

Article

Altitudinal Genetic Variation of *Pinus oocarpa* Seedling Emergence in the Southern Mountains, Oaxaca, Mexico

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Abstract: *Pinus oocarpa* is the most important conifer for resin production in Mexico, so superior resin trees were selected in the Southern Mountains of Oaxaca, Mexico. The objective was to determine the variation and differences among provenances and among trees according to the parameters of seedling emergence and the number of cotyledons, and their relationship with elevation and climatic variables. The seedling emergence of four replicates of 20 seeds from 80 trees was counted daily. For the emergence parameters, provenance contributed 42.02% to the total variance, tree 29.19% and error 28.79%. Only tree (11.71%) and error (88.29%) contributed to the total variance of the cotyledon number. The effect of provenance ($p \leq 0.0006$) and tree ($p \leq 0.0001$) was significant for all variables evaluated. Higher-elevation provenances and trees had higher emergence values. The emergence parameters were positively associated with tree elevation. Climatic variables related to precipitation and temperature were negatively related to the emergence parameters. The results allow for the selection of phenotypes without emergence problems to establish seed orchards.

Keywords: provenances; superior trees; resin production; cotyledons; total variance; association with elevation; climatic variables



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1. Introduction

Pinus oocarpa Schiede ex Schltdl. is the most widely distributed conifer in the Americas; its natural populations are found from northeastern Mexico (29° LN) to northwestern Nicaragua (12° LN), at altitudinal ranges from 200 to 1700 m asl [1,2]. The wood of this pine, due to its hardness, is used in construction, furniture, pulp, particleboard and plywood [2]. However, in Mexico, this pine is of greater importance due to the high production and quality of its resin, which is produced in natural forests [2–5]. In Mexico, pine resin is the main non-timber forest product; however, demand is greater than production [3]. An alternative to reverse the resin production deficit in Mexico is to develop a forest genetic improvement program to increase its production. In this regard, in 2010, government agencies and producers initiated research to improve *P. oocarpa*, *P. pringleii* Shaw and *P. pseudostrabus* Lindl. in Michoacán, Mexico [4,6–8]. However, in 2019, the National Institute of Forestry, Agricultural and Livestock Research initiated a broader research program for the genetic improvement of *P. oocarpa*, which includes the selection of superior resin trees in natural stands and the establishment of seed orchards in three states of the Mexican Republic (Michoacán, State of Mexico, and Oaxaca) [9].

In the forest genetic improvement cycle, the selected superior resin trees constitute the selection population [10,11] and may eventually provide seeds for urgent planting and reforestation programs, so it is necessary to know the physical and physiological quality of their seeds. On the other hand, to advance in the breeding cycle, it is necessary to establish production populations (seed orchards), which are established with germplasm from the

selection population after additional selection [10,11]. The trees that will constitute the seed orchards must produce abundant and good-quality seeds; therefore, it is essential to evaluate the physical and physiological quality of the seeds of the superior resin trees of *P. oocarpa*.

Information on the physical and physiological seed quality of resin pines is scarce. In resin trees of *P. pseudostrobus* Lindl., provenance influences the physical seed quality, and there is variation among trees; this is not the case for physiological quality [8]. In other species such as *P. cembroides* Zucc., *P. orizabensis* D. K. Bailey and Hawksworth and *P. patula* Schiede ex Schltdl. and Cham, the variation in physiological seed quality is due to the effects of geographical origin [12,13]. For *P. oocarpa*, the variation in physiological seed quality is not known; however, in the growth characteristics and wood density, there is wide variation and genetic control [6,14,15]. Therefore, there may be variation in the seed germination and seedling emergence traits. The study of the number of cotyledons is relevant because it is a trait with high genetic control; its variation does not depend on environmental factors and has a high genetic correlation with growth height in *P. oocarpa* [15]. Considering that variation is the raw material for genetic improvement [10,11], it is important to quantify and determine whether this variation is mostly concentrated between or within provenances to guide selection efforts.

On the other hand, the altitude and climatic factors of geographic origin influence the physiological quality of seeds of the genus *Pinus* L. [13,16,17], generating patterns of clinal variation as an adaptive response [18–20]. Although the relationship between the physiological quality of seeds and elevation and environmental variables has not been studied in *P. oocarpa*, it has been demonstrated that the growth in the diameter and height of seedlings has a quadratic relationship with the elevation of the sites of origin [18,19]. Study of the association between elevation and environmental factors is important for the selected *P. oocarpa* resin trees since these factors can define the capacity and speed of seedling emergence. The speed of seedling emergence can benefit the establishment and initial growth of seedlings [13,21]; in addition, it could reduce the exposure of plants to random mortality events such as frost and drought [13,22].

Therefore, the objectives of this study were: (1) To determine the level of variation and differences in the parameters of emergence and number of cotyledons among provenances and among superior *P. oocarpa* resin trees. (2) To determine the level of association between the parameters of emergence and number of cotyledons with the elevation and climatic variables of the trees. From the previous objectives, the following hypotheses were proposed: (1) The provenance and tree factor will influence the parameters of emergence and number of cotyledons due to environmental variations in the site of origin. (2) The association between the parameters of emergence and number of cotyledons with altitude and environmental variables will be significant because these factors determine adaptive traits.

2. Materials and Methods

2.1. Collection and Cleaning of Seeds

In 2019, between 6 and 28 resin trees (80 total superior trees) of *P. oocarpa* were selected from six provenances in the Southern Mountains of Oaxaca, Mexico (Table 1, Figure 1). The selection indicator was the high resin production of the trees, which was defined as follows: (1) pre-selection of trees was carried out based on the characteristics influencing resin production [23]; (2) the resin production for each tree was estimated using previously determined allometric equations for *P. oocarpa* [23]; (3) a scatter plot was created with age and estimated resin production, and a regression line was obtained; (4) trees that were above the regression line were selected as superior resin-producing trees.

