Altitudinal genetic variation among native *Pinus patula* provenances: performance in two locations, seed zone delineation and adaptation to climate change

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Abstract

To select the genetic source of *Pinus patula* Shiede and Deppe seed best adapted to different native zones for reforestation, seedlings of 12 provenances native to a selected altitudinal gradient in Oaxaca, southern Mexico, were planted on two sites at contrasting altitudes (high 3000 m above sea level (masl) and low, 2500 masl) within the species natural distribution, and tested for growth in height at age 18, 24 and 36 months. Differences among provenances and between sites showed significance ($p < 0.05$). Seedling growth showed a climatic and altitudinal pattern, in which seedlings from populations originating in low-middle altitudes had higher growth than populations originating in high altitudes, and from the extreme low altitude limit. Results were used to delineate climatic and altitudinal seed zones for contemporary and future climate zones in the decade centered in year 2030, using the average results of six climate-emissions scenarios. The results indicate that splitting the region into four seed zones is appropriate under contemporary climate scenarios with the following altitude limits: Zone 1, from 2300 to 2500 masl; Zone 2, 2500 to 2700 masl; Zone 3, 2700 to 2900 masl and Zone 4 from 2900 to 3100 masl. The equivalence on climatic intervals was also defined for mean annual temperature, precipitation and an annual aridity index (AAI). Values of contemporary AAI occurring at a given altitude by year 2030 were determined, with results suggesting that populations should be shifted 200 to 250 m attitudinally upward, through a program of assisted migration, to realign them to the future climate for which they are adapted.

Key words: Assisted migration; climate change; genetic differences; seed zoning; provenances; annual aridity index; *Pinus patula*.

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Introduction

Pinus patula Shiede and Deppe is an endemic species of Mexico (PERRY, 1991). The species is extensively grown in several countries due to its high growth rates and potential for timber, pulpwood and plywood production (WRIGHT et al., 1995; KARIUKI, 1998; DVORAK et al., 2000).

Ixtlán de Juarez, in the north of the state of Oaxaca, southern México, is an indigenous Zapotec community, in which sustainable forest management and associated industry provides employment for most of the economically active population. *P. patula* is one of the predominant species used in production and reforestation programs in the north of the state of Oaxaca (OVIEDO, 2002; CASTELLANOS-BOLAÑOS et al., 2008; MATSUZAKI and WONG, 2010).

Provenance tests of forest trees, offer an excellent method for predicting growth changes associated with environmental and climatic change (MATYAS, 1994; SCHMIDTLING, 1994). A multitude of provenance tests set in several countries on pine species show that populations tend to respond differently to selection pressures, in terms of climatic and altitudinal gradients (REHFELDT et al., 2014). Populations at higher altitudes are generally adapted to colder climates, show lower growth potential, shorter periods for shoot elongation and better tolerance to cold environments, compared with those from warmer sites at lower altitudes (WEINSTEIN, 1989; REHFELDT, 1995; SÁENZ-ROMERO et al., 2012b; CARRASQUINHO and GONÇALVES, 2013). An understanding of the adaptation of tree populations to different environments through accurate site-population matching, together with predicted environmental risks for profitable site and scheduling actions, may enhance decision making and improve reforestation efficiency under both present and future climatic scenarios (LOUW and SCHOLES, 2006; DVORAK et al., 2008; REHFELDT et al., 2014).

There is new evidence that in some mountain habitats climate change is inducing an upward elevation shift of range distribution for some species (PEÑUELAS et al., 2007; LENOIR et al., 2008). However, the speed at which this natural migration is happening might not be enough to keep coupled the tree populations to the climate for which they are adapted (JUMP et al., 2009). Evolutionary processes such as natural migration require time periods that might be longer than the time available, considering the speed at which global warm-

ing is occurring (AITKEN et al., 2008). Thus, tree populations will likely be decoupled from their ecological niches (REHFELDT et al., 2002; MÁTYÁS, 2010; MÁTYÁS et al., 2010; SÁENZ-ROMERO et al., 2012a; REHFELDT et al., 2014). It is then necessary for mankind to participate in the adaptation process, and assure that appropriate species and genotypes track their optimum climate in a timely manner, through seed transfer programs from their sites of current location, to places where future optimum climates will occur (SCHMIDTLING, 1994; TCHEBAKOVA et al., 2006; AITKEN et al., 2008). In the present circumstances, this could be done by shifting *P. patula* populations to altitudinal climates for which they will become genetically adapted, taking into consideration the change in altitude and corresponding change in temperature (0.5 °C) with each increase of 100 m of altitude, that occurs in general in the mountains of Mexico (SÁENZ-ROMERO et al., 2010).

Climatic change is expected to impact the present natural distribution and abundance along latitude and elevation gradients of *P. patula*, mainly at the margins (VAN ZONNEVELD et al., 2009). According to the projected distribution of suitable climatic habitats for conifers, a complete redistribution of genotypes could be required in order to avoid large losses of forest cover due to climatic change, as has been documented for several forest species (REHFELDT et al., 2002; JOHNSTONE and CHAPIN, 2003; MCLACHLAN et al., 2007; REHFELDT and JAQUISH, 2010; REHFELDT et al., 2012).

