Table 2. *P*-values for germination parameters of *Pinus hartwegii* Lindl. seeds from different populations and altitudes.

Source of variation	Germination capacity	Peak value	Germination value
Population	0.3791	0.0001	0.62
Altitude	0.0034	0.0004	0.0001

In general, the average percentage of germination capacity for the seeds of the three populations studied was above 85 %. Although there were no significant differences among the populations, those of *Nevado de Toluca* registered approximately 10 % less germination in relation to the other two populations (Table 3). The peak value showed differences between populations: the *PV* of the seeds from the *Cofre de Perote* were 11 % higher than those of the other two populations (Table 3). In any case, the favorable germination parameters with no statistically significant differences in the three populations suggest good seed quality. Similar results were recorded by Ortega-Mata *et al.* (2003) with germination values above 90 % for several populations of *P. hartwegii* located in the State of Mexico.

Table 3. Comparison of means (±standard error) of germination parameters of *Pinus hartwegii* Lindl. seeds from different populations and altitudes.

Altitude (m)	Germination capacity %	Peak value	Germination value
CP 3 400	95.45±2.82 a	3.08±0.11 c	10.79±0.14 e
CP 3 600	91.81±2.12 a	3.46±0.28 b	12.02±0.70 d
CP 3 800	94.54±2.82 a	3.12±0.17 c	10.97±0.68 e
CP 4 000	87.27±1.60 b	3.90±0.15 a	12.46±0.70 c
CP mean	92.27±3.67 a	3.52±0.38 a	11.56±0.79 a
MT 3 600	84.54±2.82 b	2.55±0.65 d	9.11±0.07 e
MT 3 700	98.18±2.12 a	3.71±0.22 b	13.58±0.70 b
MT mean	91.36±9.64 a	3.13±0.77 b	11.34±2.35 a
NT 3 900	90.90±1.97 b	3.55±0.28 b	12.17±0.41 c
NT 4 000	95.45±2.73 b	4.08±0.11 a	14.21±0.70 a
NT 4 100	50.90±2.75 a	1.65±0.21 e	3.31±0.07 f
NT mean	79.08±24.51 a	3.11±1.15 b	9.89±5.04 a
Overall	87.6	3.2	10.9

CP = *Cofre de Perote*; MT = Mount *Tlaloc*; NT = *Nevado de Toluca*. Different letters in the same column (populations and altitudes) indicate significant differences ($p \leq 0.05$).

In the *Cofre de Perote*, there are germination percentages of 80 to 95 % (Tejeda-Landero *et al.*, 2019). Germination percentages above 75 % indicate good seed quality (Capilla-Dinorin *et al.*, 2021). In this regard, studies on seeds from a seed orchard of *Pinus leiophylla* Schiede ex Schltdl. & Cham. showed that both the peak and germination values are good indicators of the quality of the seed produced (Gómez *et al*., 2010). In the present investigation, the germination capacity recorded in the three populations may reflect good site quality. Nutrient-rich sites favor seed production by providing the necessary resources for seed development (Lauder *et al.*, 2019).

In the selection and establishment of seed areas, the quality of the site is a factor to be considered, since it ensures the production, in quantity and quality, of the seeds of the trees conserved in these areas (Manzanilla-Quiñones *et al.*, 2019). In addition to the site, certain environmental variables —particularly precipitation— influence the reproductive processes that define seed formation and production (Andrade-Gómez *et al*., 2021). Therefore, it will be necessary to monitor these factors in order to obtain more information regarding their impact on the reproduction and conservation of the species.

Although the results obtained could rule out any problem affecting seed quality by reduced populations of *P. hartwegii* (Capilla-Dinorin *et al.*, 2021), a more detailed study of various forest census aspects is required in the three populations in order to analyze the status of reproductive indices and their relationship with natural regeneration processes.

Regarding the altitudinal levels, all the values of the parameters studied were also high and indicative of a good quality of the seeds produced in stands located at different altitudes, except for the *Nevado de Toluca* stand, in the highest region (4 100 m). At this site, the germination capacity, the peak value, and the germination value of seeds decreased by more than 42, 36, and 63 %, respectively, compared to the values recorded at the other altitudes (Table 3).

A study on the relationship between environmental variations at different altitudes and the reproductive attributes of *Pinus pseudostrobus* Lindl. determined that seed germination of populations located at their upper altitudinal limits decreased in a ratio of approximately 3:1 with respect to those at intermediate limits; drought stress was identified as one of the causes of the decrease (López-Toledo *et al.*, 2017).

Poor seed quality has been explained by poor soil moisture (Lauder *et al.*, 2019). At higher altitudinal gradients, such as at 4 100 m, soils are stony and have low water retention capacity; in addition, at these altitudes, frost and cold winds in the off-season cause damage and loss of reproductive buds (Trant *et al.*, 2018). Therefore, these abiotic stress factors could cause the poor quality of seed produced at the 4 100 masl site.

