Differential responses to drought in varieties of *Pinus pseudostrobus* sensu lato: An analysis of genetic variation and phenotypic plasticity

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Abstract

The increasing drought frequency and intensity requires a deeper understanding of adaptive mechanisms in forest trees. This study investigated drought responses in *Pinus pseudostrobus* sensu lato, by analyzing genetic variation and phenotypic plasticity across 60 progenies from five varieties (*pseudostrobus, oaxacana, apulcensis, coatepecensis*, and *estevezii*). Growth, survival, and morpho-anatomical traits of seedlings were assessed under two watering regimes in a common garden trial. Drought reduced seedling growth and altered leaf structure with pronounced effects on height and diameter growth, needle length and thickness, stomatal characteristics, and specific leaf area. Response varied both among and within varieties, with *estevezii* and *apulcensis* showing better performance under drought, expressing more conservative, xerophytic traits and adaptive plastic responses. Correlations between these traits and seedling performance strengthened under drought conditions, highlighting their adaptive value and suggesting that water deficit enhances expression of functional trait-performance relationships. Importantly, differences in trait expression and performance were linked to the climatic conditions of origin, highlighting the role of environmental niche in shaping genetic differentiation and adaptive strategies. Phenotypic plasticity was most evident in foliage developed during drought and varied by trait and family, suggesting genetic effects. Plasticity tended to be negatively associated with seedling performance under drought, but positively under well-watered conditions, indicating a trade-off between productivity and stress tolerance. These results provide strong support for ecotypic differentiation in *P. pseudostrobus* and emphasize the importance of incorporating genetic diversity and local adaptation into forest management, breeding, and conservation strategies to address challenges posed by climate change.

Keywords: Adaptation, genetic differentiation, genetic resources, morpho-anatomical traits, performance, water deficit.

Introduction

Pinus pseudostrobus Lindl. is a species of significant economic and ecological value (Cambrón-Sandoval et al. 2013). Its main distribution range is in Mexico, extending into Guatemala, Honduras, and northern El Salvador (Perry 1991). In Mexico, it is found in the central (Neovolcanic Axis) and southern regions (Sierra Madre del Sur), up to the north in the states of Durango and Nuevo Leon (Farjon and Styles 1997). Its wide and fragmented distribution, combined with hybridization and introgression with other phylogenetically related taxa (Delgado et al. 2007), has led to considerable intraspecific variability, observed in various studies that have analyzed different traits (e.g. Viveros-Viveros et al. 2006, Barrera-Ramírez et al. 2024, Escobar-Alonso et al. 2024). Consequently, the species has been the subject of taxonomic debates, particularly regarding the delimitation of intraspecific variation and characterization of its varieties (Stead 1983, Escobar-Alonso et al. 2023).

The Mexican botanist Maximino Martínez (1948) identified five morphologically distinct varieties (*pseudostrobus, oaxacana, apulcensis, coatepecensis,* and *estevezii*). Currently, the World Checklist of Vascular Plants (WCVP) only recognizes *P. pseudostrobus* var. *pseudostrobus* and *P. pseudostrobus* var. apulcensis (Lindl.) Shaw, treating the other varieties as synonyms. The *oaxacana* variety was considered a distinct species (*Pinus oaxacana* Mirov) due to differences in cone morphology and resin chemistry (Mirov 1958), although it was later reaffirmed as a *P. pseudostrobus* variety (Harrison 1965); Perry (1991), on the other hand, accepted it as the species proposed by Mirov, though later, Farjon and Styles (1997) considered it a synonym of the apulcensis variety. The *coatepecensis* variety, accepted by Perry (1991) and later rejected by Farjon and

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Styles (1997), is currently considered a synonym of the pseudostrobus variety according to the WCVP. Likewise, the estevezii variety is considered a synonym of the typical variety, although in 1982 was considered a distinct species (*Pinus estevezii* (Martínez) J.P. Perry), with an isolated distribution range restricted to northern Mexico (Perry 1982).

Despite the taxonomic controversy, the varieties initially proposed by Martínez are still used operationally and in research conducted in Mexico (e.g. Viveros-Viveros et al. 2006, Barrera-Ramírez et al. 2021, Baeza-Guzmán 2023). Studies have demonstrated intervarietal differentiation using both morphological (Escobar-Alonso et al. 2023) and molecular markers (García-Valencia et al. 2022), supporting their taxonomic distinction. As these differences may have an adaptive significance, it is necessary to assess the extent of inter- and intravarietal variation in *P. pseudostrobus* sensu lato to better manage its genetic resources, especially in the context of ongoing global climate change.

Global climate change models predict more frequent and intense droughts in many regions this century (Cook et al. 2018). For central Mexico, Carrillo-Arizmendi et al. (2022) highlight rising average annual temperature in forest ecosystems and identify adaptive responses associated with drought stress. In this context, genetic variation (GV) and phenotypic plasticity (PP) are key evolutionary mechanisms enabling forest species to cope with environmental fluctuations (de la Mata et al. 2022); they are critical for modeling species' responses to drought events, expected to become more frequent and intense in the coming decades (Anderegg et al. 2022). However, data on GV and PP in *P. pseudostrobus*, particularly at early developmental stages, remain limited, making it crucial to conduct more experimental studies (Valladares et al. 2014).

In a previous study, we identified significant GV and PP in annual shoot growth pattern and morpho-anatomical traits related to drought adaptation in *P. pseudostrobus*, primarily associated with the aridity index of progeny origin (Escobar-Alonso et al. 2024). We also found considerable genetic variation in the phenotypic plasticity of progenies grown under different climatic and edaphic conditions. The present study evaluates drought effects under controlled conditions on the early-life response of progenies within the five varieties of P. pseudostrobus proposed by Martínez (1948). The initial development stage is critical for plant survival and future adaptability, making it easier to detect genetic variation in stress response (Moran et al. 2017). We estimated phenotypic plasticity using two different approaches to address the following objectives: (1) assess drought effects on survival, growth and adaptative morpho-anatomical traits; (2) estimate inter- and intravarietal genetic variation in drought response; (3) evaluate how water availability influences structure of phenotypic correlations between performance and morpho-anatomical traits; (4) examine the relationship between phenotypic response to drought and climatic conditions at origin location; and (5) determine inter- and intravarietal variation in phenotypic plasticity in response to drought.

Materials and Methods

Genetic material

The study included seedlings from 60 progenies of the five varieties (Martínez 1948) of *Pinus pseudostrobus* [24 from *pseudostrobus* (typical variety), 16 from oaxacana, 11 from apulcensis, 5 from *coatepecensis*, and 4 from *estevezii*]. Seeds were collected from natural populations in the states of Chiapas, Oaxaca, Puebla, Veracruz, Hidalgo, State of Mexico, Michoacan, and Nuevo Leon (Figure 1). For the first four varieties, progenies came from individual open-pollinated families; for estevezii, seeds were mixed from several mother trees per location. However, for the purposes of this study, all progenies were treated as open-pollinated families to ensure consistency across varieties. The origin locations spanned from 16.67° to 25.36º N latitude, 92.16° to 102.19º W longitude, and 1507-2980 m in elevation. Basic geographical and climatic information is provided in Table S1.

Trial establishment

The common garden trial was established at the Colegio de Postgraduados forest nursery in Montecillo, State of Mexico (19°27'37.5" N, 98°54'23.7" W, 2240 m). On January 25, 2021, the seedlots were sown in 310 cm^3 containers filled with a substrate composed of sphagnum peat, composted pine bark, perlite, and vermiculite (2:1:1:1 ratio). The mix was enriched with 7 g L^{-1} of Multicote 8® (slow-release fertilizer) and 1 g L^{-1} of *Trichoderma* spp. spores in a rice matrix per liter of water, to reduce the risk of fungal diseases. Peat and pine bark were heat-sterilized (105°C) for 7 hours prior to use. After nine months, seedlings (~20 cm tall) were transplanted into raised nursery beds (10 x 1.5 x 1.2 m) at 20 x 20 cm spacing between plants. Nursery beds were filled with local agricultural soil (loamy sand texture) to 90 cm depth, with a 10 cm layer of volcanic rock at the bottom for drainage. A randomized complete block design (five blocks per bed) with single-tree plots was used.

In June 2022, two watering regimes were applied, with two beds (10 blocks) assigned to each treatment. In the well-watered (WW) regime, irrigation was applied periodically to maintain the soil's volumetric moisture content near field capacity (~23 %). In contrast, for the water-stress (WS) regime, a transparent plastic cover was installed 1.8 m above the plants to exclude rain, and irrigation was suspended for several weeks until the soil's volumetric moisture content reached the permanent wilting point (~8 %). Thereafter, light irrigations kept moisture content between 7-13 %. The drought treatment ended on December 8, 2022, with rewatering to field capacity, six months after the treatments began.

Soil moisture at 20-cm depth was periodically recorded in both treatments using a portable FieldScout® TDR-300 (Figure 2). Measurements were taken at four sampling points per block in each watering regime (40 points per treatment), just before the well-watered (WW) treatment was irrigated, reflecting the minimum moisture content for that treatment

throughout the experiment. Due to dense foliage in the WW treatment, monitoring ceased after day 120, under the assumption that weekly irrigations maintained soil moisture close to field capacity (Figure 2).

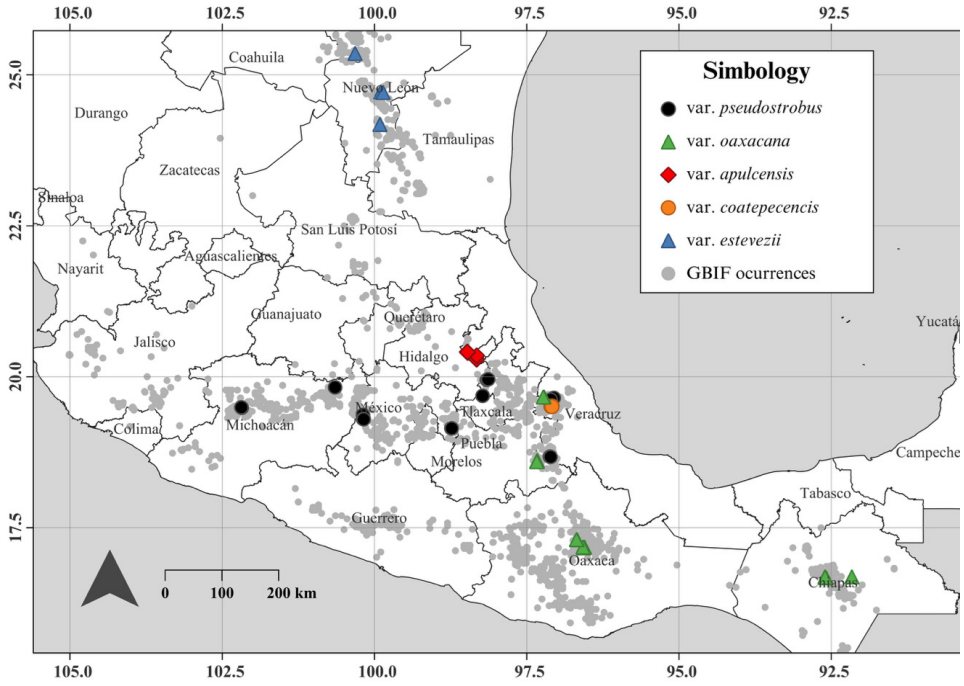

Figure 1
Geographical location of the origin sites of progenies for the varieties included in the study and information on the occurrences of *Pinus pseudostrobus* sensu lato in the Global Biodiversity Information Facility. Source: GBIF.org. Some points may overlap due to the map scale.