In previous provenance testing in a nursery (SÁENZ-ROMERO et al., 2011b) and in growth-chambers similar *Pinus patula* provenances (SÁENZ-ROMERO et al., 2011a), significant genetic differentiation among populations was evident along an altitudinal gradient.

The objectives of this work were to (1) study the genetic variation for growth traits among populations in field conditions along altitudinal gradients; (2) to delineate zones for seed collection and planting and, (3) to evaluate the potential impact of climate change on the persistence of the natural populations of these species at their contemporary locations, in order to take appropriate conservation action and develop reforestation guidelines.

Our study combined provenances collected along an altitudinal transect, tested at field sites with contrasting altitudes, together with estimated contemporary and future climates for the provenances and test sites, using a climatic spline model. Climate projections considered three General Circulation Models (GCMs) under each of two greenhouse-effect gas emission scenarios, thus aiming to provide a common basic impact and adaptation assessment for climate change.

The results may be used in selecting the appropriate sources for seeds collection and transfer along an altitudinal gradient through reforestation guidelines, by matching genotypes with the appropriate planting site in the forests, in the context of climatic change.

Material and methods

Seedling sources and establishment

Open-pollinated seeds from 13 native *P. patula* populations were collected along their natural altitudinal range of distribution in Ixtlán de Juárez, Oaxaca Mexico – one population every 50 meters of altitudinal difference, from 2400 to 3000 masl, 11 trees per population, randomly identified from the forest as those bearing mature cones. The trees represented by these samples are termed 'populations', while the location of a population is termed 'provenance'. Seed was sown in pots in a randomized block design in a nursery at Ixtlán de Juárez (SÁENZ-ROMERO et al., 2011b). When seedlings were nine months old with average seedling height of 28 cm, they were transplanted to two locations within the natural forests of Ixtlán de Juárez at contrasting altitudes; one site close to the highest and the other to the lowest natural limits of the species in the area. The 'high site' was located at 3000 altitudinal meters (17°23.157'LN, 96°27.309'LW), and the 'low site' at 2500 altitudinal meters (17°22.480'LN, 96°28.580'LW) (*Table 1, Figure 1*). The provenance test was established

Table 1. – Geographic location of 13 provenances of Pinus patula collected in the forest of the native Indian community of Ixtlán de Juárez, Oaxaca, México, and the two experimental field sites.

| Provenance	Altitude (m. a. s. l.)	Lat N	Long W
1	3000	17°22.270'	96°26.987'
2	2950	17°23.154'	96°27.402'
3	2900	17°23'185'	96°27.839'
4	2850	17°21.793'	96°27.399'
5	2800	17°21.795'	96°27.562'
6	2750	17°23.004'	96°28.577'
7	2700	17°21.749'	96°27.880'
8	2650	17°23.162'	96°28.938'
9	2600	17°23.059'	96°28.861'
10	2550	17°22.902'	96°28.902'
11	2500	17°22.808'	96°29.067'
12	2450	17°22.679'	96°28.986'
13	2400	17°22.353'	96°28.971'
Experimental field sites			
Site "Low"	2500	17°22.480'	96°28.580'
Site "High"	3000	17°23.157'	96°27.309'
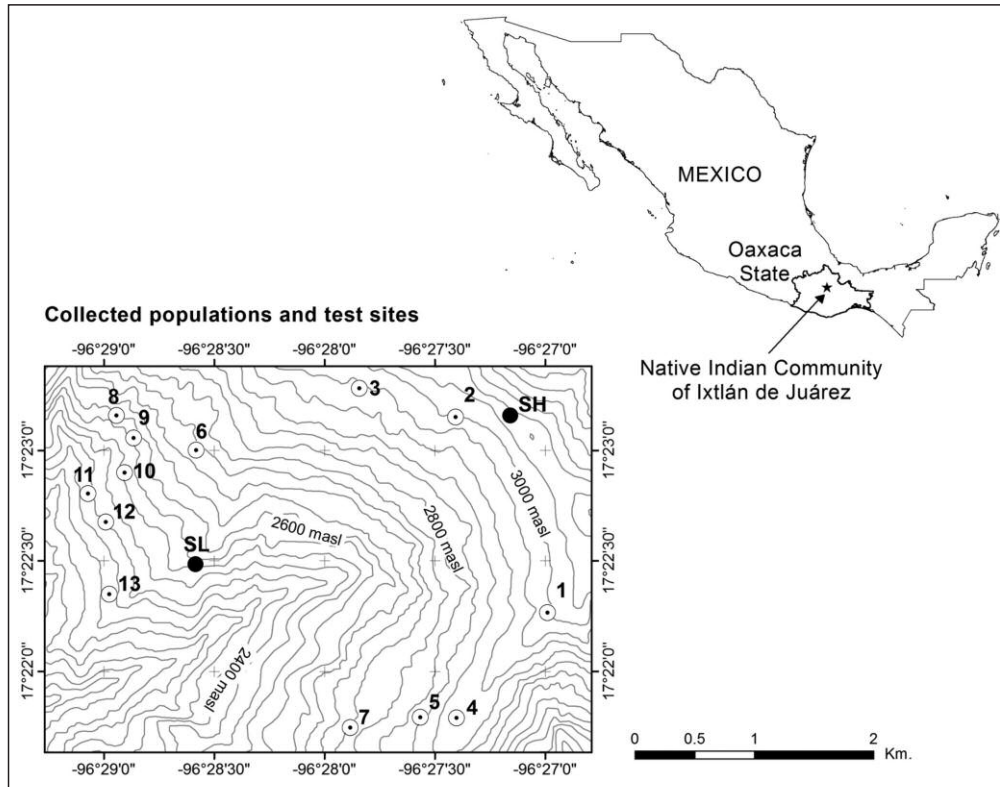
Figure 1. – Geographic location of the collected *Pinus patula* populations and tests sites in the forest of the native Indian community of Ixtlán de Juárez, Oaxaca, México. Population number in the panel insert as coded in *Table 1*. SH and SL indicate tests sites at high (3000 m) and low (2500 m) altitude, respectively.