The fruits and seeds were collected in March 2020 (Table 1), by tree climbing. The fruits of each tree were placed in cloth sacks and labeled with the identification code of the tree, origin and date of collection, then transported for processing to the facilities of the Campo Experimental Valles Centrales de Oaxaca of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (National Institute of Forestry, Agriculture and Livestock

Research). The cones were placed in plastic trays duly labeled and exposed to the open air inside a transparent plastic greenhouse for drying, thus provoking the opening of scales and extraction of seeds. Afterward, impurities (empty and damaged seeds, pieces of scales and plant material) were removed. Finally, seeds from each tree were placed in properly labeled paper envelopes and stored in a cold room (5 °C) until planting in October 2021. Throughout the process, the identity of the seeds was preserved (tree key, origin, date of collection).

Table 1. Number and identification key of superior resin trees of six provenances of *Pinus oocarpa* in the Southern Mountains of Oaxaca, Mexico.

Provenance	Community	Number of Trees	Altitude (m asl)	Collection Date	Identification Key of Superior Trees
Rosario	Constancia del Rosario	27	902	14 to 15 March 2020	CDR01, CDR02, ..., CDR28
Sesteadero	Putla Villa de Guerrero	6	905	16 March 2020	SES01, SES02, SES03, SES06, SES07, SES11
San Pedro	Santo Domingo Coatlán	18	1130	23 to 24 March 2020	SDC30, SDC31, ..., SDC47
El Tizne	Santo Domingo Coatlán	8	1288	19 to 20 March 2020	SDC11, SDC12, ..., SDC18
Las Tejas	Santo Domingo Coatlán	10	1302	21 to 22 Marzo 2020	SDC01, SDC02, ..., SDC10
El Nanche	Santo Domingo Coatlán	11	1325	17 to 18 Marzo 2020	SDC19, SDC20, ..., SDC29

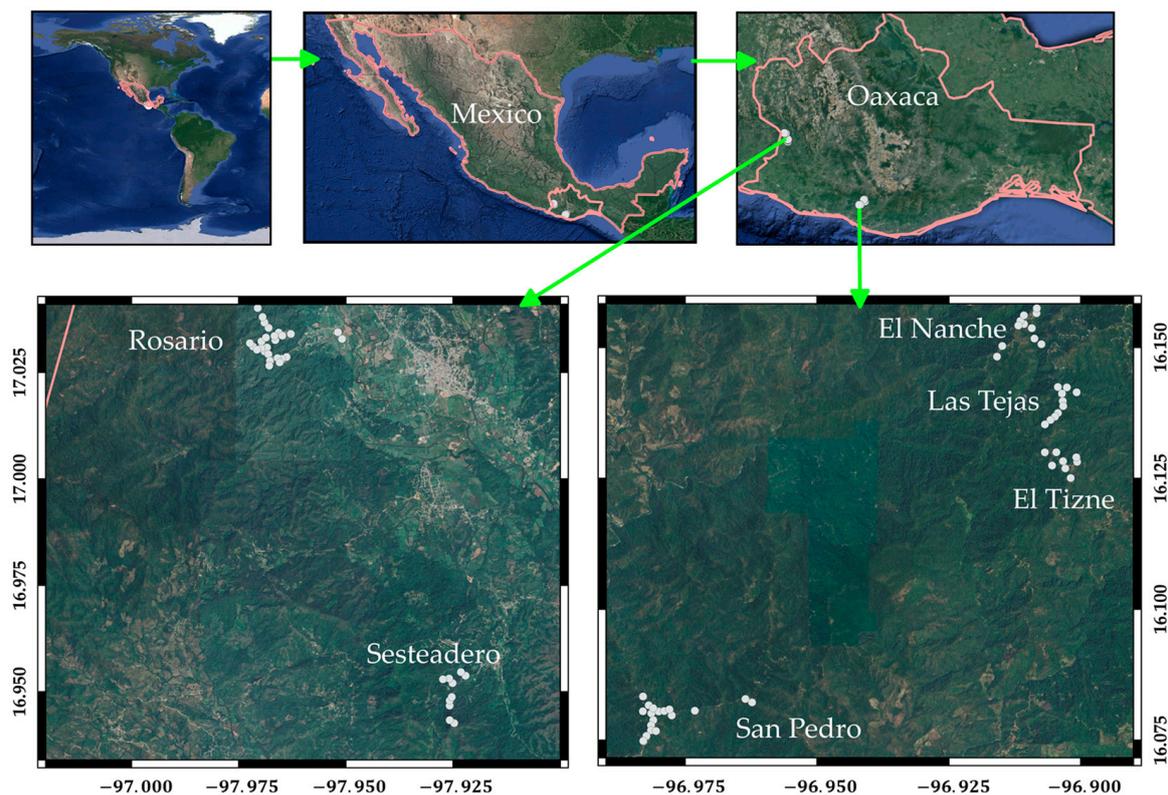


Figure 1. Location of superior *Pinus oocarpa* trees (white dots) in six provenances in the Southern Mountains of Oaxaca, Mexico.

2.2. Sowing and Experimental Design

Seeds from each tree were soaked in a solution composed of clean water with Captan 50WP[®] fungicide (2.0 g L⁻¹ of water) for 12 h to separate the full seeds from the empty ones. Due to the low seed production of some trees, 80 viable seeds per tree were selected. Each individual seed was sown in a 310 mL rigid container with commercial substrate composed of Peatmoss[®] (30%), Perlite[®] (35%) and Vermiculite[®] (35%).

After planting, four groups of 20 containers (with seeds sown) were made for each tree; the 20 containers formed the experimental unit, so each tree had four replicates. The experimental units were distributed in growth beds inside a greenhouse covered with white Raschel shade netting at 50%, using a completely randomized design. During the first three days after planting, irrigation was carried out daily, then every two days, depending on the environmental conditions. The average daily greenhouse temperature was 25 °C.

2.3. Emergence Parameters Evaluated

After seed sowing, daily counts of seedling emergence were made, which occurred between day 10 and 33 after sowing. With the daily count data, the emergence parameters evaluated were the emergence capacity (EC), peak emergence value (PEV), average daily emergence (EMD), emergence energy (EE) and emergence value (EV). The emergence capacity was the final emergence percentage, the peak value was the maximum accumulated emergence, the average daily emergence was obtained by dividing the daily accumulated emergence percentages by the number of day in the period of each evaluation [24], the emergence energy was the number of days in which 50% of emergence was achieved (higher values indicate lower emergence energy) [25] and the emergence value was the product of multiplying the peak value by the average daily emergence [24]. In addition, the number of cotyledons (NCOT) of each seedling was counted.