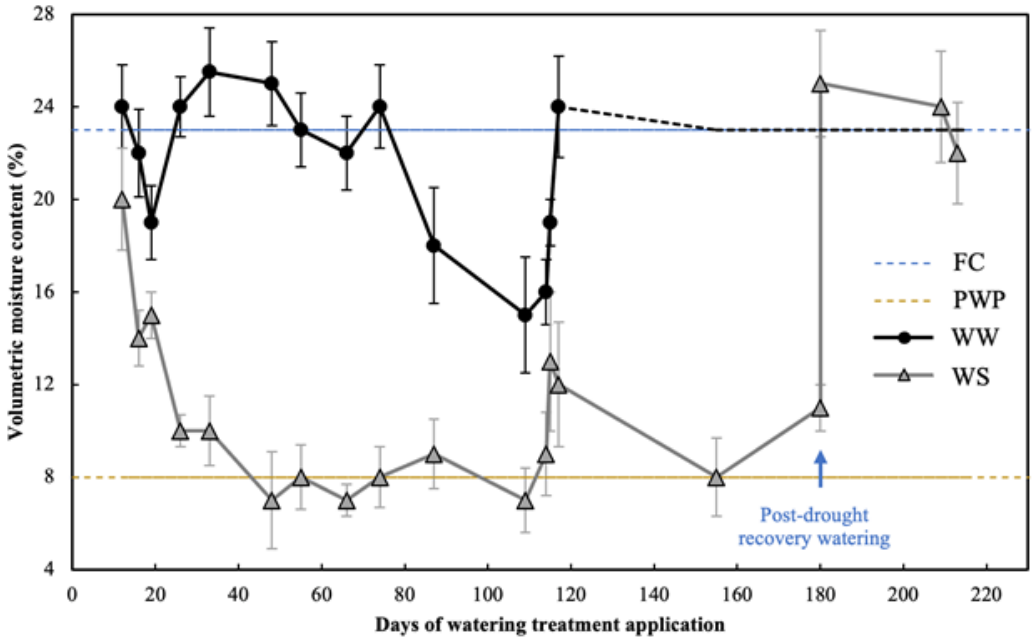

Figure 2
Changes in the average (± standard deviation) volumetric moisture content at 20-cm soil depth recorded during the two watering treatments in the drought response trial of progenies from different varieties of *Pinus pseudostrobus* sensu lato. The black dashed line indicates the assumed volumetric moisture content in the control treatment after 120 days. The acronyms stand for: FC = Field capacity, PWP = Permanent wilting point, WW = Well-watered treatment, WS = water stress treatment (drought).

Traits evaluated

We evaluated seedling survival and growth, and morpho-anatomical foliage traits. Height and basal stem diameter were measured when treatments started. A mark was placed at the seedling tip to identify the foliage formed before (section 1) and during (section 2) the application of watering regimes. At the end of the drought period, height and diameter were measured again to determine height growth (ΔH), diameter growth (ΔD) and survival (Surv).

To evaluate the morpho-anatomical foliage traits, measurements were taken 40-days after post-drought watering. In each seedling, the number of fascicles present in 3-cm of the middle part of stem sections 1 and 2 (previously marked) was counted, and based on this, the mean stem-unit length (MSLU, mm) in each section was estimated. Six fascicles per section were sampled (two each from the lower, middle, and upper parts). For each fascicle, the diameter at the base was measured with a caliper (Jiavarry IP54), and the length of the longest needle (NL, cm) was measured with a millimeter ruler. Needle thickness (NT, mm) was estimated as half the fascicle diameter, considering its circular cross-section at the base. Specific leaf area (SLA, $cm^2\ g^{-1}$) was estimated from a 5-cm length sample in the middle zone of each stem section. Leaf surface area (cm^2) was calculated by adding the surface areas of segments per fascicle, based on diameter, length, and needle geometry; dry weight (g) of needles was obtained with an analytical balance (Chyo JK200) after drying the samples in an oven at 70°C for 72 hours.

Two needles per section, each taken from a different fascicle in the midportion of sections 1 and 2, were used to determine the number of stomata lines (LS, n.) and the number of stomata within 3 mm of the central line on the dorsal needle side, using a stereoscopic microscope (Olympus SZ51). From these counts, the number of stomata per millimeter of line [NS, (n. mm^{-1})] and the total number of stomata per millimeter on the dorsal side [TS, n. mm^{-1} = (NS * LS)] were calculated.

Statistical analysis

To evaluate drought effects and estimate variance components among and within varieties, a linear mixed-effects model was used, and variance analysis was performed using the MIXED procedure with the REML method of Statistical Analysis System (SAS). The statistical model used was:

$$Y_{ijkl}=\mu+T_i+B_{j(i)}+Vk+T_i\times V_k+F_{l(k)}+T_i\times F_{l(k)}+\boldsymbol{\varepsilon}_{ijkl}$$

where Y_{ijkl} is the observation of *ijkl*-th tree, μ is the overall mean, T_i is the fixed effect of *i*-th watering treatment, $B_{j(i)}$ is the fixed effect of *j*-th block nested within *i*-th treatment, V_k is the random effect of $_k$-th variety ~NID (0, σ^2_{var}), $F_{l(k)}$ is the random effect of *l*-th family nested within *k*-th variety ~NID (0, $\sigma^2_{fam(var)}$), $T_i\times V_k$ and $T_i\times F_{l(k)}$ are the random effects of the corresponding interactions ~NID (0, $\sigma^2_{trat\times var}$ and 0, $\sigma^2 trat\times_{fam(var)}$ respectively), and εijkl is the experimental error term. In a second analysis to determine the varieties response to moisture treatments, the variety factor (V_k) and its respective interaction with drought ($T_i\times V_k$) were considered as fixed effects. Means were estimated using the LSMEANS statement in PROC MIXED.

For growth traits, ΔH and ΔD, initial height was included as a covariate in the model to separate the effect of differences in initial plant size. Count variables (SL, NS, TS) were square root transformed to meet the normality assumptions. Means were calculated using the original values.

To evaluate the effect of moisture conditions on phenotypic correlations between performance and morpho-anatomical traits, the family-mean values for traits in each set of variables and watering regime was calculated. Subsequently, the degree of correlation was determined using PROC CORR in SAS. Correlation values and their significance were graphically represented using the RStudio program, employing the *corrplot* library.

To relate progeny drought response and climatic conditions at their place of origin, several climatic variables were selected. Initially, 24 climatic variables from the 1991-2020 climate normal were extracted from the ClimateNA v7.30 model (Wang et al. 2016); additionally, the aridity index (Rehfeldt et al., 2009) was included. To avoid multicollinearity, variables were filtered to ensure that correlation coefficients (r) between them remained below 0.80. In cases of high correlation ($r \geq 0.80$), the variable with greater biological relevance to drought stress and plant survival was retained. This selection resulted in five variables: mean annual temperature (MAT), relative humidity (RH), reference evapotranspiration (EREF), continentality (CONT, temperature difference between average temperature of the hottest and the coldest months), and aridity index (AI). Principal component analysis was performed using the prcomp function from the stats package and the *ggbiplot* function from the *ggplot2* package in R (v 4.0.3) to describe the climatic variation at the origin location of progenies included in the trial. A canonical correlation analysis was also conducted using the CANCORR procedure in SAS between the set of average trait values for progenies in the water stress treatment and the set of selected climatic variables.

Estimation of phenotypic plasticity

Phenotypic plasticity was evaluated using two distinct approaches, to distinguish between "spatial plasticity" and "temporal plasticity" of traits.

Spatial plasticity: This approach evaluates plasticity as the relative change in phenotypic values between seedlings in the WS treatment and those of the same family in the WW treatment. To quantify this plasticity, the relative distance plasticity index was calculated for each individual ($RDPI_i$) of each family in the WW treatment, as the mean of the absolute differences in the phenotypic trait value evaluated in that individual and each of its half-siblings established in the WS treatment ($d_{ij}\rightarrow i'j' = |\ x_{i'j'} - x_{ij}\ |$), divided by the sum of the respective pairs of values ($x_{i'j'} + {}_{xij}$), as shown in the following equation (Valladares et al. 2006):

$$\mathrm{RDPI}_i = \sum \frac{(d_{ij} \rightarrow i'j')/(x_{i'j'} + x_{ij})}{n}$$

Table 1
Mean values ($\bar{X}$) by watering treatment (well-watered [WW] and water-stress [WS]) and their standard error, and proportion of variance components among and within varieties, and their significance in the common garden trial of *Pinus pseudostrobus* sensu lato progenies

	X		Variance components (%)				ε
Trait	WW	WS	σ^2_{var}	$\sigma^2_{trat \times var}$	$\sigma^2_{fam(var)}$	$\sigma^2_{trat \times fam(var)}$	σ^2
Performance-related traits							
Surv (%)	94.4 ± 6.7[a]	77.7 ± 6.7[b]	8.6	11.2*	0.4	2.5	77.3
ΔH (cm)	26.4 ± 1.5[a]	7.4 ± 1.5[b]	2.2	7.6	8.1**	10.4***	71.7
ΔD (mm)	4.1 ± 0.4[a]	1.9 ± 0.4[b]	18.3	4.3	5.3**	1.7	70.4
Morpho-anatomical traits in stem section 1 (formed before the watering treatments)							
$MSLU_1$ (mm)	2.36 ± 0.12[a]	1.84 ± 0.13[b]	9.8	1.3	3.1*	0.0	85.6
NL_1 (cm)	22.7 ± 0.82[a]	17.4 ± 0.83[b]	19.7	1.1	19.9***	0.0	59.2
NT_1 (mm)	0.84 ± 0.04[a]	0.67 ± 0.04[b]	44.7*	1.7	17.7***	0.1	35.7
LS_1 (n.)	4.6 ± 0.29[a]	3.9 ± 0.29[b]	29.0*	0.0	9.5***	1.4	60.1
NS_1 (n. mm^{-1})	20.2 ± 0.38[a]	19.4 ± 0.39[b]	8.0	0.0	10.2***	1.8	80.1
TS_1 (n. mm^{-1})	91.4 ± 4.6[a]	75.3 ± 4.6[b]	21.3*	0.1	7.6**	0.7	70.3
SLA_1 (cm2 g^{-1})	171.0 ± 10.2[b]	190.0 ± 10.2[a]	41.2*	2.9	13.2***	1.0	41.6
Morpho-anatomical traits in stem section 2 (formed during the watering treatments)							
$MSLU_2$ (mm)	2.61 ± 0.06[a]	1.59 ± 0.07[b]	1.4	0.8	0.0	4.4*	93.3
NL_2 (cm)	20.3 ± 0.8[a]	13.1 ± 0.9[b]	15.0	0.0	12.6***	0.0	72.4
NT_2 (mm)	0.77 ± 0.03[a]	0.62 ± 0.04[b]	34.1*	1.6	17.5***	0.0	46.8
LS_2 (n.)	4.7 ± 0.3[a]	3.5 ± 0.3[b]	32.6*	0.0	12.7***	0.0	51.1
NS_2 (n. mm^{-1})	18.7 ± 0.5[a]	18.8 ± 0.5[a]	18.7	0.3	6.1*	2.7	72.3
TS_2 (n. mm^{-1})	87.9 ± 4.0[a]	64.9 ± 4.1[b]	20.4*	0.0	5.2**	0.0	74.4
SLA_2 (cm2 g^{-1})	176.5 ± 12.8[b]	221.9 ± 12.9[a]	34.9*	4.3	12.3***	5.5**	42.9

Means and standard errors with different letters as superscripts are significantly different with $p < 0.05$. Asterisks indicate the significance of the respective variance component where *$p \leq 0.10$; **$p \leq 0.05$; ***$p \leq 0.01$. Surv = Survival, ΔH = Height increase, ΔD = Base stem diameter increase, MSLU = Mean stem length unit, NL = Needle length, NT = Needle thickness, LS = Lines of stomata, NS = Number of stomata in the central line of the dorsal side, TS = Total stomata in 1 mm length on the dorsal side, SLA = Specific leaf area

Table 2
Average values of *Pinus pseudostrobus* sensu lato varieties in the well-watered treatment (WW) and relative change (%) in mean value at the water-stress treatment (WS) compared to WW.