in the rainy season (early August) of 2008. Each site had an identical design: randomized complete block design with 12 blocks, 13 provenances and four seedlings per plot in a row (48 seedlings per provenance per field site).

Assessment and data analysis

Total seedling heights (cm) were measured at 18, 24 and 36 months (age from seed germination), equivalent to 9, 15 and 27 months post-planting. The significance of differences among sites and among populations was determined by an analysis of variance using PROC GLM (SAS 2011), and model [1]. Blocks and populations were considered as random effects and site was considered as fixed. We used the option TEST to specify the appropriate denominator for F-statistics. The proportion of variance compared with the total variance was estimated using PROC VARCOMP METHOD = REML (SAS, 2011). It was conducted a mean multiple comparison using Tukey tests, to identify where significant differences existed between population means.

$$Y_{ijkl} = \mu + S_i + P_j + B_k (S_i) + \qquad [1]$$
$$S_i * P_j + P_j * B_k (S_i) + e_{ijkl}$$

Where Y_{ijkl} is the individual-tree observation, μ the overall mean, S_i the effect of the ith site, P_j the effect of the jth population, $B_k (S_i)$ the effect of the kth block nested in the ith site , $S_i * P_j$ the interaction of the ith site by the jth population, $P_j * B_k(S_i)$ the interaction of block nested in site by population and e_{ijkl} is the error term.

An analysis of variance and estimation of the components of variance was conducted for each separate site, using the same model [1], but without the site term and its interaction.

We determined a pattern of altitudinal differentiation through conducting a regression analysis (linear and quadratic) between provenance performance and the climate or altitude of origin, using PROC REG (SAS, 2011).

Simple linear regression:

$$Y_{ij} = \beta_0 + \beta_1 X_i + e_{ij} \qquad (2)$$

Quadratic regression:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X^2_i + e_{ij} \qquad (3)$$

Where Y_{ij} = mean seedling height of the ith provenance, β_0 = intercept, β_1 and β_2 = regression parameters, X_i = climate or altitude (masl) of the ith provenance and e_{ij} = the error.

Climate estimations

Contemporary climate estimations (average of years 1961–1990) were made for each of the 13 collection sites and the two field test sites with a spline climate model, such as in SÁENZ-ROMERO et al. (2010). It was assumed that contemporary populations are adapted to the contemporary climate and not to the average for 2000–2010, and thus already affected by global warming.

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We estimated for each site mean annual temperature, annual degree days >5°C (DD5), mean annual precipitation (MAP), and an annual aridity index (AAI, ratio of square root of DD5 to MAP). The index indicates the association between the temperature suitable for plant growth (daily temperatures above 5°C), and moisture availability (precipitation) throughout the year. Climate variables were selected on the basis of having demonstrated relevance and association with forest tree population genetic differentiation and performance (REHFELDT, 2006) and association with biome distributions (REHFELDT et al., 2006). Climatic values were obtained by querying a spline climatic model (SÁENZ-ROMERO et al., 2010) uploaded on a web site interface (http://forest.moscowfsl.wsu.edu/climate/; REHFELDT, 2006; CROOKSTON and REHFELDT, 2013), in terms of latitude, longitude and elevation of each collection and field test site.

We selected the decade centered on the year 2030 as the target for realigning the tree populations to the future climate for which they were adapted, to avoid damage from frost on recently planted seedlings; if contemporary genotypes were transferred to higher, colder altitudes where suitable weather is expected to occur by the decade centered in the year 2060 or further, it is likely that severe frost damage could occur in the present, after planting (SÁENZ-ROMERO et al., 2012a; LOYA-REBOLLAR et al., 2013).