These results show that the altitude influences the reproductive processes of *P. hartwegii*, which must be associated with the environmental variables that occur at each altitude and that cause stress directly impacting the populations. At altitudinal limits, environmental stress is more extreme, in addition, populations are more exposed (to snow, frost, and wind), and soils are generally poor in nutrients. Therefore, the conservation of *P. hartwegii* populations occurring near the alpine grassland, such as the one located at 4 100 m, requires implementing, in the medium and long term, management programs that will include prevention of logging, programmed fertilization in reproductive trees to improve the quality and quantity of the seed production, a study of the stand seed bank dynamics, and the protection of natural regeneration, as well as considering assisted regeneration.

Growth in *Pinus hartwegii* plants

The growth characteristics evaluated in *P. hartwegii* plants, except for the number of cotyledonary leaves, indicated significant differences between populations and altitudes (Table 4).

Table 4. ANOVA *p*-values estimated for the growth of *Pinus hartwegii* Lindl. plants from different populations and altitudes.

Source of variation	***NCL***	***Total height***	***Diameter at base***	***Apical bud diameter***	***PNOCS***
Population	0.06	0.0001	0.0001	0.0001	0.0001
Altitude	0.05	0.0001	0.0001	0.0117	0.0001

NCL = Number of cotyledonary leaves; *PNOCS*= Plants not in the grass stage.

The number of cotyledonary leaves, with an average of 5 cotyledons per seedling, did not vary between populations or altitudes. The lower altitudes (3 400 and 3 600 m) had slightly lower average values of cotyledons (Table 5). The lack of variation in the number of cotyledons is unusual, because in conifer species, during the early stages of development there is a large variation in the number of cotyledons: this is considered an important characteristic for the detection of genetic variation and it is related to the initial growth of the seedling (Romanovskii and Morozov, 2019).

Table 5. Means (±standard error) morphological and growth characteristics of *Pinus hartwegii* Lindl. seedlings from different populations and altitudinal levels.

Population /Altitude	*NCL*	*TH* (cm)	*DB* (mm)	*ABD* (mm)	*PNOCS* (%)
CP 3 400	4.83±0.11 a	9.21±0.40 a	6.76±0.20 de	4.42±0.17 a	51.00±2.50 c
CP 3 600	4.96±0.14 a	8.81±0.26 a	6.48±0.13 e	4.18±0.06 b	63.00±1.06 b
CP 3 800	5.29±0.21 a	9.82±0.32 a	7.07±0.15 d	4.30±0.09 ab	84.00±1.41 a
CP 4 000	5.09±0.08 a	8.76±0.27 b	7.17±0.22 d	4.37±0.14 a	81.00±1.42 a
CP mean	5.03±0.07 a	9.15±0.17 a	6.87±0.10 c	4.30±0.06 a	69.75±7.78a
MT 3 600	5.07±0.03 a	5.71±0.50 d	8.71±0.14 a	3.90±0.14 c	19.00±0.50 ef
MT 3 700	5.27±0.06 a	6.84±0.31 c	9.01±0.12 a	3.95±0.15 c	24.50±1.50 e
MT mean	5.19±0.04 a	6.32±0.33 b	8.86±0.10 a	3.97±0.10 b	22.10±2.75 c
NT 3 900	5.75±0.31 a	7.27±0.29 bc	8.06±0.19 b	3.93±0.06 c	45.50±1.63 c
NT 4 000	5.81±0.26 a	6.66±0.28 cd	8.52±0.09 b	4.12±0.17 bc	39.50±1.22 d
NT 4 100	5.55±0.05 a	5.48±0.24 d	7.95±0.21 bc	3.81±0.09 c	17.50±0.81 f
NT mean	5.75±0.43 a	6.65±0.24 b	8.18±0.11 b	4.28±0.07 a	34.20±8.51 b

CP = *Cofre de Perote*; MT = Mount *Tlaloc*; NT = *Nevado de Toluca*; *NCL* = Number of cotyledonary leaves; *TH* = Total height; *DB* = Diameter at the base; *ABD* = Apical bud diameter; *PNOCS* = Plants not in the grass stage. Different letters in the same column (populations and altitudes) indicate significant differences ($p \leq 0.05$).

In general, plants started to break the grass stage (epicotyl elongation) when they reached five months. At the end of the experiment, the percentage of breakage differed between populations and altitudes. Among the populations, the highest breakage percentage (approximately 70 %) was obtained with plants from the *Cofre de Perote*, exceeding by over 30 % those from Mount *Tlaloc*, which exhibited the lowest breakage percentage (Table 5). On the other hand, in regard to the altitudes, there was no trend in breakage between plants from lower and those located at higher altitudes; however, there was a significant difference between them. The lowest breakage percentages occurred at 3 600 masl on Mount *Tlaloc* and at 4 100 m on *Nevado de Toluca*. At both altitudes, the percentage of breakage was reduced by more than 70 %, compared to those of the *Cofre de Perote* (3 800 and 4 000 m), which had the highest values (Table 5).