Trait	Varieties				
	pseudostrobus	*oaxacana*	*apulcensis*	*coatepecensis*	*estevezii*
Performance-related traits					
Surv (%)	95.0 (-38.6)	96.9 (-6.5)	98.2 (-7.4)	84.0 (-37.5)	97.5 (-0.6)
ΔH (cm)	28.6 (-79.1)	31.3 (-72.4)	26.7 (-66.7)	21.5 (-78.3)	20.8 (-61.1)
ΔD (mm)	3.43 (-54.0)	4.64 (-53.7)	5.73 (-54.0)	2.59 (-66.3)	3.82 (-38.7)
Morpho-anatomical traits in stem section 1 (formed before the watering treatments)					
$MSLU_1$ (mm)	2.15 (-20.5)	2.56 (-28.0)	2.45 (-14.1)	2.02 (-37.6)	2.61 (-18.3)
NL_1 (cm)	22.1 (-29.0)	23.7 (-22.1)	24.3 (-21.0)	19.9 (-28.1)	23.0 (-18.2)
NT_1 (mm)	0.76 (-21.3)	0.88 (-21.3)	0.92 (-19.0)	0.73 (-27.3)	0.88 (-12.3)
LS_1 (n.)	4.2 (-11.8)	4.3 (-16.6)	5.2 (-15.1)	3.8 (-9.9)	5.6 (-16.5)
NS_1 (n. mm^{-1})	20.6 (-3.4)	19.7 (-5.2)	19.8 (-3.7)	21.8 (-6.3)	19.1 (-0.8)
TS_1 (n. mm^{-1})	85.0 (-14.6)	82.7 (-21.0)	102.5 (-18.8)	83.5 (-15.9)	105.2 (-17.0)
SLA_1 (cm^2 g^{-1})	188.4 (13.6)	161.7 (13.8)	157.6 (9.2)	193.7 (15.7)	154.6 (1.1)
Morpho-anatomical traits in stem section 2 (formed during the watering treatments)					
$MSLU_2$ (mm)	2.70 (-44.6)	2.63 (-37.9)	2.70 (-35.6)	2.28 (-41.2)	2.47 (-33.4)
NL_2 (cm)	19.5 (-38.0)	21.9 (-33.8)	22.3 (-30.9)	18.2 (-48.6)	19.5 (-36.1)
NT_2 (mm)	0.69 (-16.8)	0.82 (-21.7)	0.86 (-17.8)	0.67 (-22.4)	0.81 (-15.7)
LS_2 (n.)	4.2 (-27.4)	4.4 (-26.2)	5.3 (-24.0)	3.9 (-32.8)	6.1 (-29.6)
NS_2 (n. mm^{-1})	19.6 (-1.3)	18.7 (-1.1)	18.4 (1.6)	20.0 (4.5)	16.5 (6.9)
TS_2 (n. mm^{-1})	82.5 (-27.4)	80.8 (-27.7)	96.8 (-24.7)	80.7 (-29.3)	100.2 (-24.9)
SLA_2 (cm^2 g^{-1})	196.4 (30.2)	164.6 (29.2)	159.6 (19.7)	202.2 (32.6)	161.2 (16.1)

In parentheses, the relative change (%) in the mean value per variety in WS compared to the mean value in WW. Surv = Survival, ΔH = Height increase, ΔD = Diameter increase at the stem base, MSLU = Mean of stem length unit, NL = Needle length, NT = Needle thickness, LS = Stomatal lines on the dorsal side, NS = Number of stomata per 1 mm of the central line on the dorsal side, TS = Total stomata per 1 mm of length on the dorsal side, SLA = Specific leaf area

The RDPI can take values ranging from 0 (no plasticity) to 1 (maximum plasticity) for each individual ($RDPI_i$) and family mean. The $RDPI_i$ values were subjected to analysis of variance to estimate the genetic variation in the expression of phenotypic plasticity among and within varieties for each trait. The statistical model used was similar to the one previously described, but without including the effects of drought treatments (T_i). Subsequently, variety-mean values were calculated and compared, and the correlation between family-means for the various traits was determined, as well as their relationship with average family performance.

Temporal plasticity: This approach measures the change in phenotypic attributes of foliage developed during the watering regimes compared to foliage formed before them. Phenotypic plasticity was estimated as the relative change of traits in stem section 2 (foliage formed during the watering treatments) with respect to those in section 1 (foliage formed before the watering treatments). This comparison allowed to evaluate phenotypic modifications within each individual at two different phenological times. These relative changes, expressed on a decimal scale, were subjected to analysis of variance using the complete statistical model described earlier. Additionally, variety means were estimated for each treatment to determine differences in plasticity due to phenology (in WW) and the combined effect of phenology and drought (in WS).

Results

Effect of watering regime

A significant effect ($p < 0.10$) of the drought treatment was found for all traits, except for the number of stomata in section 2 (NS2) (Table 1). Lower water availability in the WS regime reduced seedling survival by 17 % and generally caused decreased trait values, with greater effects in ΔH (72 %) and ΔD (54 %), followed by most of the morpho-anatomical traits in foliage formed during the drought period (stem section 2). On the other hand, specific leaf area of foliage formed before drought (SLA1) increased by 11 % under WS, but the effect was more pronounced in the foliage formed during drought, which showed a 20 % increase (Table 1).

Genetic variation among and within varieties in response to drought

For most performance and morpho-anatomical traits, variation among varieties (σ^2_{var}) was higher than within varieties ($\sigma^2_{fam(var)}$). On average, variance among varieties explains about 21 % of the total variance, while genetic variation within them contributed an average of 9 % (Table 1). The variation among varieties represents a large portion of the total variation in most traits, except for Surv, ΔH, NS_1, and $MSLU_2$, where it contributed less than 10 %. Within-variety variance was considerable for some traits, such as needle length (NL_1) and needle thickness (NT_1 and NT_2), contributing about 20 % of the total variance. Although the water deficit effect on morpho-anatomical traits was greater in foliage formed during the drought period, variance distribution for each trait was relatively similar in both stem sections (Table 1).

Though the relative contribution of variance among varieties was higher than that of family variance within them, in some cases it was not significant (Table 1), partly due to the strong imbalance in the number of families within each variety. The estimated σ^2_{var} was significant only for NT, LS, TS, and SLA in the foliage of both stem sections; on the other hand, the genetic variance within varieties was significant for most traits, except Surv and $MSLU_2$. Interaction variance was generally non-significant at both levels (Table 1); however, at the variety level, interaction was significant for Surv and contributed substantially to total variance in ΔH, ΔD, and SLA. At the family level, interaction variance was significant only for ΔH, $MSLU_2$, and SLA_2.

Response of varieties to watering regimes

Significant differences ($p < 0.10$) were found among variety-mean values for all traits under both watering treatments. Despite differences in the phenological stage, mean values in the two stem sections in WW treatment showed a similar pattern across varieties. Foliage traits in section 1 were strongly correlated with those in section 2 ($0.88 < r < 0.99$, data not shown), except for LMU ($r = 0.37$). For most traits, the relative change between watering regimes was smaller in the *apulcensis* and *estevezii* varieties (Table 2).

The performance trait with the greatest differentiation in relative change among varieties was survival (Surv), with a greater effect of drought on *pseudostrobus* and *coatepecensis* varieties. The *estevezii* variety had the highest survival under water-stress conditions (WS), with 97 % of seedlings alive by the end of the drought period, followed by the *oaxacana* and *apulcensis* varieties, both with over 90 %. In contrast, the effect of water shortage on height growth (ΔH) was greater in *pseudostrobus* and *coatepecensis*, with the latter also being the most affected in diameter growth (ΔD) (Table 2). Under WW conditions, *oaxacana* and *pseudostrobus* performed well in height growth (ΔH); however, under drought conditions, *estevezii* and *apulcensis* were least affected (Table 2). Drought effects on morpho-anatomical traits were greater in foliage formed during the drought period (Table 2), except for NT_2 and NS_2. The *coatepecensis* variety showed greater relative changes for most traits, while *pseudostrobus* and *coatepecensis* exhibited thinner needles and higher SLA in both stem sections. In contrast, *estevezii* exhibited the thickest needles and the lowest SLA under WS treatment.

The interaction between watering regimes and varieties was significant ($p < 0.05$) for Surv, ΔH, ΔD, needle thickness (NT_1 and NT_2), and specific leaf area (SLA_1 and SLA_2). However, this interaction generally showed as a change in magnitude of the drought effect across varieties, with few ranking changes, mostly involving the *estevezii* variety (Figure 3).