Future climate estimates were assessed with the average output data across six climate model-scenarios, being the results from the three global circulation models (GCMs). Each GCM was combined with each of the two greenhouse-effect gas emission scenarios: (1) CGCM3 (T63 resolution), from Canadian Center for Climate Modeling and Analysis (CCCMA), scenarios A2 and B1; (2) HadCM3, from Met Office, Hadley Centre (UKMO), scenarios A2 and B2; and (3) CM2.1, Geophysical Fluid Dynamics Laboratory (GFDL), scenarios A2 and B1.

The A2 emissions scenarios assume in general continued high emissions from an increasing human population, but dissimilar economic and technological development according to country, and are thus regionally oriented, sometimes referred to as the 'business as usual' or 'pessimistic scenario'. Alternatively the B1 and B2 scenarios combine social and economic solutions to sustainability, a lower rate of increase in global population and slower, more diverse technological development with emphasis on environmental protection and social equity, than the A2 scenarios, thus referred to as the 'optimistic scenario' (IPCC, 2013).

Clines were developed to estimate the upward altitudinal shift needed to realign the *P. patula* population to the sites in which they would occur under a future climate (decade centered in the year 2030). Regression models were fitted for a contemporary and future climate variable AAI, adjudged as the single climatic variable best representing the climate, incorporating degree days and precipitation available for plant growth, against the altitude of the provenance of origin, using Proc REG (SAS 2011), to assess climatic clines among

populations in genetic responses to climate of provenances.

Seed zoning

We delineated climatic and altitudinal zones, using the least significant difference (LSD, $\alpha = 0.20$) among populations of 36 month-old seedling height. The LSD value was translated to the climatic or altitudinal difference that makes two populations statistically different. The regression model fit between population performance against climate or altitude of the population of origin was used to delineate the zones, as well the association between altitude and climate values of the provenances (REHFELDT, 1991; SÁENZ-ROMERO et al., 2006). It is noteworthy that use of 0.2 alpha error on this zone delineation process leads to a more conservative set of management rules, because the resulting altitudinal intervals, making two population significantly different, are narrower and therefore there will be more restrictions on seed movement (SÁENZ-ROMERO et al., 2006; SOTO-CORREA et al., 2012; CASTELLANOS-ACUÑA et al., 2014).

Results

Differences between test locations and populations

Evaluations on seedling growth indicate highly significant statistic differences between locations ($P \leq 0.05$) at all ages tested. Genotype by environment interactions (site x population) were also significant. Populations growing at the low site (2500 m) had, on average, more than twice the seedling height than seedlings at the high altitude site (100 cm vs. 48 cm at 24 months; and 220 vs. 93 cm at 36 months old) (*Table 2, Figure 2*).

Differences in seedling height among populations were not significant at any age, when the analysis was conducted across both sites using the full statistical model. However, in the separate site analyses, significant statistical differences were identified for mean seedling height among populations, except for the site at high altitude at 36 months of age (*Table 2*).

The generally poorer growth at the high altitude test site (*Figure 2*) and the lack of significance among populations at age 36 months (*Table 2*), might be the result of stress induced by the cold conditions of this site, located at 3000 m of altitude. Several seedlings exhibited very short needles and dead or damaged leader buds. Damaged terminal buds on such short seedlings in all likelihood increased the variation within the provenance, thus causing a reduction in statistical power to reflect significant differences among populations.

Altitudinal and climatic pattern of genetic differentiation

Population means for seedling height were significantly different between provenances and related to provenance altitude of origin or provenance climate in the low altitude test site (2500 m; *Figure 3a*). At the low altitude test site, the best regression fit, for the three ages measured, was obtained by quadratic modeling of seedling height against altitude: $r^2 = 0.65$, $P = 0.005$ at 18 months;

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Table 2. – Individual-site and pooled site analyses of variance for 18, 24 and 36 month-old *Pinus patula* provenance tests. Percentage contribution to total variance (%) and significance values (P).