2.4. Climatic Variables

With the location data (latitude, longitude and altitude) of each tree, ecologically important variables (Table S1) for the plants were obtained from the Moscow Forest Sciences Laboratory [26,27]. In addition, annual and summer aridity and moisture indices (Table S1) were obtained [19,28]. These data were used to understand the association with the parameters of emergence and number of cotyledons.

2.5. Data Analysis

The assumption of normality and homogeneity of variance in the data was verified using the Shapiro–Wilk and Levene tests, respectively. None of the variables met both assumptions ($p \leq 0.0253$). Therefore, to assess differences among origins and among trees according to the emergence parameters and the number of cotyledons, non-parametric variance tests and multiple comparisons of ranks RT1 [29] were conducted. The following statistical model was employed:

$$Y_{ijk} = \mu + P_i + T_j(P_i) + \epsilon_{ijk} \quad (1)$$

where Y_{ijk} is the value of the ijk -th observation, μ is the population mean, P_i is the effect of the i -th provenance, T_j is the effect of the j -th tree nested within the i -th provenance and ϵ_{ijk} is the experimental error.

With the aforementioned model, the variance components associated with each source of variation were obtained using the VARCOMP procedure with the REML option in the SAS 3.4 statistical program [20].

The grouping of provenances and trees based on the emergence values and the number of cotyledons was performed using Ward's hierarchical clustering and Euclidean distance [15]. To determine the association between the emergence and cotyledon number parameters and the altitude and environmental variables at the tree locations, Spearman's correlation coefficients were obtained using the CORR procedure in SAS 9.4 [30].

3. Results

3.1. Variation between Provenances and between Trees

Regarding the emergence parameters, the average contribution of provenance to the total variance was 42.02%, that of trees was 29.19%, and errors contributed 28.79%. The highest contribution of origin to the total variance occurred in the peak emergence

value (48.65%), while the lowest contribution happened for the emergence energy (33.85%). Conversely, the highest and lowest contributions of trees to the total variance occurred in emergence energy (40.63%) and daily mean emergence (22.52%), respectively. The greatest contribution of error to the total variance was in the daily mean emergence (37.82%), and the lowest contribution occurred in the peak emergence value (21.15%).

As for the number of cotyledons, error had the highest contribution to the total variance (88.29%), the tree contributed only 11.71%, while the provenance had no contribution (Table 2).

Table 2. Variance components (%) and significance level (*p*) of emergence parameters in seedlings of *Pinus oocarpa* in the Southern Mountains of Oaxaca, Mexico.

Variable ¹	Mean	Contribution to Total Variance (%) and Significance (<i>p</i>)					Total Variance
		Provenance	<i>p</i>	Tree (Provenance)	<i>p</i>	Error	
CE	66.88	46.85	<0.0001	28.59	<0.0001	24.55	413.03
VPE	3.84	48.65	<0.0001	30.20	<0.0001	21.15	1.82
EMD	3.45	39.66	<0.0001	22.52	<0.0001	37.82	1.71
VE	14.71	41.07	<0.0001	24.00	<0.0001	34.93	84.04
EE	14.28	33.85	<0.0001	40.63	<0.0001	25.52	2.16
NCOT	5.91	0.00	0.0006	11.71	<0.0001	88.29	0.66

¹ CE: emergence capacity, VPE: peak emergence value, EMD: average daily emergence, VE: emergence value, EE: emergence energy, NCOT: number of cotyledons.

The onset of emergence in the *P. oocarpa* seedlings occurred between day 9 (provenance El Tizne) and day 12 (provenance Sesteadero). The maximum emergence capacity was reached between days 15 and 20 for all provenances, with no significant increases thereafter (Figure 2). All emergence parameters and the number of cotyledons showed significant differences between provenances ($p \leq 0.0006$). The El Tizne and San Pedro provenances exhibited the highest values for peak emergence, daily mean emergence and emergence value, while the Sesteadero and Rosario origins had the lowest values. Additionally, El Tizne and Sesteadero showed the highest and lowest germinative energy, respectively (Table 3).

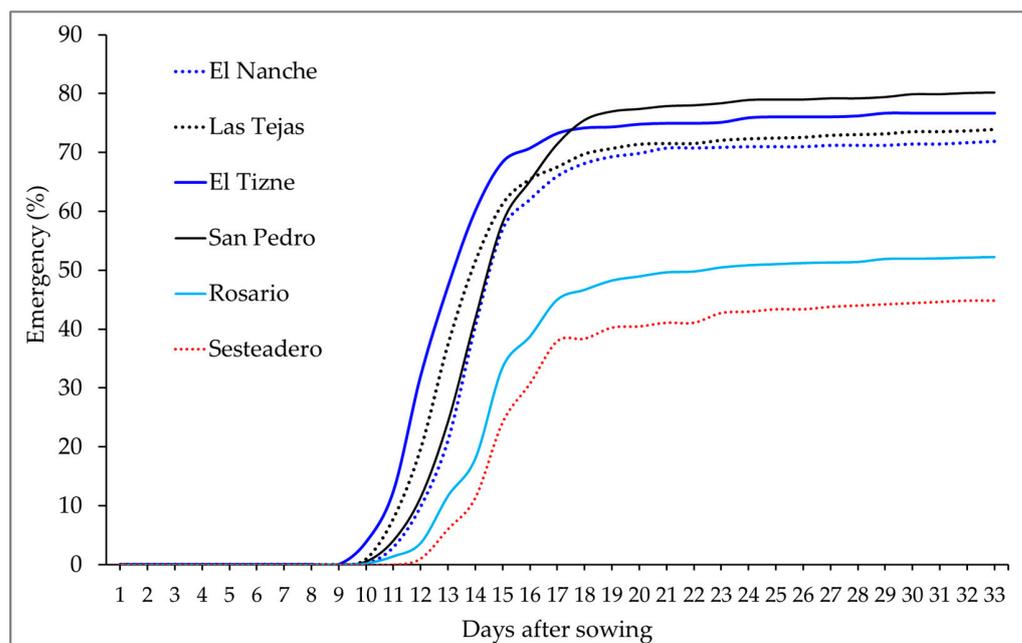


Figure 2. Seedling emergence from six provenances of *Pinus oocarpa* from the Southern Mountains of Oaxaca, Mexico.