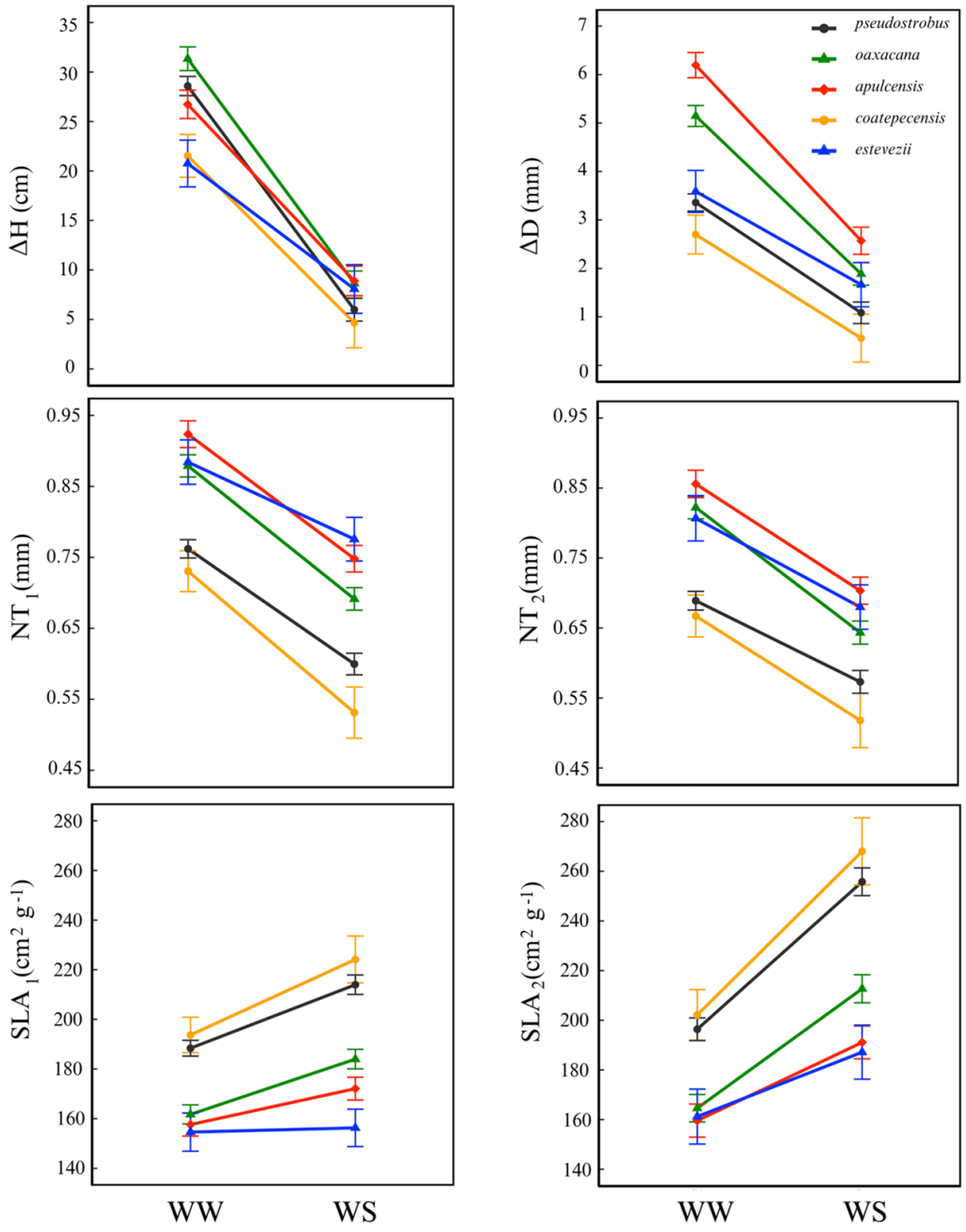

Figure 3
Genotype-environment (GxE) interaction for mean values of morpho-anatomical traits of *Pinus pseudostrobus* sensu lato varieties in the watering treatments (WW and WS). ΔH = Height increase, ΔD = Diameter increase at the stem base, NT = Needle thickness, SLA = Specific leaf area.

Effect of drought on phenotypic correlations

The watering regimes had an important effect on phenotypic correlations between morpho-anatomical traits and progeny performance. Correlations were much stronger under WS than under WW treatment (Figure 4), showing the adaptive role of the morpho-anatomical traits in supporting progeny performance under water deficit conditions. The mean stem-length unit, needle length (NL), needle thickness (NT), as well as the total number of stomata per unit length, particularly in section 2 (TS_2), showed a positive correlation with survival and growth, while specific leaf area showed a negative correlation with these traits (Figure 4).

Relationship between progeny performance and climatic conditions at the origin site

The principal component analysis of climatic variables showed that the first two components explain 75.4 % of the total variance. The first component (PC_1), accounting for 54.4 % of the total variation, was associated with mean annual temperature (MAT), reference evapotranspiration (E_{REF}), aridity index (IA), and continentality (CONT). The second component (PC_2), contributing 21.0 % of the total variance, was mainly associated with relative humidity (HR). In the PC_1-PC_2 plane (Figure 5), the origin sites of *estevezii* and *coatepecensis* varieties occupied the extremes of higher and lower temperature and potential evapotranspiration, respectively. Meanwhile, the other varieties were located in intermediate climatic habitats, with some overlap, although with different gradients relative to the two main components. For example, *apulcensis* families originated from cooler, more humid (mesic) sites, while *oaxacana* progenies followed a gradient in relative humidity, and *pseudostrobus* families cover an extensive range of temperature and humidity, located at the drier extreme relative to *oaxacana*.

The canonical correlation analysis between progeny-mean traits under WS treatment and climatic variables at the origin sites of families showed significance ($p < 0.05$) in the first canonical correlation, with a value of $r_c = 0.81$ ($r^2_c = 0.65$). Redundancy analysis showed that the first canonical variable (CV_1) of performance and morpho-anatomical traits explains 26 % of the variance of the original traits, while the CV_1 of climatic variables explains 17 %. The correlations between CV_1 and its traits indicate that the highest values of this canonical variable are mainly associated with higher survival, MSLU, NT, LS, TS, and lower SLA (Table S2). The highest values of the CV_1 for climatic variables were mainly associated, in order of contribution to their canonical variable, with higher CONT, AI, and MAT (Table S2). In the plane formed by the first canonical variables (CV_1) (Figure 6), the phenotypic and climatic differentiation of the *estevezii* and *apulcensis* varieties was most pronounced. Interestingly, CONT was prioritized in the climatic component of *apulcensis*, positioning it closer to *estevezii* in terms of both climatic and phenotypic drought response, despite contrasting AI and MAT values (Figure 6). Meanwhile, *pseudostrobus*, *oaxacana*, and *coatepecensis* shared several climatic and phenotypic traits, but also showed broad intravarietal variation with respect to the CV_1 of morpho-anatomical traits.

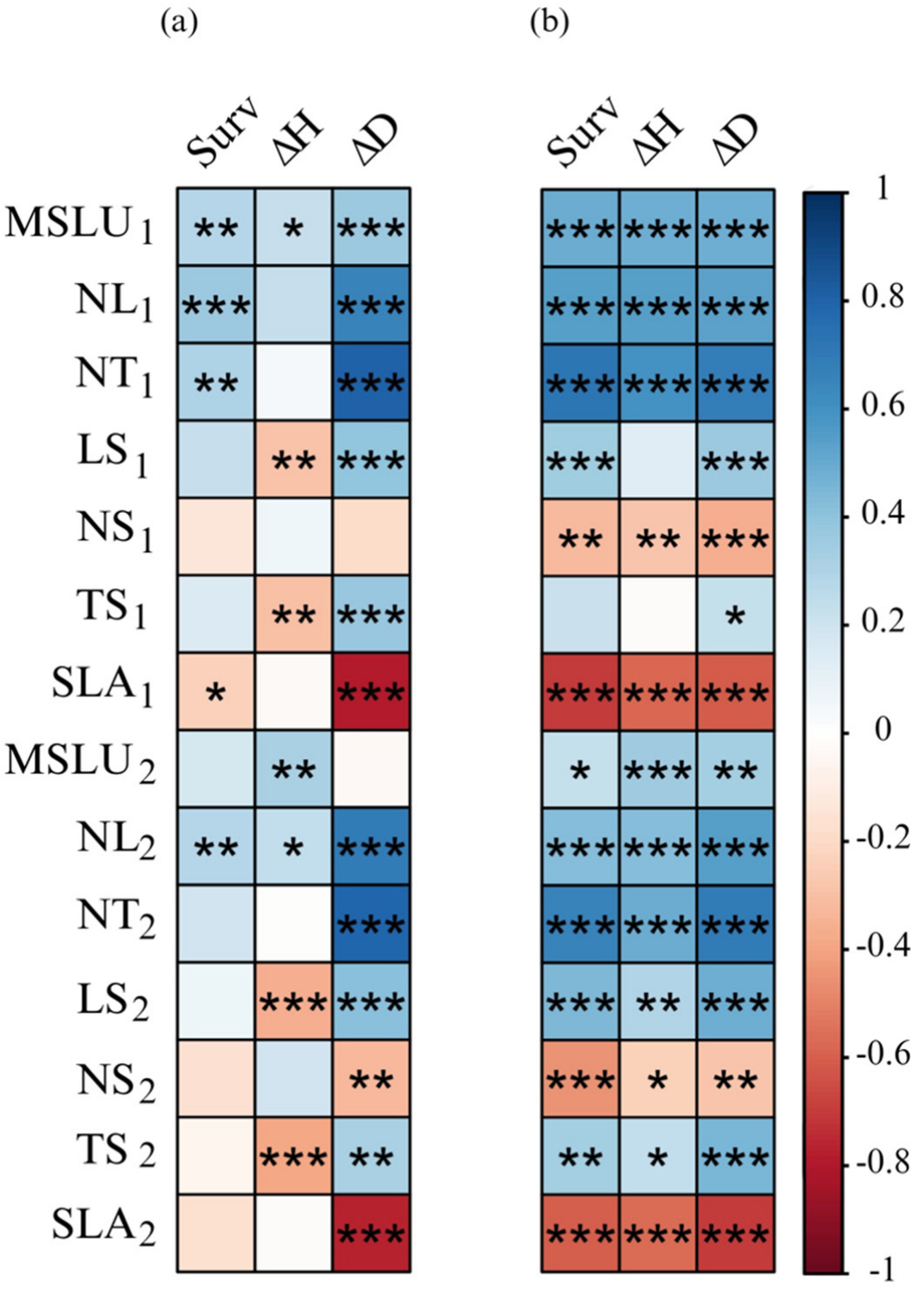

Figure 4
Family-mean correlations between morpho-anatomical traits of foliage formed before (stem section 1) and during the drought period (stem section 2) and seedling performance traits under (a) well-watered (WW) and (b) water-stress (WS) conditions, where *p < 0.10; **p < 0.05; ***p < 0.01.

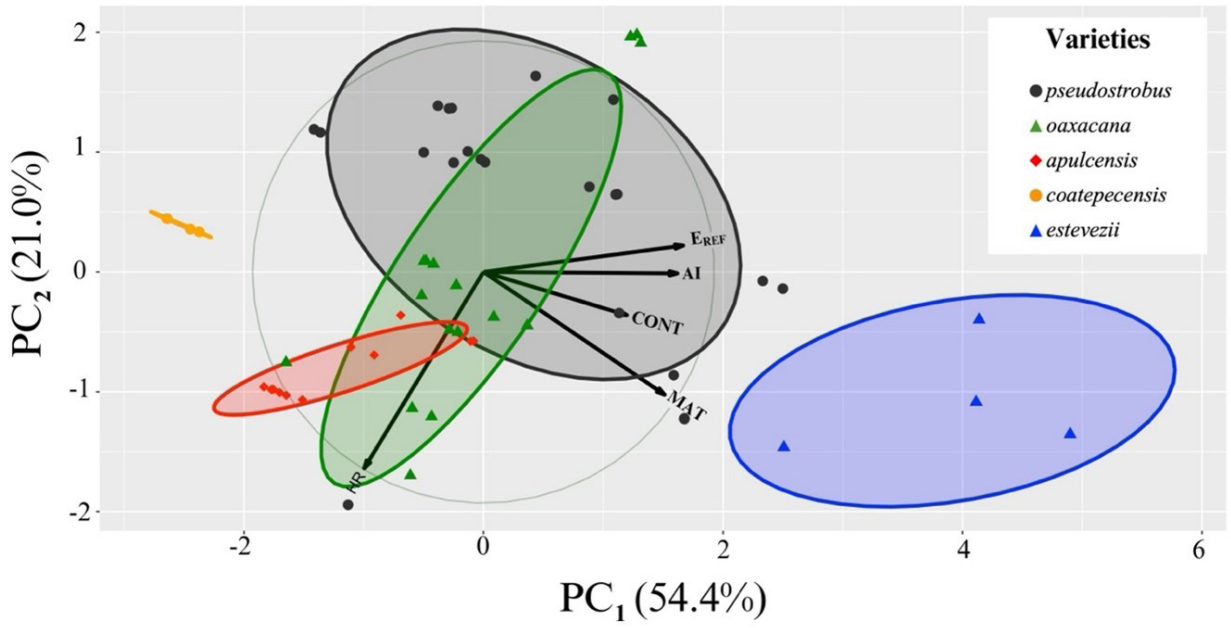

Figure 5
Graphical representation of the principal component analysis for the climatic habitat of progenies within the five *Pinus pseudostrobus* sensu lato varieties included in the trial.