| Source of Variation	18 months		24 months		36 months	
	%	P	%	P	%	P
Overall						
Site	51.1	<.0001	72.1	<.0001	77.9	<.0001
Population	2.6	0.191	0.7	0.272	0.4	0.287
Block (Site)	1.6	0.002	2.5	<.0001	4.0	<.0001
Site * Population	3.2	0.001	1.3	0.012	1.1	0.010
Pop * Block (Site)	2.7	0.089	3.2	0.010	2.2	0.005
Error	38.7		20.1		14.5	
Low Site 2500 masl						
Population	8.6	0.001	7.4	0.006	7.9	0.001
Block	4.5	0.008	10.7	<.0001	24.3	<.0001
Population * Block	6.6	0.020	14.4	0.001	10.0	0.003
Error	80.4		67.5		57.8	
High Site 3000 masl						
Population	23.8	<.0001	6.5	0.002	0	0.535
Block	1.2	0.321	6.6	0.001	3.0	0.026
Population * Block	5.3	0.283	0	0.713	11.7	0.061
Error	69.7		86.9		85.3	
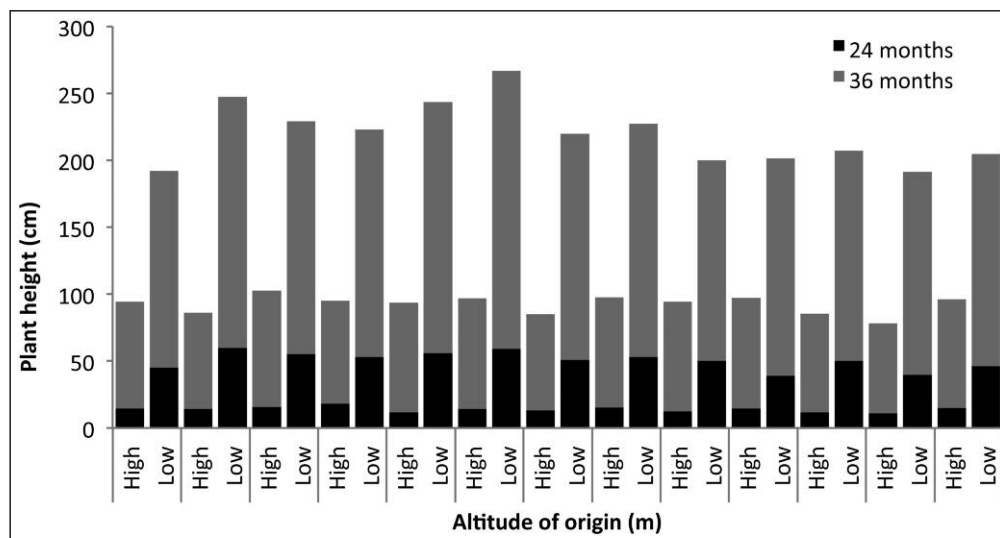
Figure 2. – Average seedling height at age 24 and 36 months for 13 populations of *Pinus patula* in a provenance test. High = High altitude provenance test site (3000 m). Low = Low altitude provenance test site (2500 m).

$r^2 = 0.56$, P = 0.019 at 24 months and $r^2 = 0.42$ P = 0.067 at 36 months respectively. In general, populations from low-mid and mid altitudes achieved better growth (*Figure 3a*), than populations from the extreme low and high altitudinal limits. This altitudinal trend was similar at the high altitude site (3000 m; *Figure 3b*), although less pronounced and not significant. At both field sites, the population from altitude 2650 m (the mid-low part of the altitudinal distribution) demonstrated the best growth by far (*Table 2, Figure 3*).

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Similar growth trends were found at early ages (6-month-old at nursery stage) in the same provenances (SÁENZ-ROMERO et al., 2011b), as well as in the growth chambers (SÁENZ-ROMERO et al., 2011a).

A very similar and significant clinal pattern was found when regressing average 36-month-old seedling height from the low altitude site, against climate of the provenances, in particular against Annual Aridity Index values: quadratic model: $r^2 = 0.58$ P = 0.019. In general, populations from high-mid and mid annual aridity index values achieved better growth (*Figure 3a*), than populations from the annual aridity index extreme low index values (too cold and humid) and the extreme high index values (too dry and warm). The similarity between altitudinal and climatic patterns for seedling performance are due to the strong association between Annual Aridi-ty Index (AAI) and altitude, indicated by a highly significant regression analysis ($r^2 = 0.98$, P = <.0001). This strong association can be explained by the strong altitudinal cline of growing degree days > 5 °C. At the high elevation site, however, regressions between climate or altitude of provenance against seedling height average per population, were not statistically significant at any age (*Figure 3b*), most likely due to the generally poor growth across populations.