Thus, it can be inferred that site conditions may have a greater influence than altitude on the breaking of the cespitose state of *P. hartwegii*. The cespitose state is considered to be an adaptive feature, acquired to survive fires and drought conditions (Robles-Gutiérrez *et al.*, 2016). The variation observed in the breaking of the cespitose state between populations and altitudes evidences the existence of different levels of genetic control in each population, probably influenced by the different conditions (temperature, humidity, fire incidence) prevailing at each altitude (Harsch and Bader, 2011).

The advantages and disadvantages of the rapid breaking of the grass stage should be evaluated under field conditions, where the vulnerable *P. hartwegii* cespitose plants are exposed to changes in temperature, drought and competition between species in the ecosystem. Although, the cespitose condition confers resistance to abiotic stress factors (Dixit *et al.*, 2020), it has also been documented that, if the grass stage is prolonged for a few years, the survival of the plants will be at risk due to ecological competition from fast-growing species (Jin *et al.*, 2019). It was also demonstrated that under drought conditions, *Pinus palustris* Mill. plants at the grass stage were very vulnerable because their water potential, cell turgor and photosynthetic capacity declined rapidly when the water stress was prolonged (Hart *et al.*, 2020). Plants with a prolonged grass stage at 3 600 m on Mount *Tlaloc* and at 4 100 m on *Nevado de Toluca* could be in trouble if exposed to frequent drought and competition events.

P. hartwegii plants from the *Cofre de Perote* stood out for their height growth. These were 25 % taller, and their apical bud size, over 85 % larger, than those of

Mount *Tlaloc* (the shortest among the populations), but their diameter was smaller by over 20 % compared to those of *Nevado de Toluca* (Table 5). Benavides *et al.* (2011) indicate a similar difference in diameter and height growth in *P. hartwegii* plants from the *Cofre de Perote* with respect to populations of the State of Mexico and Mexico City. This is explained by the rapid breaking of the grass state of the plants of the *Cofre de Perote*, which allowed them to grow faster and take better advantage of their resources. In plants that emerge more quickly from the grass stage, most of the reserve substances are destined for growth in length (epicotyl elongation) and apical bud development, while plants at the grass stage with little or no growth in height allocate their resources to the growth of the diameter (at stem base) and root (O'Brien *et al.*, 2008).

A study on the grass stage of *P. palustris* seedlings concluded that the plants decrease in vigor if the grass stage remains for several years (due to competition with other species and to diseases), with the consequent threat to their survival; in addition, stress factors such as drought prolong the cespitose condition for several years (Jin *et al.*, 2019).

As for the diameter of the apical bud, the plants from Mount *Tlaloc* were slightly smaller (3.9 mm) than those of the other two populations (>4.0 mm). This coincides with the grass state breakage parameter, which was lowest in the plants of Mount *Tlaloc*. Apical bud size may indicate increased mitotic activity prior to hypocotyl elongation, both of which are processes influenced by the environment. In particular, conifer populations tend to be genetically differentiated, and their growth patterns, such as bud formation and elongation, adapt to changes in ambient temperature ranges to avoid severe damage and cell death (Dixit and Kolb, 2020).

Therefore, the populations of *Nevado de Toluca*, located at higher extreme altitudes (4 100 m), would be at greater risk, not only because of poor germination, low cespitose state breakage, and slow growth, but also because of the predictions of the climatological models for this location, which forecast low rainfall and high temperature conditions (Manzanilla *et al*., 2019).

Conclusions

Pinus hartwegii seeds produced by the trees of the three populations studied exhibit a good quality, regardless of the altitudinal level of the site. Low-quality seeds were produced only by trees located at the highest altitude site (4 100 m) on *Nevado de Toluca*, which suggests that more attention should be paid to the analysis of the causes of seed deterioration among these populations. The acceleration of the breaking of the grass stage and the onset of height growth of *P. hartwegii* seedlings depend on the population and the altitudinal level and reflect an adaptive variation to the conditions of the site.

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Conflict of interest

The authors declare that they have no conflict of interest.

Contribution by author

José Luis Sánchez Mendoza: development of the research, data collection, statistical analysis, structure and design of the manuscript; Marcos Jiménez Casas: design, supervision of the experiment, results analysis, drafting and editing of the manuscript; Carlos Ramírez Herrera: design, supervision of the experiment, advice and editing of the manuscript; Héctor Viveros-Viveros: supervision, advice and editing of the manuscript.

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