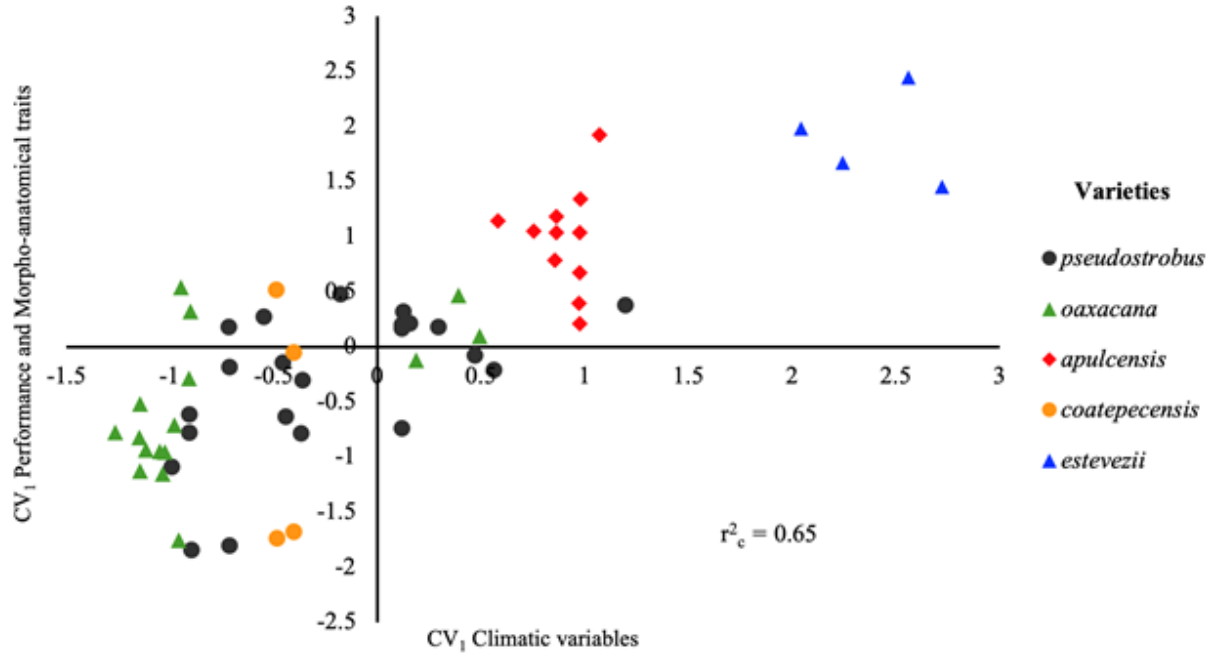

Figure 6
Graphical representation of the canonical correlation analysis using climatic information from the origin site of progenies of five *Pinus pseudostrobus* sensu lato varieties and their phenotypic means in performance and morpho-anatomical traits under water-stress conditions

Phenotypic plasticity

The variance component of the relative distance plasticity index (RPDI) at the variety level was not significant for any trait. However, for ΔH, it explained 22 % of the total variance, and for NL_1, NT_1, and SLA_2, it contributed between 4 % and 7.4 % of variance in phenotypic plasticity (data not shown). In general, plasticity was greater for traits in stem section 2, which coincides with the greater drought effect on this foliage. The *estevezii* and *apulcensis* varieties consistently showed the lowest RDPI values in both sections (Table 3). On the other hand, a wide and significant variation was observed among families within varieties ($\sigma^2_{fam(var)}$) for all traits, contributing between 11 % and 71 % of total variance (Table 3).

The family-mean plasticity indices were positively correlated in most cases (Figure 7). Families with greater plasticity in one trait also tended to exhibit it for most of them, particularly in stem section 2, which formed during the drought period (Figure 7b). Plasticity in performance was positively correlated with plasticity in NL, NT, TS, and SLA. For SLA, this pattern was common in both stem sections, implying the adaptive value of this trait for seedling performance. Although plasticity in $MSLU_2$ was positively associated with plasticity in performance traits, it did not correlate with other morpho-anatomical traits. Average RDPI values for some traits were significantly correlated with family performance in both treatments (Figure S1). However, correlations with height growth (ΔH) were generally positive under WW and negative under WS, while correlations with ΔD were negative in both environments. Under favorable moisture conditions, families with higher phenotypic plasticity in NL_1, NT_1, SLA_1, and SLA_2 showed greater height growth, whereas under water deficit conditions, the pattern was reversed. Water availability significantly affected the temporal phenotypic plasticity for all foliage traits except for NT ($p > 0.10$, Table 4). Different plasticity patterns were observed for foliage traits formed in stem section 2 depending on the watering treatments. In MSLU and LS, values in section 2 increased under WW conditions but decreased under WS conditions, whereas NL and TS decreased in both treatments, with a larger reduction under WS. For NS, the decrease was smaller under WS, while in SLA, values increased in both conditions, with a greater increase under WS. The contrasting plasticity patterns of stomatal traits LS and NS resulted in a compensatory effect on TS under WW conditions but caused a >10 % reduction under WS.

As with RDPI, the variance among varieties and its interaction with watering treatments were not significant for temporal plasticity, contributing less than 4 % to the total variance (Table 4). The among-family variance ($\sigma^2_{fam(var)}$) was generally not significant (Table 4), except for SLA, which showed a marginal significance ($p = 0.096$). Similarly, only a marginal significant effect ($p < 0.10$) of the GxE interaction at the family level ($\sigma^2_{trat \times fam(var)}$) was found in temporal plasticity for NL and NT. The effect of water availability on the temporal phenotypic plasticity patterns of foliage traits was also reflected at the variety level (Table 5). Under WS conditions, variety *coatepecensis* was the only one showing an increase in growth units length, while others, particularly *estevezii*, showed a decrease. Variety *estevezii* also exhibited the greatest temporal plasticity in NL and NT, with reductions of 35 % and 12 %, respectively. *Pseudostrobus* and *coatepecensis* were more plastic in LS and TS, with reductions of 12 % to 14 % under WS. On the other hand, variety *apulcensis* showed the lowest temporal plasticity in SLA, with the smallest increase, especially under WS conditions.

Discussion

Effect of drought on plant performance and morpho-anatomic traits

The reduction in water availability generally decreased the mean trait values, particularly height and diameter growth (by 72 % and 54 %, respectively), reflecting the stress caused by water deficit (Bhargava and Sawant 2013). Growth occurs through cell division and elongation, involving genetic and physiological mechanisms (Pantin et al. 2012). These processes are inhibited under drought conditions by the loss of cell turgor due to reduced xylem water flow, depending on the intensity and duration of the stress (Sharp et al. 2004). In two-year-old *Pinus ponderosa* seedlings subjected to prolonged drought, a reduction in growth of similar traits was observed: growth in height, diameter, and needle length and thickness were reduced, leading to an increase in specific leaf area compared to well-watered seedlings (Olivas-García et al. 2000). While stomatal density in P. ponderosa did not change significantly, the number of stomata per unit length in this study decreased by 26 %, primarily due to a reduction in stomatal rows (Table 1). Drought had a more pronounced impact on younger needles formed during the water restriction period. Their greater sensitivity can be attributed to the higher dependance on water for growth and cellular differentiation during early development (Pantin et al. 2012), as well as morphological differences such as reduced cuticle thickness (Pardos et al. 2009).

Inter- and intravarietal genetic variation in drought adaptation

Pinus pseudostrobus seedlings exhibited a wide intervarietal variation in the morpho-anatomical traits (Table 1). This finding suggests the presence of genetic differences among them that may influence their performance and adaptative capacity, as in the white pine species complex (Moreno-Letelier and Barraclough 2015). In *P. pseudostrobus*, these genetic differences appear to confer greater drought adaptation to some varieties, contributing to the phenotypic differences observed in the response to water deficit (Table 2). Thus, varieties *estevezii* and *apulcensis* showed greater drought adaptation, with smaller relative changes (i.e., less phenotypic plasticity) in traits under water deficit compared to their performance under favorable watering conditions, while *coatepecensis* and *pseudostrobus* exhibited greater plastic responses, reflected as larger relative reductions in key performance traits like survival (Surv), height increase (ΔH), and diameter increase (ΔD) (Table 2). The intravarietal variation was also relevant for most traits (Table 1).

Table 3
Average, minimum, and maximum values in phenotypic plasticity for performance and morpho-anatomical traits of *Pinus pseudostrobus* sensu lato varieties, estimated from the relative distance plasticity index (RDPI), and proportion (%) of the variance component of families within varieties ($\sigma^2_{fam(var)}$) with their significance