Seed zoning and guidelines for seed movement

Least significant difference (LSD $\alpha = 0.20$) among provenances was equal to 18 cm for seedling total height. When using that LSD value for delimiting the altitudinal or climatic zone interval limits, by finding its equivalence on an altitudinal or climate interval making
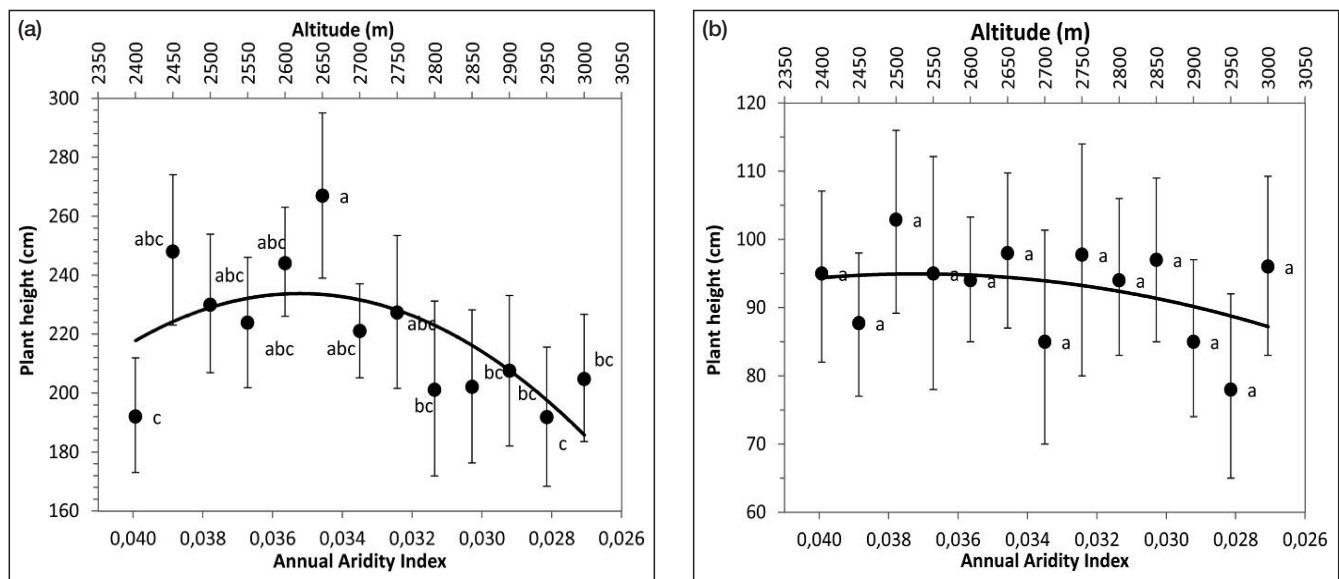

Figure 3. – Regression between average seedling height per population against Annual Aridity Index (AAI; larger index values = warmer and less moisture, more arid; lower values = colder and more moisture, less arid) or altitude (m) of seed source, using a quadratic model (solid line, using all populations). a) Low altitude site (2500 m). b) High altitude site (3000 m). Vertical bars indicate population average confidence intervals (95 %). Different letters indicate mean grouping according to Tukey ($\alpha = 0.05$) test.

Table 3. – Proposed altitudinal and climatic limits of the four seed zones of *P. patula*, established for the region of Ixtlán de Juarez, Oaxaca, southern México.

Zone	Altitude (m) Limits			Mean Annual Temperature (°C) Limits			Annual Precipitation (mm) Limits			Annual Aridity Index (index) Limits		
	Lower	Upper	Range	Lower	Upper	Range	Lower	Upper	Range	Lower	Upper	Range
1	2300	2500	200	14.7	13.5	1.2	1411	1499	88	0.041	0.037	0.004
2	2500	2700	200	13.5	12.3	1.2	1499	1587	88	0.037	0.033	0.004
3	2700	2900	200	12.3	11.1	1.2	1587	1675	88	0.033	0.029	0.004
4	2900	3100	200	11.1	9.9	1.2	1675	1763	88	0.029	0.025	0.004

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any two populations significantly different, we found different interval sizes, due to the quadratic shape of the curve (*Figure 4*). Zone delimitation commenced in such a manner that one zone could be centered approximately
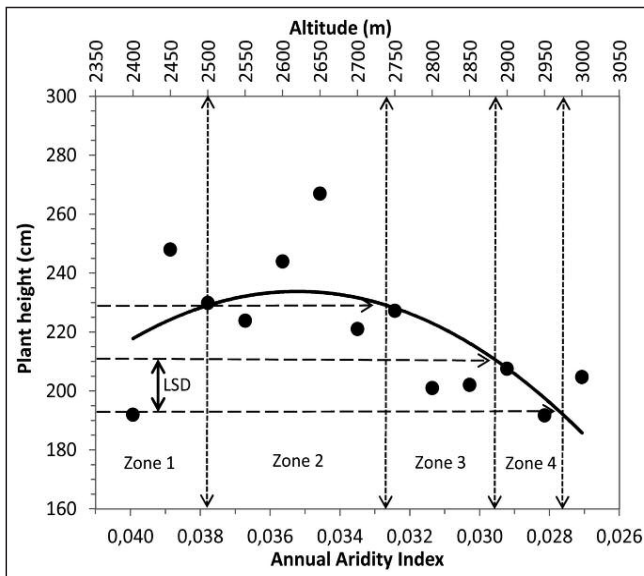

Figure 4. – Delineation of climatic or altitudinal seed zones. The quadratic regression line between average seedling height per population against Annual Aridity Index of seed source is intersected by an interval whose width is defined by seedling height least significant difference (LSD, indicated by the double solid arrow) that makes two populations genetically different. The associated vertical broken lines (double arrows) show the equivalence of such difference on the Y axis, either for annual aridity index or (approximately) for altitude (m).