Table 3. Averages with comparison of means of emergence parameters and number of cotyledons in six provenances of *Pinus oocarpa* from the Southern Mountains of Oaxaca, Mexico.

Provenances	Emergence Capacity (%)	Peak Emergence Value	Average Daily Emergence	Emergence Value	Emergence Energy (Day)	Number of Cotyledons
San Pedro	81.10 ^a	4.59 ^{ab}	4.12 ^a	19.53 ^{ab}	14.34 ^b	5.90 ^{bc}
El Tizne	76.63 ^b	4.76 ^a	4.20 ^a	20.51 ^a	13.10 ^a	5.90 ^b
Las Tejas	74.55 ^{bc}	4.36 ^b	3.93 ^{bc}	17.99 ^{bc}	13.49 ^c	5.81 ^c
El Nanche	71.93 ^c	4.11 ^c	3.75 ^c	16.11 ^c	14.14 ^c	5.94 ^{ab}
Rosario	52.22 ^d	2.86 ^d	2.61 ^d	8.42 ^d	15.01 ^d	5.94 ^{ab}
Sesteadero	44.84 ^e	2.36 ^e	2.12 ^e	5.71 ^e	15.63 ^e	6.00 ^a

Different letters in the same column indicate statistically significant differences ($p < 0.05$).

The clustering analysis revealed the existence of two groups of provenances. The first group consists of the provenances El Tizne, Las Tejas, San Pedro and El Nanche, while the second group comprises the provenances Sesteadero and Rosario (Figure 3).

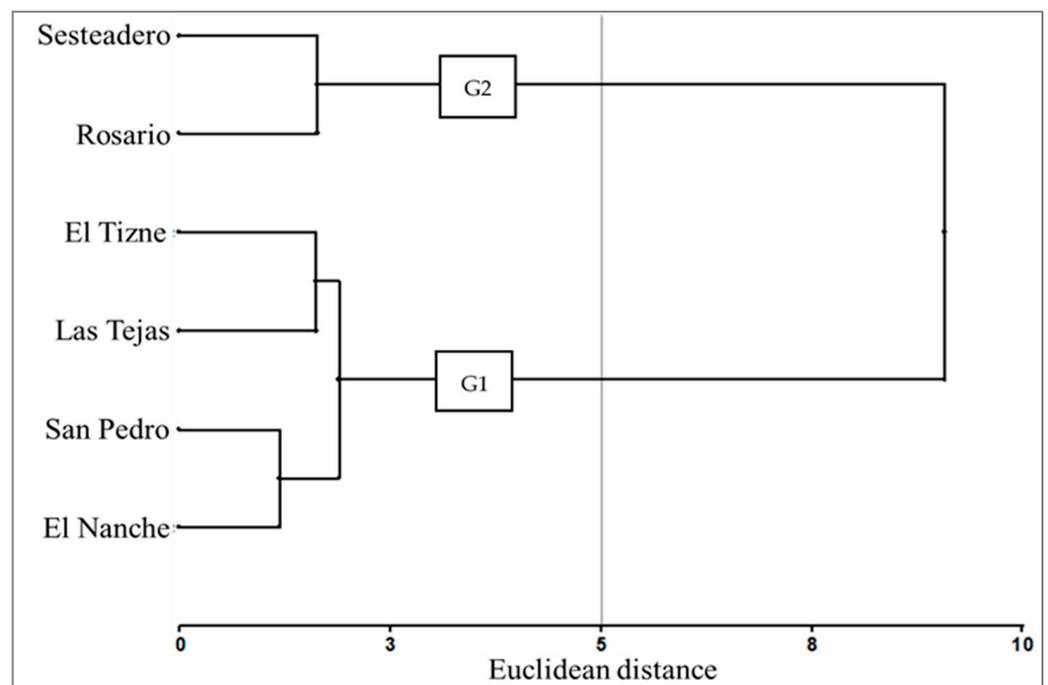


Figure 3. Grouping of provenances *Pinus oocarpa* in the Southern Mountains of Oaxaca, Mexico.

All emergence parameters and the number of cotyledons showed significant differences among trees ($p \leq 0.0001$). Concerning the emergence capacity, 28 trees exhibited the highest values statistically equal (76.90 to 90.00%) to the highest value, and 19 were statistically equal to the lowest value (21.25 to 50.30%), while the rest showed intermediate values (52.5 to 6.25%) (Table S2). Regarding the peak emergence value, 26, 35 and 19 trees presented high values (5.66 to 4.59), intermediate values (2.74 to 4.51) and low values (1.19 to 2.62), respectively. The daily mean emergence was high (12.00 to 13.50) in 28 trees, low in 18 (1.16 to 2.40) and the rest had intermediate values (2.54 to 4.04). The emergence value was high (19.89 to 31.49) for 29 trees, intermediate (8.06 to 19.66) for 30 trees and low (8.06 to 19.66) for 20 trees. In 18 trees, the emergence energy was high (12.00 to 13.50), intermediate (13.75 to 15.67) in 46 trees and low for the rest (15.75 to 17.5). Overall, trees with lower values in all emergence parameters belonged to the Sesteadero and Rosario origins (Table S2). As for the number of cotyledons, 13 trees had higher values (6.14 to 6.77),

51 trees had intermediate values (5.67 to 6.12) and 18 trees had lower values (5.13 to 5.66) (Table S2).

The clustering analysis revealed the formation of two groups of trees. Each group consisted of half (40 trees) of the total evaluated trees. The first group comprised trees with high values for the emergence parameters, while trees with low values for the emergence parameters were grouped into the second group (Figure 4).