Trait	Varieties					$\sigma^2 f_{am(var)}$(%)
	pseudostrobus	*oaxacana*	*apulcensis*	*coatepecensis*	*estevezii*	
Performance-related traits						
ΔH	**0.68^{a} (0.42 – 0.90)**	**0.61^{ab} (0.51 – 0.80)**	**0.52^{b} (0.36 – 0.60)**	**0.68^{a} (0.62 – 0.81)**	**0.49^{b} (0.48 – 0.52)**	36.6 ***
ΔD	**0.57^{a} (0.28 – 0.90)**	**0.55^{a} (0.39 – 0.71)**	**0.48^{b} (0.29 – 0.60)**	**0.65^{a} (0.48 – 0.90)**	**0.48^{b} (0.39 – 0.53)**	51.7 ***
Morpho-anatomical traits in stem section 1 (formed before the watering treatments)						
$MSLU_1$	0.21^{a} (0.10 – 0.39)	0.21^{a} (0.12 – 0.27)	0.18^{a} (0.10 – 0.26)	0.19^{a} (0.06 – 0.32)	0.19^{a} (0.14 – 0.27)	16.9 ***
NL_1	**0.17^{a} (0.06 – 0.30)**	**0.14^{ab} (0.08 – 0.22)**	**0.13^{b} (0.08 – 0.16)**	**0.15^{ab} (0.07 – 0.18)**	**0.11^{b} (0.07 – 0.16)**	46.6 ***
NT_1	**0.12^{a} (0.06 – 0.20)**	**0.12^{a} (0.07 – 0.16)**	**0.11^{ab} (0.09 – 0.15)**	**0.13^{a} (0.04 – 0.19)**	**0.08^{b} (0.06 – 0.11)**	32.5 ***
LS_1	0.12^{a} (0.02 – 0.28)	0.12^{a} (0.02 – 0.19)	0.12^{a} (0.06 – 0.21)	0.14^{a} (0.07 – 0.20)	0.14^{a} (0.09 – 0.18)	39.0 ***
NS_1	0.06^{a} (0.02 – 0.10)	0.07^{a} (0.04 – 0.10)	0.07^{a} (0.04 – 0.10)	0.06^{a} (0.04 – 0.08)	0.06^{a} (0.05 – 0.07)	10.7 **
TS_1	0.14^{a} (0.07 – 0.29)	0.15^{a} (0.11 – 0.23)	0.14^{a} (0.08 – 0.22)	0.15^{a} (0.13 – 0.17)	0.14^{a} (0.08 – 0.17)	17.1 ***
SLA_1	0.12^{a} (0.04 – 0.17)	0.12^{a} (0.04 – 0.14)	0.12^{a} (0.04 – 0.10)	0.15^{a} (0.05 – 0.10)	0.14^{a} (0.05 – 0.06)	39.0 ***
Morpho-anatomical traits in stem section 2 (formed during the watering treatments)						
$MSLU_2$	0.31^{a} (0.07 – 0.55)	0.26^{a} (0.18 – 0.40)	0.24^{a} (0.10 – 0.35)	0.28^{a} (0.07 – 0.51)	0.25^{a} (0.17 – 0.30)	51.7 ***
NL_2	0.26^{a} (0.09 – 0.63)	0.22^{a} (0.16 – 0.34)	0.21^{a} (0.14 – 0.28)	0.29^{a} (0.19 – 0.39)	0.23^{a} (0.22 – 0.25)	70.6 ***
NT_2	0.11^{a} (0.08 – 0.15)	0.13^{a} (0.06 – 0.26)	0.11^{a} (0.09 – 0.17)	0.12^{a} (0.07 – 0.15)	0.09^{a} (0.08 – 0.10)	22.7 ***
LS_2	0.19^{a} (0.06 – 0.31)	0.17^{a} (0.10 – 0.26)	0.15^{a} (0.07 – 0.22)	0.24^{a} (0.15 – 0.33)	0.18^{a} (0.12 – 0.21)	35.0 ***
NS_2	0.07^{a} (0.04 – 0.10)	0.07^{a} (0.03 – 0.13)	0.06^{a} (0.03 – 0.09)	0.07^{a} (0.04 – 0.09)	0.06^{a} (0.05 – 0.07)	23.3 ***
TS_2	0.20^{a} (0.11 – 0.31)	0.18^{a} (0.09 – 0.32)	0.16^{a} (0.12 – 0.21)	0.21^{a} (0.12 – 0.28)	0.16^{a} (0.14 – 0.20)	20.7 ***
SLA_2	**0.19^{ab} (0.07 – 0.27)**	**0.17^{ab} (0.08 – 0.21)**	**0.15^{b} (0.05 – 0.15)**	**0.24^{a} (0.09 – 0.20)**	**0.18^{ab} (0.07 – 0.12)**	35.0 ***

In parentheses, the minimum and maximum family-means in the relative distance plasticity index (RDPI). Means with different superscript letters in bold are significantly different with $p < 0.05$. Asterisks indicate the significance of the variance component in the joint analysis and the significance of the family variance ($\sigma^2_{fam(var)}$) in the site-specific analysis, where **$p \leq 0.05$; ***$p \leq 0.01$. ΔH = Height increase, ΔD = Diameter increase at the stem base, MSLU = Mean stem length unit, NL = Needle length, NT = Needle thickness, LS = Stomatal lines on the dorsal side, NS = Number of stomata per 1 mm of the central line on the dorsal side, TS = Total stomata per 1 mm of the dorsal side, SLA = Specific leaf area

Table 4
Mean values ($\bar{X} \pm SE$) by watering treatment (well-watered [WW] and water-stressed [WS]), and variance components among and within varieties for the relative change in foliage traits between stem sections in progenies of *Pinus pseudostrobus* sensu lato

Trait	$\bar{X}$		Variance components (%)				
	WW (%)	WS (%)	σ^2_{var}	$\sigma^2_{trat \times var}$	$\sigma^2_{fam(var)}$	$\sigma^2_{trat \times fam(var)}$	σ2
MSLU (mm)	20.8 ± 6.1^{a}	-5.7 ± 6.4^{b}	1.3	3.1	5.0	2.2	88.4
NL (cm)	-10.6 ± 1.8^{b}	-24.0 ± 1.9^{a}	4.0	0	0.6	7.2*	88.2
NT (mm)	-7.9 ± 0.8^{a}	-6.2 ± 0.9^{a}	0	0.5	1.3	9.7*	88.6
LS (n)	4.3 ± 1.5^{a}	-8.1 ± 1.7^{b}	0	0	1.3	0	98.7
NS (n mm^{-1})	-6.1 ± 1.3^{a}	-2.3 ± 1.4^{b}	3.3	0	0	1.0	95.7
TS (n mm^{-1})	-0.2 ± 1.4^{b}	-10.6 ± 1.6^{a}	0	0	2.8	0	97.2
SLA ($cm^2\ g^{-1}$)	3.5 ± 1.3^{a}	16.2 ± 1.4^{b}	2.0	0.1	4.7*	2.2	90.9

Means and standard error on the same line followed by different superscript letters are significantly different between treatments with $p \leq 0.05$. Asterisks indicate the significance of the respective variance component where *$p \leq 0.10$; **$p \leq 0.05$. MSLU = Mean stem length unit, NL = Needle length, NT = Needle thickness, LS = Stomatal lines on the dorsal side, NS = Number of stomata per 1 mm of the central line on the dorsal side, TS = Total stomata per 1 mm of the dorsal side, SLA = Specific leaf area

Table 5
Average values ($\bar{X}$) by variety and watering treatment (well-watered [WW] and water-stress [WS]) of the relative change (%) in phenotypic values of foliage traits in stem section 2, relative to those in section 1 (temporal plasticity), in the five varieties of *Pinus pseudostrobus* sensu lato

Trait	Varieties									
	pseudostrobus		*oaxacana*		*apulcensis*		*coatepecensis*		*estevezii*	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
MSLU (mm)	43.4	-3.3	9.1	-4.5	18.7	-9.2	20.9	11.3	1.9	-16.9
NL (cm)	-11.6	-23.6	-7.9	-21.4	-8.3	-20.3	-8.7	-28.9	-14.9	-33.5
NT (mm)	-9.1	-4.7	-6.5	-6.7	-7.0	-5.8	-8.6	0.5	-8.4	-12.0
LS (n)	2.8	-12.2	3.8	-6.9	4.6	-7.8	6.9	-11.7	11.4	-2.3
NS (n mm^{-1})	-3.7	-1.7	-4.4	-0.7	-6.1	-1.4	-7.5	2.1	-13.0	-6.9
TS (n mm^{-1})	0.8	-14.3	-0.1	-7.9	-1.5	-10.4	-0.1	-12.4	-2.4	-10.7
SLA ($cm^2 g^{-1}$)	4.9	20.0	2.1	15.5	1.8	11.0	4.0	17.8	4.5	19.7

MSLU = Mean stem length unit, NL = Needle length, NT = Needle thickness, LS = Stomatal lines on the dorsal side, NS = Number of stomata per 1 mm of the central line on the dorsal side, TS = Total stomata per 1 mm of the dorsal side, SLA = Specific leaf area

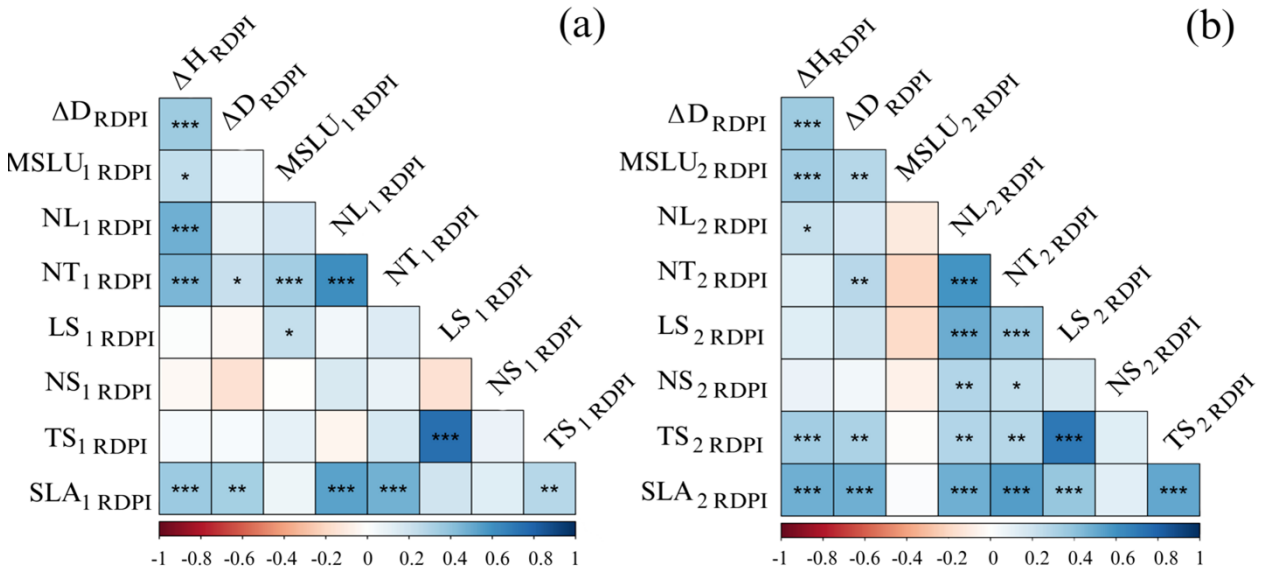

Figure 7
Correlation between the average relative distance plasticity index (RDPI) for progenies of *Pinus pseudostrobus* sensu lato for traits in (a) stem section 1 and (b) stem section 2, where *p < 0.10; **p < 0.05; ***p < 0.01. ΔH = Height increase, ΔD = Diameter increase at the stem base, MSLU = Mean stem length unit, NL = Needle length, NT = Needle thickness, LS = Stomatal lines on the dorsal side, NS = Number of stomata per 1 mm of the central line on the dorsal side, TS = Total stomata per 1 mm of the dorsal side, SLA = Specific leaf area.

This may be the result of divergent selection among populations within each variety, developing local adaptations to distinct conditions in their native environments (Scotti et al. 2023), and showing this as differences in progeny performance at the common garden trial.

Under prolonged water deficit, drought-related adaptive traits tend to be expressed more prominently; this may be due to the activation of gene complexes related to response to water stress, leading to physiological and morphological changes allowing plants to adjust their processes to water shortage (Bhargava and Sawant 2013, Samarah 2016). Additionally, under drought conditions, the main limiting factor becomes water availability, reducing the influence of other environmental variables compared to more favorable conditions. This environmental "stringency" can increase the strength of correlations by eliminating or reducing the influence of other environmental factors that could mask or weaken the relationships between adaptive traits and seedling performance (Soro et al. 2023).