on those populations with the best growth (population from 2650 m of altitude), and was then continued the towards the downward and upward altitudinal cline. The resulting climatic or altitudinal zones were named Zone 1, with a width of 0.0030 of annual aridity index value (AAI) or 150 m of altitudinal difference; Zone 2, the one centered at the best populations, with a width of 0.0045 of AAI or 240 m; Zone 3: 0.0040 AAI or 135 m; Zone 4: 0.0020 or 90 m (*Figure 4*). However, seed zones should not only be scientifically sound, but also practical for management (SÁENZ-ROMERO et al., 2006). In the present case, zone 4 undoubtedly is too narrow to be useful for management purposes. Consequently, we suggest the use of a fixed zone of 200 m width in altitudinal difference – the average width across zones was 154 m, with the centered zone 240 m, which is approximately equivalent to 0.004 units of AAI. Further, maintaining the practice of centering one seed zone on the best performing populations, we suggest a seed zone delimitation as demonstrated in *Table 3*, which for practical management of seed zones, can be converted from contemporary climate variables to altitude, or *vice versa*, due to the significant association, as mentioned above, between altitude and Annual Aridity Index. Seed zone delimitation based on AAI, mean annual temperature and precipitation followed the same procedure, as used in terms of elevation. This process was based on the results of the low altitudinal site only, because at the high altitude there were no significant differences among provenances at age 36 months (*Table 2*), nor was the altitudinal trend significant (*Figure 3b*).

These zones are to be used for guiding seed and seedling transfer through selection of the best seed source according to the objectives of reforestation prac-
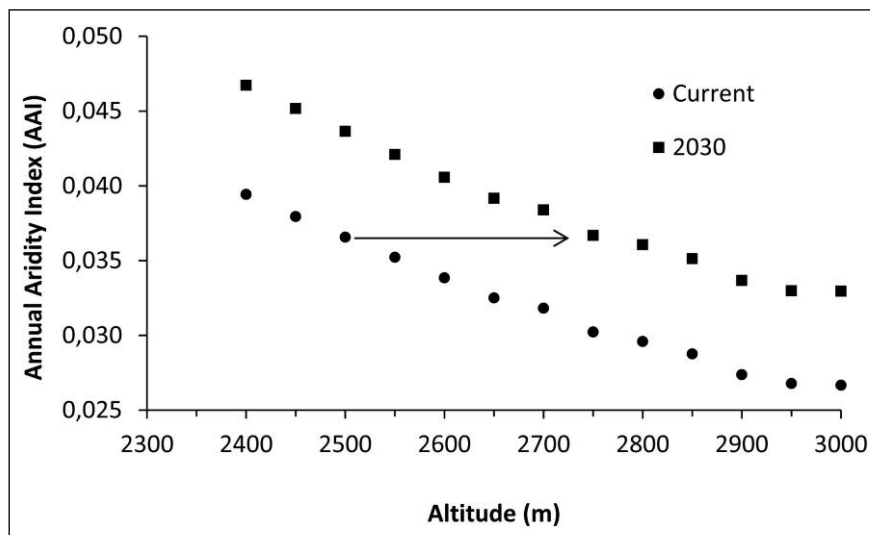

Figure 5. – Average annual aridity index (AAI, larger values are dryer and warmer) for contemporary (average 1961-1990, circle symbols) and future climate projections (decade centered in the year 2030; average across six model-scenario; square symbols), for 13 *Pinus patula* provenances along an altitudinal cline. The arrow shows the altitude shift required (approximately 200 to 250 m) to match contemporary AAI in 2030 for the provenance originating at 2550 m of altitude, as an example.

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tices for: (a) Ecological restoration and conservation of genetic resources, in which case the guideline is to use seed or seedlings originating within the same seed zone or within ± 100 m in altitude of the seed source; (b) tree breeding for wood production in commercial plantations, in which the guideline is to select sources from the best growing provenance or populations within the seed zone with the best growth performance; and (c) adaptation to climatic change effects, in which the guideline is to collect seeds from a given seed zone, produce seedlings in the nursery and plant in the adjacent altitudinal zone, higher than the current zone. In terms of the ability of *P. patula* populations to cope with the climate projected for the decade centered around 2030, populations should be shifted attitudinally between approximately 200 and 250 m upwards, to expected similar climatic conditions (AAI) in which populations are currently adapted (*Figure 5*). Such an upward altitudinal shift is roughly equivalent to the width of a delineated seed zone. Notice that seed zones differ by about 0.004 AAI, 1.2 °C in mean annual temperature and 88 mm precipitation (*Table 5*), and that estimates for 2030 show an AAI increase of 0.006, mean annual temperature of 1.5 °C and a decrease in precipitation of approximately 120 mm. Thus, a simplified guide for field management, in at least partially coping with these projected climatic change, would be to collect seeds from a given seed zone, produce seedlings in the nursery and plant in the adjacent altitudinal zone, higher than the current zone.

Discussion

The altitudinal pattern of differentiated growth among populations of *P. patula* is likely to be the result of local adaptation to environmental conditions. Environmental differences along the altitudinal gradient, even over short linear distances, are a characteristic of steep landscapes and typically generate genetic variation in populations distributed along the gradient, in response to differential selection pressures (THOMAS, 2011; MORI et al., 2013).