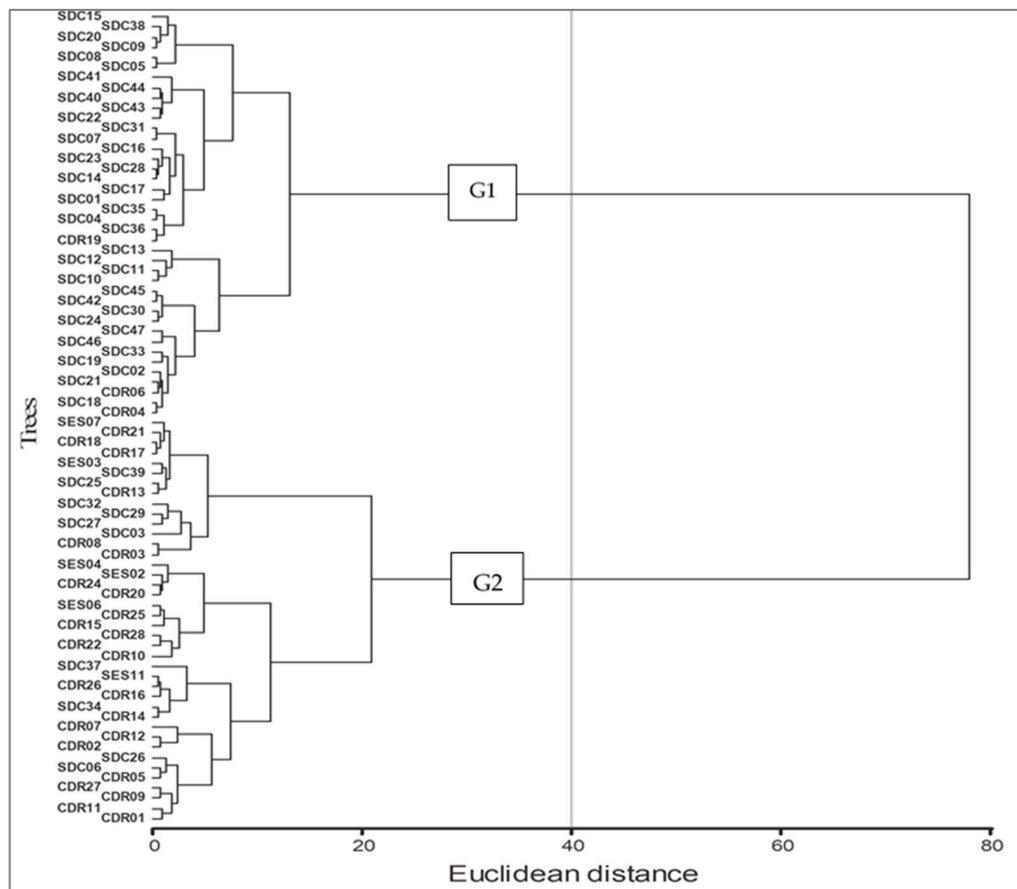


Figure 4. Dendrogram of clustering for superior resinous trees of *Pinus oocarpa* in the Southern Mountains of Oaxaca, Mexico.

3.2. Association between Emergence and Cotyledons and Elevation and Climatic Variables

The association between all emergence parameters and the elevation of the trees was significant ($p < 0.0001$). The association was positive ($r = 0.52$ to 0.52) between elevation and the emergence capacity, peak emergence value, daily mean emergence and emergence value, while the association between elevation and the emergence energy was negative ($r = -0.50$). On the other hand, no association was found between the elevation of the trees and the number of cotyledons ($r = -0.13$, $p = 0.239$) (Figure 5).

As for the climatic variables, all were significantly associated ($p \leq 0.0485$) with all the emergence variables of the *P. oocarpa* seedlings, except for the annual aridity index (sdi) and the summer moisture index (smi) with the emergence capacity, peak emergence value, daily mean emergence and emergence value ($p \geq 0.1261$) (Table S3). Additionally, the association between the emergence capacity and the Julian date of the last spring frost (sday) was not significant ($p = 0.2449$). The association between all environmental variables and the number of cotyledons was not significant ($p \geq 0.1184$) (Table S3).