The structure of phenotypic correlations revealed that needle length and thickness, as well as number of stomata per unit length, showed positive correlations with plant performance under water deficit conditions, whereas specific leaf area showed a negative correlation. In other words, progenies with greater drought adaptation developed longer and thicker needles, resulting in lower SLA. These traits enhance gas exchange efficiency, in terms of photosynthetic capacity and water retention per unit of leaf area, allowing seedlings to maintain higher tissue hydration and productivity under drought (Greenwood et al. 2017). On the other hand, the positive relationship between number of stomata per unit length and progeny performance under drought indicates that, in addition to an indirect effect due to its relationship with needle length and thickness, this trait is important for maintaining a higher gas exchange capacity (Franks and Farquhar 2007).

Most traits allowing greater discrimination among progenies, both at the inter- and intravarietal levels, also showed a strong correlation with climatic conditions at their native location. The close relationship between progeny performance under drought conditions and local climatic conditions indicates that *P. pseudostrobus* varieties differ in their environmental niche, implying potential ecotypic differentiation. The breadth of the environmental niches was evident in the principal component analysis, with clear climatic differences among the native locations of progenies tested, particularly for varieties *estevezii* and *coatepecensis*, which represent the extremes of the aridity gradient for the species range, aligning with the observations made by Perry (1991).

On the other hand, the canonical correlation analysis confirmed the phenotypic and environmental differences of *estevezii* and *apulcensis* varieties, compared to the others (Figure 6). Variety *estevezii* acquires particular adaptive relevance due to its marked differentiation in morpho-anatomical traits and outstanding performance under drought conditions. These results support earlier observations on its chemical differentiation from other varieties, particularly on the resin terpenes profile (Perry 1991); some studies show the importance of these compounds in plant responses to various biotic and abiotic stress factors, including drought (Kumar et al. 2021). As in *Pinus greggii* Engelm. ex Parl. (Hernández-Pérez et al. 2001), we also found that northern populations of the species have greater drought resistance than southern populations.

Even though variety *apulcensis* occurs in less arid regions than *estevezii*, it also exhibited xerophytic traits and good drought performance, which might be related to greater continentality (Tabakova et al. 2020), implying that this variety is particularly adapted to drier and warmer conditions in the summer. This could also lead to greater drought resistance, as plants from these origins may have developed adaptations to conserve water and withstand prolonged summer droughts. On average, progenies of varieties *coatepecensis*, *oaxacana*, and *pseudostrobus* showed lower performance under drought conditions and less xerophytic traits; however, the wide intravarietal genetic variation was reflected in the broad distribution along the canonical variable of morpho-anatomical traits, with a wide range of phenotypic values that should be considered when selecting for performance and drought adaptation.

Phenotypic plasticity in response to drought

Significant differences were observed among varieties in the magnitude of "spatial plasticity" (i.e., the first approach used to estimate plasticity), especially for traits with a significant genotype x environment interaction. The varieties *apulcensis* and *estevezii* exhibited lower phenotypic plasticity in response to drought, showing less pronounced phenotypic shifts. However, intravarietal genetic variation in the expression of plasticity was also evident, suggesting an important hereditary component in this trait, as observed for the phenotypic plasticity of shoot growth traits (Escobar-Alonso et al. 2024). Additionally, phenotypic plasticity of foliar traits was strongly interrelated, so progenies with higher plasticity in one trait were also more plastic in most of them.

Since "spatial plasticity" in this study reflects the effect of water deficit, it was negatively correlated with seedling performance under water restriction. However, the most plastic progenies (i.e., those with greater growth reduction under drought) were the most productive under favorable watering conditions. This behavior has also been observed for other conifer species, such as *Pinus ponderosa* (Kerr et al. 2015) and *Pseudotsuga menziesii* (Mirb.) Franco (Montwé et al. 2015), where generally the most productive genotypes were also the most sensitive to water deficit. In our study, variety estevezii was one of the most conservative in terms of height and diameter growth, aligning with the theory of the compensatory effect between productivity and drought adaptation identified in other pine species (de la Mata et al. 2014).

In the "temporal plasticity" approach, the basic concept of phenotypic plasticity (Sultan 2000) was applied by comparing the changes in foliar traits on the stem section formed during the drought period with those in the stem section formed before within the same genotype. This approach allowed separation of the ontogenic effect from the combined effect of drought on plasticity of foliar traits and provided an overview

of the different responses among and within varieties to cope with water stress. The ontogenic effect between the two stem sections was observed under well-watered (WW) conditions; this was shown by phenotypic changes associated with phenological differences between the two stem sections. However, under drought conditions, these changes differed significantly for most traits, both in magnitude and/or direction.

"Temporal plasticity" in average length of growth units, as well as in needle length and thickness, was greater in *estevezii*, allowing this variety to adjust these traits toward smaller values. Efficient regulation of these traits more efficiently, *estevezii* seedlings could reduce resource allocation to elongation of growth units (Bongarten 1986) and produce shorter, thinner leaves, thus reducing the gas exchange area (Wang et al. 2019). Conversely, *coatepecensis* seedlings allocated more resources to elongation of growth units and needle thickness under water deficit conditions, a response possibly representing a compensatory effect to their reduced capacity for growth unit formation resulting from water deficit. Meanwhile, *apulcensis* seedlings appeared to follow similar conservative responses to *estevezii*, but maintained a low specific leaf area, which may reduce transpiration and improve water balance (Zhong et al. 2022). The varieties most affected by water shortage, *coatepecensis* and *pseudostrobus*, showed a greater reduction in stomatal frequency during drought. A decrease in stomatal frequency, which is closely related to stomatal density, can reduce the gas exchange area and minimize water loss. However, it would also result in decreased productivity due to a lower photosynthetic rate.

Management implications in the context of increased drought

The evidence gathered in this research demonstrated that *P. pseudostrobus* varieties differ in their environmental niche concerning water availability, particularly the *estevezii* variety, which showed superior performance under drought conditions. This differentiation in environmental niche highlights the risks of indiscriminately moving and utilizing the germplasm across different geographical regions, which may lead to maladaptation and/or outbreeding depression. It is important to reconsider the taxonomic distinction and expand the operational use of the different *P. pseudostrobus* varieties proposed by Martínez (1948) and supported by other studies (García-Valencia et al. 2022, Escobar-Alonso et al. 2023). However, more information about the specific requirements for each variety and their behavior in relation to water availability is needed to use and move germplasm more efficiently. Such strategies may be implemented in assisted migration and enrichment of natural populations (Sáenz-Romero et al. 2020), or for supporting reforestation, ecological restoration, and commercial plantation programs in the context of climate change (Harris et al. 2006).

Intervarietal differentiation offers a valuable opportunity to implement management strategies that leverage the desirable traits of varieties with greater drought adaptability within the *P. pseudostrobus* group. At the same time, the wide intravarietal variation allows for the identification and use of seed sources within each variety that demonstrate better performance under water deficit conditions. In this way, different forest programs for the species can take advantage of both levels of variation to select and develop more resistant genotypes, with the appropriate phenotypic plasticity to modulate traits and optimize their performance in an environmental scenario of more frequent and intense droughts (Cook et al. 2018). With future research, the adaptive value of varieties *estevezii* and *apulcensis* could be crucial when implementing domestication strategies considering intervarietal hybrids. This technique, which allows the combination of desirable traits from different related taxa to create progenies with a unique combination of adaptive and productive traits, has been used in management plans for other conifers, such as *Pseudotsuga menziesii* (Rehfeldt 1977). On the other hand, the wide variation within varieties also offers opportunities to generate intravarietal hybrids, thus promoting combinational hybridization in the offspring resulting from these crosses and enhancing genetic diversity. With these approaches, the strategic opportunity to effectively combine complementary genetic traits between and within *P. pseudostrobus* varieties can lead to the creation of genotypes with an optimized set of drought-adaptive traits.

Additionally, the effective management of *P. pseudostrobus* genetic resources also requires identifying and protecting key habitats for each variety, such as the restricted distribution of variety *estevezii* in northern Mexico. Preserving the integrity of these habitats and promoting connectivity between populations will facilitate migration and gene flow between them, in order to conserve and, when necessary, enhance intrapopulational genetic variation and evolutionary processes.

Conclusions

Drought significantly affected the performance and morpho-anatomical traits of *Pinus pseudostrobus* progenies. However, important inter- and intravarietal variation in the response to drought was also observed. Differences in performance and correlations between traits, particularly under water deficit, highlighted the adaptive value of most morpho-anatomical traits, such as average-length of growth-units, needle length and thickness, number of stomatal lines, and stomatal frequency on the dorsal side of needles, as well as specific leaf area. Furthermore, the observed phenotypic differences correlated with the climate of origin, demonstrating the relevance of the environmental niche in the genetic differentiation of each variety and reflecting evolutionary responses leading to their particular adaptations and diverse patterns of response to water deficit. As a result, differences were also identified both among and within varieties in terms of phenotypic plasticity, suggesting a possible hereditary influence on this mechanism. Among the varieties, *apulcensis* and, particularly, *estevezii* exhibited superior performance under drought conditions, with the manifestation of more xerophytic traits, as well as plastic responses that favored their performance under these conditions. These results support the implementation of management strategies to optimize the use of genetic resources within the species in an environmental scenario of more intense and

frequent droughts associated with global warming. Additionally, empirical evidence of ecological differentiation was found, which should be considered in the operational use of the different taxonomically recognized varieties.

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References

Anderegg WR, Wu C, Acil N, Carvalhais N, Pugh TA, Sadler JP, Seidl R (2022). A climate risk analysis of Earth's forests in the 21st century. Science 377(6610):1099-1103. https://doi.org/10.1126/science.abp9723

Aragón-Peralta RD, Rodríguez-Ortiz G, Vargas-Hernández JJ, Enríquez-del Valle JR, Hernández-Hernández A, Campos-Ángeles GV (2020). Selección fenotípica y características reproductivas de *Pinus pseudostrobus* var. oaxacana (Mirov) SG Harrison. Revista Mexicana de Ciencias Forestales 11(59):118-140. https://doi.org/10.29298/rmcf.v11i59.700

Baeza-Guzmán Y, Trejo Aguilar D, Montaño NM, Camargo-Ricalde SL (2023). Synergistic effects of Amanita stranella and Suillus decipiens inoculation on morphological features and phenolic compounds of *Pinus pseudostrobus* var. coatepecensis, a narrow endemic Mexican variety. New Forests 5(6):1033-1048. https://doi.org/10.1007/s11056-023-10018-6

Barrera-Ramírez R, Vargas-Hernández JJ, Escobar-Alonso S, Pérez-Luna A, López-Upton J (2024). Variación intraespecífica en tolerancia al frío en progenies de *Pinus pseudostrobus* en dos sitios de evaluación. Madera y Bosques 30(2):e3022548. https://doi.org/10.21829/myb.2024.3022548

Barrera-Ramírez R, Vargas-Hernández JJ, López-Aguillón R, Muñoz-Flores HJ, Treviño-Garza EJ, Aguirre-Calderón OA (2021). Influencia de factores externos e internos en el prendimiento inicial de injertos de *Pinus pseudostrobus* var. oaxacana (Mirov) Harrison. Revista Chapingo Serie Ciencias Forestales y del Ambiente 27(2):243-256. https://doi.org/10.5154/r.rchscfa.2020.05.037

Bhargava S, Sawant K (2013). Drought stress adaptation: metabolic adjustment and regulation of gene expression. Plant Breeding 132:21-32. https://doi.org/10.1111/pbr.12004