In this study, *P. patula* populations originating, in general, at mid-low and mid altitudes had a higher growth potential, which seems to be characteristic of this species (SALAZAR-GARCÍA, 1999). However, the population from the extreme lowest altitude (2400 m) had poorer growth performance than other populations from similar altitudes. This is possibly due to the accepted strategy for pine populations from low altitudes, tending to have a more conservative growth strategy to avoid drought stress (SÁENZ-ROMERO et al., 2006). At its low altitudinal limit, *P. patula* is interspersed with *P. oaxacana*, a species that grows in much dryer sites than *P. patula*. On the opposite altitudinal extreme, provenances from upper altitudes and colder climates, in general grew slower, since they may have evolved strategies to survive adverse conditions of lower winter temperatures, through shorter growing seasons and extended and deeper dormancy periods, than those sourced from warmer climates (DVORAK et al., 2000; SÁENZ-ROMERO et al., 2006; MITCHELL et al., 2013).

The consistency of the best performing provenance, from altitude 2650 m, is remarkable. It was further one of the best performing provenances at the nursery stage (SÁENZ-ROMERO et al., 2011b), as well as in growth chambers (SÁENZ-ROMERO et al., 2011a).

Management alternatives of altitudinal zoning

The proposed seed zones may be used for several practical applications in reforestation and forest management programs.

In ecological restoration programs, the main objective is to restore a transformed area with original elements and functions (HOBBS and NORTON, 1996). Thus, seed collected within a zone can then be used within the same zone, on the assumption that these are well adapted to local environmental conditions (ST. CLAIR, 2006; GONZALO-TURPIN and HAZARD, 2009). Similar rules apply for programs focused on conservation of forest genetic resources, where in-situ protection is vital for protecting the genetic diversity, as well as allowing the natural evolutionary forces to shape the genetic structure of populations. For genetic conservation, reforestation should capture a representative sample of the genetic diversity of *P. patula* within the zone (DVORAK et al., 2000).

In tree breeding for commercial timber production, it is advisable to select the population with greater performance in both sites, or use the zone with the best population. Hence, the population originating at 2650 m would be the ideal source, due to its superior seedling height exhibited at both sites, up to age 36 months. The results of the combined and individual site analyses suggest that these provenances are likely to perform successfully at any place along the cline, within the natural distribution.

Adapting to the effects of climatic change

In order to prevent the negative effects of poor adaptation of *P. patula* populations to future global warming scenarios, we suggest following the zoning guidelines, by initially moving upwards in the slope 200 m from the collection site, sufficient to achieve or at least approach the estimated climate requirements for adaptation to 2030. Shifting seed sources 200 m upwards to a colder climate, allows populations from warmer climates to adapt and grow more than local seed sources (SCHMIDTLING, 1994). In nature, populations are likely to reduce the differences between optimum and occupied climate by inhabiting environments colder than ideal (REHFELDT et al., 2002). However, an excessive upward altitudinal shift, such as expected by migrating populations in adapting to projected climates in the more distant future, such as the decades centered in the years 2060 or 2090, would increase the risk of frost damage (SÁENZ-ROMERO and TAPIA-OLIVARES, 2008).

Gene exchange between assisted migrated populations and surrounding local populations would be expected, considering the wide gene flow of an open pollinated species such as this. The resulting next generation of such gene exchange might have on average an intermediate performance between the local and the planted, as

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well as a large genetic variation that might serve as a wide array of genotypes to be selected under future climatic conditions, which would have a positive effect (KREMER et al., 2012).

The proposed zonification ideally should be confirmed for additional tests from seed collected along altitudinal gradients from other regions of the *P. patula* distribution (states of Tamaulipas, Veracruz, Hidalgo, Querétaro and Puebla).

Conclusions

There is genetic differentiation among populations of *Pinus patula* for seedling growth in terms of seedling height. The altitudinal pattern demonstrated that mid and low provenances performed better than populations from higher altitudes. The provenance from 2650 m showed the best growth and could be preliminarily selected as a source of seed for commercial plantations of this species, in the region of this study. Based on the altitudinal difference that causes any two populations to differ genetically, we propose an altitudinal and climate zoning, for decision-making in regard to management of seed and seedlings of *P. patula* for reforestation purposes in Ixtlán de Juárez, Oaxaca. Our suggested seed zoning should have the following altitudinal limits: Zone 1, from 2300 to 2500 m; Zone 2, from 2500 to 2700 m; Zone 3, from 2700 to 2900; and Zone 4, from 2900 to 3100 m. Those limits have their climatic equivalence in mean annual temperature, mean annual precipitation and in annual aridity index (ratio of square root of degree days to mean annual precipitation). For management of natural resources for adaptation to climate change, we suggest an attitudinal upward shift of the seed sources, of maximum range of 200 m from the collection site.

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