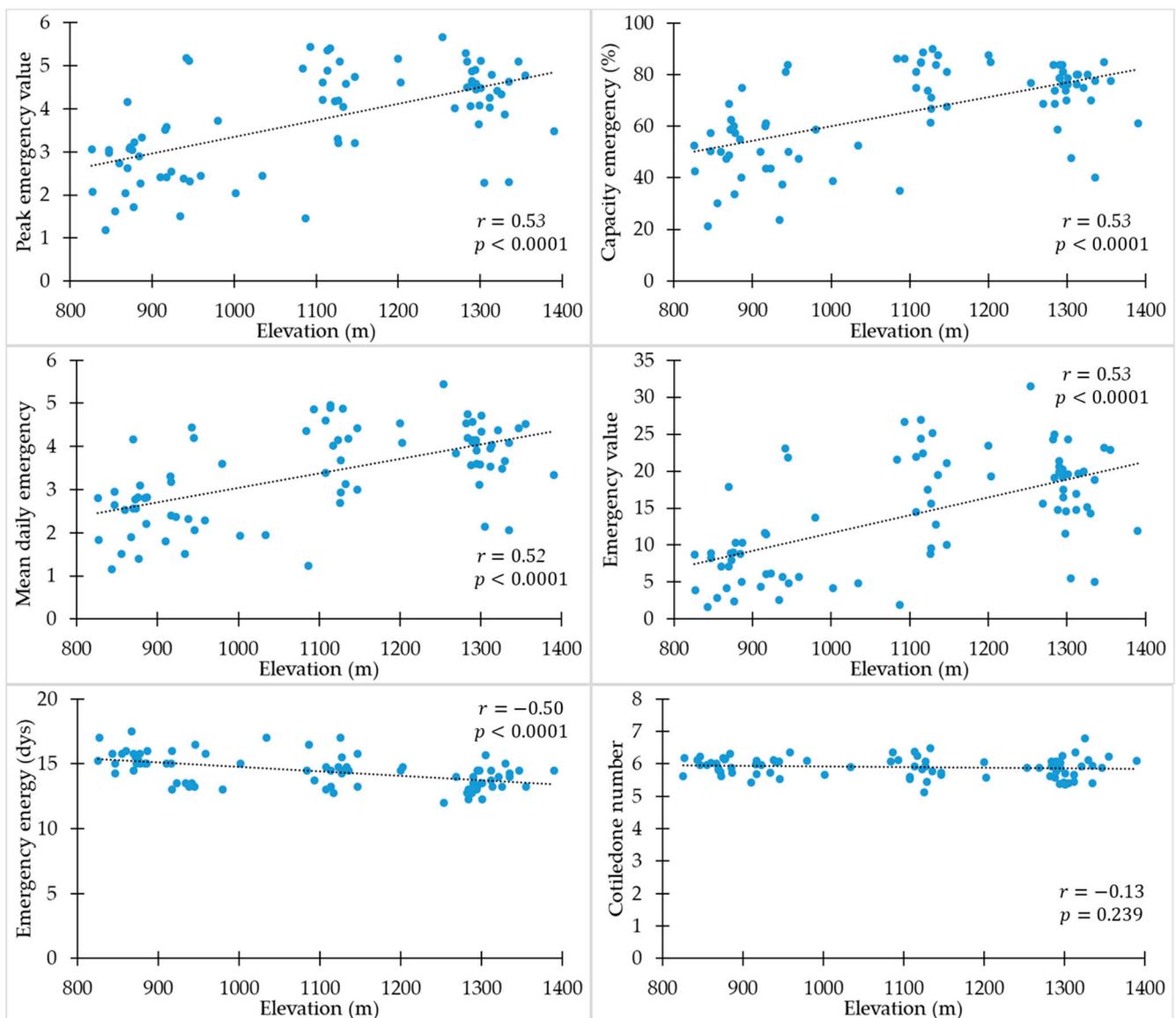


Figure 5. Association between emergence parameters and the number of cotyledons and the elevation of *Pinus oocarpa* trees in the Southern Mountains of Oaxaca, Mexico. The dotted lines represent the linear regression line.

The emergence capacity, peak emergence value, daily mean emergence and emergence value showed a moderate negative relationship ($r = -0.52$ to -0.56) with the annual mean temperature (mat), annual mean precipitation (map), growing season precipitation (gsp), mean temperature in the coldest month (mtcm), mean minimum temperature in the coldest month (mmin), mean temperature in the warmest month (mtwm), mean maximum temperature in the warmest month (mmax), degree days $> 5^{\circ}\text{C}$ (dd5), degree-days $> 5^{\circ}\text{C}$ accumulating within the frost-free period (gsdd5), summer precipitation balance (smrpb), summer/spring precipitation balance (smrsprpb) and summer precipitation (smrp). Conversely, the association with the annual aridity index was moderately negative ($r = 0.35$ to 0.41). On the other hand, the association between these seedling emergence variables was moderately positive ($r = 0.47$ to 0.61) with the Julian date of the first freezing date of autumn (fday), length of the frost-free period (ffp), Julian date on which the sum of degree days $> 5^{\circ}\text{C}$ reaches (d100), spring precipitation (sprp) and winter precipitation (winp) (Table S3). The correlation between the emergence energy and environmental variables exhibited the exact opposite behavior in terms of negative or positive association (Table S3).

4. Discussion

4.1. Variation between Provenances and between Trees

The knowledge of the amount of variation existing among trees and among provenances is crucial for forest genetic improvement [10], as it allows for the direction of selection efforts. The high average contribution of the provenance factor to the total variance indicates that, to have *P. oocarpa* resinous trees with a greater emergence capacity, the selection effort should be focused on selecting the best provenance. The greater contribution of provenance to the total variance indicates a high degree of geographic differentiation, which may result from environmental variation or genetic drift due to population fragmentation and isolation [24]. However, the values of the contribution of trees to the total variance also indicate strong genetic control; therefore, within each provenance, selection among trees to achieve genetic gains [15] in emergence parameters is feasible.

For all the emergence parameters of *P. oocarpa*, except for emergence energy, provenance had a greater contribution to the total variance, contrary to what was observed for other species, where there was greater variation within families, meaning a higher contribution of error to the total variance. For example, error had a greater contribution (66.31%) to the total variance in the germination capacity of *Pseudotsuga menziesii* (Mirb.) Franco [24], as well as in the germination capacity (54.59%) and germinative value (32.45%) of trees from a sexual seed orchard of *P. leiophylla* Schiede ex Schltdl. and Cham. [31]. On the other hand, for the emergence energy of *P. oocarpa*, the tree had a greater contribution to the total variance, which was also contrary to what was reported for *P. greggii* Engelm. ex Parl. and *P. menziesii*, where the provenance had a higher contribution (59.0% and 46.24%, respectively) [24,32].

Regarding the number of cotyledons, error had the greatest contribution to the total variance, which was consistent with what has been reported for other populations of *P. oocarpa* (error contribution of 74.4%) [15] and for *P. menziesii* (error contribution of 83.47%) [24]. The wide variation in the number of cotyledons within trees is possibly due to the fact that this trait depends on embryo viability and is independent of environmental effects [18,33]; this could explain the lack of contribution of provenance to the total variance, as reported in other populations of *P. oocarpa* [15]; Additionally, the number of cotyledons is not affected by the age of the parent trees [33].

The wide variation levels among provenances and among trees resulted in differences between provenance and between trees in the germinative parameters and the number of cotyledons. Generally, the higher-elevation provenances (San Pedro, El Tizne, Las Tejas and El Nanche) did not exhibit issues in seedling emergence compared to the lower-elevation ones (Sesteadero and Rosario). This aligns with the reports for *P. patula* and *P. chiapensis* (Martínez) Andresen, where higher-elevation populations showed the greater capacity and speed of seedling emergence [13,20]. According to the results of this study, the hypothesis that the provenance and tree factor influence the parameters studied is accepted because the environmental variations of the site of origin shape the genetic component. Therefore, there is strong genetic control over the germination parameters, as has been demonstrated for the morphological variables and initial growth of this species [6,15]. In other species such as *P. cembroides*, *P. orizabensis* and *P. patula*, significant effects of the provenance on seed quality have been demonstrated [12,13]. However, it is important to note that the physiological quality of seeds also depends on factors such as provenance, seed maturity, harvest season, the age of harvested trees and the seed year [34,35].