Bongarten B (1986). Relationships between shoot length and shoot length components in Douglas-fir and blue spruce. Canadian Journal of Forest Research 16(2):373-380. https://doi.org/10.1139/x86-064

Cambrón-Sandoval VH, Sánchez-Vargas NM, Sáenz-Romero C, Vargas-Hernández JJ, España-Boquera ML, Herrerías-Diego Y (2013) Genetic parameters for seedling growth in *Pinus pseudostrobus* families under different competitive environments. New Forests 44:219-232. https://doi.org/10.1007/s11056-012-9312-1

Carrillo-Arizmendi L, Pérez-Suárez M, Vargas-Hernández JJ, Rozenberg P, Martínez-Campos AR (2022). Warming effects on tree-ring variables in *P. hartwegii* Lindl. at the extremes of its natural elevational distribution in central Mexico. Agricultural and Forest Meteorology 324:109109. https://doi.org/10.1016/j.agrformet.2022.109109

Cook BI, Mankin JS, Anchukaitis KJ (2018). Climate change and drought: From past to future. Current Climate Change Reports 4:164-179. https://doi.org/10.1007/s40641-018-0093-2

de la Mata R, Merlo E, Zas R (2014). Among-population variation and plasticity to drought of Atlantic, Mediterranean, and interprovenance hybrid populations of Maritime pine. Tree Genetics & Genomes 10(5):1191-1203. https://doi.org/10.1007/s11295-014-0753-x

de la Mata R, Zas R, Bustingorri G, Sampedro L, Rust M, Hernandez-Serrano A, Sala A (2022). Drivers of population differentiation in phenotypic plasticity in a temperate conifer: A 27-year study. Evolutionary Applications 15(11):1945-1962. https://doi.org/10.1111/eva.13492

Delgado P, Salas-Lizana R, Vázquez-Lobo A, Wegier A, Anzidei M, Alvarez-Buylla ER, Vendramin GG, Pinero D (2007). Introgressive hybridization in *Pinus montezumae* Lamb. and *Pinus pseudostrobus* Lindl. (Pinaceae): morphological and molecular (cpSSR) evidence. International Journal of Plant Sciences 168(6):861-875. https://doi.org/10.1086/518260

Escobar-Alonso S, Vargas-Hernández JJ, López-Upton J (2023). Ability of morphological traits from needles and cones to identify *Pinus pseudostrobus* Lindl. varieties. Revista Chapingo Serie Ciencias Forestales y del Ambiente 29(1):99–116. https://doi.org/10.5154/r.rchscfa.2022.05.038

Escobar-Alonso S, Vargas-Hernández JJ, López-Upton J, García-Campusano F, Jiménez-Casas M, Cruz-Huerta N (2024). Genetic variation and phenotypic plasticity in the seasonal shoot growth pattern of *Pinus pseudostrobus*. New Forests 55:1379-1398. https://doi.org/10.1007/s11056-024-10040-2

Farjon A, Styles BT (1997). Flora Neotropica. *Pinus* (*Pinaceae*). Botanical Garden, New York, US.

Franks PJ, Farquhar GD (2007). The mechanical diversity of stomata and its significance in gas-exchange control. Plant physiology 143(1):78-87. https://doi.org/10.1104/pp.106.089367

García-Valencia LE, Pérez-García J, Vallejo-Reyna MÁ, Reynoso-Santos R, Vargas-Hernández JJ, García-Campusano F (2022). cpSSR and high-resolution melting analysis (HRM) for *Pinus pseudostrobus* Lindl. variety genotyping and discrimination. Forests 13(2):200. https://doi.org/10.3390/f13020200

Greenwood S, Ruiz-Benito P, Martínez-Vilalta J, Lloret F, Kitzberger T, Allen CD, Fensham R, Laughlin DC, Kattge J, Bönish G, Kraft NJB, Jump AS (2017). Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. Ecology letters 20(4):539-553. https://doi.org/10.1111/ele.12748

Harris JA, Hobbs RJ, Higgs E, Aronson J (2006). Ecological restoration and global climate change. Restoration Ecology 14(2):170-176. https://doi.org/10.1111/j.1526-100X.2006.00136.x

Harrison SG (1965). Note on gymnosperm nomenclature. Taxon 14(7):247.

Hernández-Pérez C, Vargas-Hernández JJ, Ramírez-Herrera C, Muñoz-Orozco A (2001). Variación geográfica en la respuesta a la sequía en plántulas de *Pinus greggii* Engelm. Revista Mexicana de Ciencias Forestales 26(89):61-79.

Kerr KL, Meinzer FC, McCulloh KA, Woodruff DR, Marias DE (2015). Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. Tree Physiology 35(5):535-548. https://doi.org/10.1093/treephys/tpv034

Kumar M, Kumar Patel M, Kumar N, Bajpai AB, Siddique KH (2021). Metabolomics and molecular approaches reveal drought stress tolerance in plants. International Journal of Molecular Sciences 22(17):9108. https://doi.org/10.3390/ijms22179108

Martínez M (1948). Los pinos mexicanos. Universidad Nacional Autónoma de México, México.

Mirov NT (1958). *Pinus oaxacana*, a new species from Mexico. Madroño 14(5):145-150.

Montwé D, Spiecker H, Hamann A (2015). Five decades of growth in a genetic field trial of Douglas-fir reveal trade-offs between productivity and drought tolerance. Tree Genetics & Genomes 11:1-11. https://doi.org/10.1007/s11295-015-0854-1

Moran E, Lauder J, Musser C, Stathos A, Shu M (2017). The genetics of drought tolerance in conifers. New Phytologist 216(4):1034-1048. https://doi.org/10.1111/nph.14774

Moreno-Letelier A, Barraclough TG (2015). Mosaic genetic differentiation along environmental and geographic gradients indicate divergent selection in a white pine species complex. Evolutionary Ecology 29:733-748. https://doi.org/10.1007/s10682-015-9785-4

Olivas-García JM, Cregg BM, Hennessey TC (2000). Genotypic variation in carbon isotope discrimination and gas exchange of Ponderosa pine seedlings un-

der two levels of water stress. Canadian Journal of Forest Research 30(10):1581-1590. https://doi.org/10.1139/x00-080

Pantin F, Simonneau T, Muller B (2012). Coming of leaf age: control of growth by hydraulics and metabolics during leaf ontogeny. New Phytologist 196(2):349-366. https://doi.org/10.1111/j.1469-8137.2012.04273.x

Pardos M, Calama R, Climent J (2009). Difference in cuticular transpiration and sclerophylly in juvenile and adult pine needles relates to the species-specific rates of development. Trees 23:501-508. https://doi.org/10.1007/s00468-008-0296-6

Perry JP (1982). The taxonomy and chemistry of *Pinus estevezii*. Journal of the Arnold Arboretum 63(2):187-198.

Perry JP (1991) The pines of Mexico and Central America. Timber Press, Portland, Oregon.

Rehfeldt GE (1977). Growth and cold hardiness of intervarietal hybrids of Douglas-fir. Theoretical and Applied Genetics 50:3-15. https://doi.org/10.1007/BF00273790

Rehfeldt GE, Ferguson DE, Crookston NL (2009). Aspen, climate, and sudden decline in western USA. Forest Ecology and Management 258(11):2353-2364. https://doi.org/10.1016/j.foreco.2009.06.005

Sáenz-Romero C, Mendoza-Maya E, Gómez-Pineda E, Blanco-García A, Endara-Agramont AR, Lindig-Cisneros R, López-Upton J, Trejo-Ramírez O, Wehenkel C, Cibrián-Tovar D, Flores-López C, Plascencia-González A, Vargas-Hernández JJ (2020). Recent evidence of Mexican temperate forest decline and the need for ex situ conservation, assisted migration, and translocation of species ensembles as adaptive management to face projected climatic change impacts in a megadiverse country. Canadian Journal of Forest Research 50(9):843-854. https://doi.org/10.1139/cjfr-2019-0329

Samarah NH (2016). Understanding how plants respond to drought stress at the molecular and whole plant levels. In: Hossain M, Wani S, Bhattacharjee S, Burritt D, Tran LS (eds) Drought stress tolerance in plants Vol 2. Springer. https://doi.org/10.1007/978-3-319-32423-4_1

Scotti I, Lalagüe H, Oddou-Muratorio S, Scotti-Saintagne C, Ruiz Daniels R, Grivet D, Lefevre F, Cubry P, Fady B, González-Martínez SC, Roig A, Lesur-Kupin I, Bagnoli F, Guerin V, Plomion C, Rozenberg P, Vendramin GG (2023). Common microgeographical selection patterns revealed in four European conifers. Molecular Ecology 32(2):393-411. https://doi.org/10.1111/mec.16750

Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT (2004). Root growth maintenance during water deficits: physiology to functional genomics. Journal of Experimental Botany 55(407):2343-2351. https://doi.org/10.1093/jxb/erh276

Soro A, Lenz P, Roussel JR, Nadeau S, Pothier D, Bousquet J, Achim A (2023). The phenotypic and genetic effects of drought-induced stress on wood specific conductivity and anatomical properties in white spruce seedlings, and relationships with growth and wood density. Frontiers in Plant Science 14:1297314. https://doi.org/10.3389/fpls.2023.1297314

Stead, J. W. (1983). A study of variation and taxonomy of the *Pinus pseudostrobus* complex. The Commonwealth Forestry Review 62(1): 25-35.

Sultan SE (2000). Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5(12):537-542. https://doi.org/10.1016/S1360-1385(00)01797-0

Tabakova MA, Arzac A, Martínez E, Kirdyanov AV (2020). Climatic factors controlling *Pinus sylvestris* radial growth along a transect of increasing continentality in southern Siberia. Dendrochronologia 62:125709. https://doi.org/10.1016/j.dendro.2020.125709

Valladares F, Matesanz S, Guilhaumon F, Araújo MB, Balaguer L, Benito-Garzón M, Cornwell W, Gianoli E, van Kleunen M, Naya DE, Nicotra AB, Poorter H, Zavala MA (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecology Letters 17(11):1351-1364. https://doi.org/10.1111/ele.12348

Valladares F, Sánchez-Gómez D, Zavala MA (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. Journal of Ecology 94(6):1103-1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Viveros-Viveros H, Sáenz-Romero C, Vargas-Hernández JJ, López-Upton J (2006). Variación entre procedencias de *Pinus pseudostrobus* establecidas en dos sitios en Michoacán, México. Revista Fitotecnia Mexicana 29(2):121-121.

Wang N, Palmroth S, Maier CA, Domec JC, Oren R (2019). Anatomical changes with needle length are correlated with leaf structural and physiological traits across five Pinus species. Plant, Cell & Environment 42(5):1690-1704. https://doi.org/10.1111/pce.13516

Wang T, Hamann A, Spittlehouse D, Carroll C (2016). Locally downscaled and spatially customizable climate data for historical and future periods for North America. PloS One 11:e0156720. https://doi.org/10.1371/journal.pone.0156720

Zhong G, Tian Y, Liu P, Jia X, Zha T (2022). Leaf traits and resource use efficiencies of 19 woody plant species in a plantation in Fangshan, Beijing, China. Forests 14(1):63. https://doi.org/10.3390/f14010063