The emergence capacity of the San Pedro, El Tizne and Las Tejas provenances was higher than the values reported for other *P. oocarpa* populations (64.06 to 73.44%) [36]. In contrast, the emergence capacity of Rosario and Sesteadero was lower, and only the El Nanche provenance had values similar to those reported for *P. oocarpa* [36]. All *P. oocarpa* provenances showed a higher emergence capacity than populations of *P. pseudostrobus* Lindl. var. *apulcensis* Farjon and Styles (26.92%) [37]. Provenances in group 1 of *P. oocarpa* had an emergence capacity similar to *P. densata* Mast. provenances (61.83 to 83.88%) [16]. Some *P. oocarpa* provenances had values similar to those of other species such as *P. devoniana*

Lindl. (64.4%), *P. pseudostrobus* (74.8%) [38], *P. patula* (54.2 to 88.3%) [13], *P. chiapensis* (50.5 to 87.0%) [20] and *P. patula* Schiede ex Schltdl. and Cham var. *longipedunculata* Loock (81.28%) [37]. On the contrary, the emergence capacity of the provenances was lower than the average for populations of selected resinous trees of *P. pseudostrobus* (85 to 98%) [8]. Additionally, higher values were reported for populations of other species such as *P. nigra* Arnold var. *salzamannii* (Dunal) Franco (90 to 94.5%) [39], *P. patula* (82.5 to 85.4%) [40], *P. cembroides* (82 to 95%), *P. orizabensis* (82.13 to 87.13%) [12] and seed production stands of *P. engelmannii* Carr. (78.5 to 98.1%) [41]. The germination capacity ranges for populations of *P. roxburghii* Sarg. (39.2 to 92.4%) [42], *P. hartwegii* Lindl. (22 to 90%) [17] and *P. menziesii* (7.7 to 84.5%) (7.7 to 84.5%) [24] were broader than those determined for the *P. oocarpa* provenances in this study.

The peak emergence value of the resinous tree provenances of *P. oocarpa* was similar to the values of *P. chiapensis* (2.26 to 4.44) [20], but lower than the values of populations of *P. cembroides* (4.35 to 5.37) and *P. orizabensis* (4.63 to 4.72) [12]. On the other hand, Las Tejas and El Nanche had peak emergence values similar to what was reported for populations of *P. hartwegii* (12.33 to 18.0), San Pedro and El Tizne had higher values while Rosario and Sesteadero had lower values [17]. The emergence value of the group 1 provenances (San Pedro, El Tizne, Las Tejas and El Nanche) was higher than that of populations of *P. chiapensis* (3.82 to 12.86); whereas, the group 2 provenances presented similar values [20]. In comparison, *P. roxburghii* showed greater variation among provenances (2.84 to 28.9) [42]. All *P. oocarpa* provenances had a higher emergence energy than populations of *P. patula* (35.4 to 45.3) [13] and similar values to populations of *P. chiapensis* (12.5 to 18.5) [20]. The emergence energy of the group 1 *P. oocarpa* provenances was similar to the germination energy of *P. cembroides* (11.13 to 14.55 days) and *P. orizabensis* (12.95 to 14.08), while the group 2 provenances had a slightly lower emergence energy [12].

Regarding the average values of the emergence parameters for superior resinous trees of *P. oocarpa*, 38 of these exhibited a higher emergence capacity than reported in other populations of the same species [37], and only 8 trees had the same value. Meanwhile, 23 trees of *P. oocarpa* had an emergence capacity similar to that of selected trees of *P. patula* (79.7 to 94.0) [43]. The variation in the average emergence capacity of *P. oocarpa* trees was similar to the average values in selected resinous trees of *P. pseudostrobus* var. *pseudostrobus* (25 to 100%) [8] and to the values of trees in a seed orchard of *P. leiophylla* (58.6 to 97.8%) [31]. Only 24 resinous trees of *P. oocarpa* had a peak emergence value similar to that of trees in a seed orchard of *P. leiophylla* (4.6 to 11.4); the rest of the *P. oocarpa* trees had lower values [31]. The variation in the germination values of trees in a seed orchard of *P. leiophylla* (11.2 to 44.2) was greater than that of selected trees of *P. oocarpa* [31]. All selected resinous trees of *P. oocarpa* had a higher emergence energy than the values of selected trees of *P. patula* [43].

Regarding the number of cotyledons, all provenances presented values close to the average reported for other populations of *P. oocarpa* (5.92) [15]; however, the average number of cotyledons per tree showed a greater difference compared to the previously reported value [15].

4.2. Association between Emergence and Cotyledons and Elevation and Climatic Variables

The correlation analysis indicates that the higher the elevation of the location of *P. oocarpa* trees, the greater the potential for seedling emergence. This proved that the hypothesis of an association between the characteristics evaluated with the elevation and environmental factors was not false. This association suggests that the altitudinal origin of *P. oocarpa* seeds in the Southern Mountains of Oaxaca, Mexico, has an effect, and there is a pattern of clinal genetic differentiation according to these characteristics, which may be an adaptive response [19,20]. This aligns with other studies; for example, elevation had a positive relationship with the germination energy in *P. brutia* Ten. [44] and *P. patula* [13], with the germination value of *P. roxburghii* [42] and with the germination energy of *P. patula* [13]. However, the relationship is not always positive in conifers; for *P. brutia* [44] and *P. mensiesii* [24], elevation's relationship with the germination percentage was negative,

which was contrary to the observations in this study. Similarly, contrary to the positive linear relationship found in *P. oocarpa*, the relationship was quadratic between altitude and the germination capacity in *P. hartwegii* [17] and *Abies religiosa* (Kunth) Schltdl. and Cham. [45]. In other *P. oocarpa* populations, the relationship between elevation and seedling height growth was also quadratic [19].

The significant association between the emergence parameters of *P. oocarpa* seedlings and climatic factors is logical due to the relationship between elevation and climatic variables [19]; however, contrary to the elevation relationship, most variables related to temperature and precipitation were negatively correlated with the emergence parameters. This indicated that trees located in wetter and warmer sites exhibit lower emergence values. Similar to this, in *P. mensiezii*, the germinative capacity was positively associated with the total annual precipitation [24]; in contrast, in *P. densata*, the germinative capacity, daily mean germination, peak value and germination value were positively associated with the average maximum temperature in December [16]. In this study, only climatic variables related to the winter precipitation, frost-free period, autumn freezing date and annual humidity index were positively related to the germinative parameters. The relationship between the humidity index and emergence parameters was contrary to what was found in other populations of *P. oocarpa*, where the correlation with juvenile height growth was negative [19].

As for the number of cotyledons, it was not associated with the elevation and climatic variables, confirming its independence from environmental effects [18,33]. The lack of association between the number of cotyledons and elevation and climatic variables coincided with the findings in *P. mensiezii*, where this characteristic was not associated with elevation, mean annual temperature or mean annual precipitation [24].

4.3. Practical Applications

The results of this study are vital to continue the forest genetic improvement cycle since these trees constitute the selection population due to their superior resin production [11]. Knowledge of reproductive capacity allows for the selection of those trees that will form the production population (seed orchards) [10,11], where the greatest seed emergence is necessary to plan and cover operational planting programs; in addition, homogeneous and early germination is also important. For example, in other species, it has been shown that early emergence increases the fresh mass of seedlings [46] and benefits growth [47]. On the other hand, it should be recognized that, due to the scant progress in genetic improvement in *P. oocarpa* for resinous purposes, these trees will eventually constitute the source of seeds for urgent planting and reforestation programs [10], so it is essential to know their reproductive capacity. Under these same approaches, the variation in the number of cotyledons of these trees becomes important due to its strong genetic control and its high genetic correlation with initial plant height [15].

The results of the distribution of variance components indicated that, in order to have trees with a good emergence capacity, the best provenances must first be selected. In this sense, according to the results of the analysis at the provenance level, firstly, seeds from San Pedro and El Tizne are recommended, and secondly, Las Tejas and El Nache. Likewise, the contribution of the tree factor to the total variance indicated that within the provenances, selection should also be made to obtain trees with better emergence values. Related to this, the analysis at the family (tree) level indicated that the families SDC13, SDC10, SDC18, SDC46, SDC31 and SDC07 presented high values for all the emergence parameters, which permits the assumption that these are the families with the best reproductive capacity (sexual), and that they are recommended for urgent needs and to form the production population (seed orchards). Likewise, although the average values of the Rosario provenance were low, the analysis at family level showed that some trees of this provenance presented values statistically equal to the highest value in the capacity of emergence (CR04 and CR06), peak value of emergence (CR04 and CR06), average daily emergence (CR04, CR06, CR19) and value of emergence (CR03, CR04, CR08, CR12), so these trees can be used

to cover the urgent needs of plantations in low-elevation sites. This shows that even within provenances with a lower performance, there are families with high emergence parameter values, which favors the objectives of forest genetic improvement.

According to the results of the multivariate analysis, to guarantee seed production, seed orchards should be established with seeds from trees belonging to group 1 (Figure 4); however, if it is desired to maintain a broader genetic base, trees with high and average values in terms of the germination parameters and number of cotyledons can be established (Table S2).

In contrast, the trees SDC06 (Las Tejas provenance) and SDC26 (San Pedro provenance), which correspond to higher-performing provenances, were statistically equal to the lowest values for the emergence capacity, peak emergence value, mean daily emergence and emergence value. Likewise, the families CR25, CR15, CR11, CR24, CR01, CR06, ES02 and CR10 were the worst in all emergence parameters; therefore, these trees are not recommended to be continued in the forestry breeding cycle, unless adequate technology for vegetative propagation is developed.

On the other hand, the results of the association between the emergence parameters and the elevation and climatic variables indicated that the provenances of *P. oocarpa* are adapted to particular environments [19,20]; therefore, the movement of seeds of these trees should be taken care of. In the Southern Mountains of Oaxaca, there are no rules for seed movement for *P. oocarpa*; however, in Michoacán, Mexico, altitudinal seed zones of up to 200 m in amplitude have been determined [19]. Therefore, until detailed studies are available, to guarantee the success of reforestation with seeds of the *P. oocarpa* trees studied, seeds from the provenances El Sestadero and Rosario should be planted in sites with an elevation between 800 and 1000 m asl. Seeds from San Pedro should be planted between 1000 and 1100 m asl and seeds from El Tizne, Tejas and El Nanche should be planted between 1200 and 1400 m asl.

5. Conclusions

The wide ranges of variation in germination parameters and the number of cotyledons among superior resinous trees of *P. oocarpa* lead to differences between provenances and between trees. At the provenance level, those at higher elevations do not face issues with seedling emergence. Even in provenances with lower performance, there are trees with high emergence values, so within provenances, individuals with a high reproductive capacity can be selected. The differentiation between provenances and trees is associated with elevation; at higher elevations, emergence parameters are better. Additionally, due to the relationship between elevation and climatic factors, the latter also influence the germination parameters; trees in warmer and wetter sites have lower emergence values. In contrast, higher winter precipitation, a longer frost-free period, a later autumn freezing date and a higher annual humidity index benefit the emergence parameters. The elevation and environmental variables of tree locations do not influence the variation in the number of cotyledons. Finally, understanding the level of variation and differences between provenances and between trees, as well as the relationship between elevation and climatic variables and emergence parameters, enables decisions regarding the selection population (selected resinous trees) to establish the production population (seed orchard establishment) and continue with the forest genetic improvement cycle.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/seeds3010001/s1>, Table S1: The environmental data of *Pinus oocarpa* trees in the Southern Mountains of Oaxaca, Mexico; Table S2. General average, minimum and maximum values of emergence parameters and number of cotyledons of superior resin trees of *Pinus oocarpa*, classified into high values (statistically equal to the highest value), intermediate and low (statistically equal to the lowest value); Table S3. Spearman's correlation coefficients of elevation and environmental variables vs. emergence parameters and number of cotyledons of *Pinus oocarpa* trees in the Southern Mountains of Oaxaca, Mexico.

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