GENETIC VARIATION AMONG *Pinus patula* POPULATIONS ALONG AN ALTITUDINAL GRADIENT. TWO ENVIRONMENT NURSERY TESTS

VARIACIÓN GENÉTICA ENTRE POBLACIONES DE *Pinus patula* EN UN GRADIENTE ALTITUDINAL. ENSAYO DE VIVERO EN DOS AMBIENTES

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SUMMARY

Aiming to determine if there is genetic differentiation among *Pinus patula* Schiede *et* Chamizo populations along an altitudinal gradient and for quantifying the genotype x environment interaction, 13 *Pinus patula* populations were sampled from forests of the Native Indian Community of Ixtlán de Juárez, Oaxaca, state of México, along an altitudinal gradient (2400 m to 3000 m), cones being collected on groups of trees on every 50 m of altitudinal step). Seedlings were grown in tree pots in two different environments: a shadehouse located at Ixtlán de Juárez, and in a greenhouse and then in a shadehouse situated at Quebec, Canada. Total seedling height was measured at 6-months of age in both locations. Results indicated that populations differed significantly (P = 0.025), but there was no significant genotype x environment interaction (P = 0.426; B type genetic correlation = 0.93). Population from 2650 m (middle altitude) exhibited the best height. Although not definitive, our study suggests the presence of a weak altitudinal pattern of variation in seedling height, where populations originating of mid-altitudes exhibit the highest growth potential while populations from the upper and lower altitudinal extremes exhibit the lowest growth potential.

Index works: *Pinus patula*, genotype x environment interaction, commercial plantations, provenances, altitudinal genetic variation.

RESUMEN

Con el objetivo de determinar si existe diferenciación genética entre poblaciones de *Pinus patula* Schiede *et* Chamizo a lo largo de un gradiente altitudinal y de cuantificar la interacción genotipo x ambiente, 13 poblaciones de *Pinus patula* se muestrearon en bosques de la Comunidad Indígena de Ixtlán de Juárez, Estado de Oaxaca, México, a lo largo de un gradiente altitudinal (2400 m a 3000 m, mediante colecta de conos en grupos de árboles por cada 50 m de intervalo altitudinal). Las plántulas crecieron en contenedores rígidos en dos ambientes diferentes: una casa de sombra en Ixtlán de Juárez, y en un invernadero y después en una casa de sombra situada en Quebec, Canadá. La altura total de plántula fue medida a los seis meses de edad en ambas localidades. Los resultados indicaron que las poblaciones difieren significativamente (P = 0.025), pero que no hay interacción genotipo x ambiente (P = 0.426; Correlación genética tipo B = 0.93). La población de 2650 m (altitud media) exhibió el mejor crecimiento en altura. Si bien no de manera definitiva, este estudio sugiere la presencia de un débil patrón altitudinal de variación en altura de planta, en donde las poblaciones originadas de altitudes intermedias exhiben el potencial de crecimiento más alto y las poblaciones de los extremos altitudinales superior e inferior, exhiben el potencial más bajo.

Palabras clave: *Pinus patula*, interacción genotipo x ambiente, plantaciones comerciales, procedencias, variación genética altitudinal.

INTRODUCTION

Pinus patula Schiede *et* Chamizo is one of the most productive pine species native to México. Its straight stem form, wood of moderate density, low extractives content, long tracheids, high fiber coarseness, low lignin content and high growth rate, confer to this species a very high potential value to be grown as intensively managed plantations in the country (Dvorak *et al.*, 2000; Velázquez-Martínez *et al.*, 2004), as well as for ecological restoration of degraded sites in the regions where it is naturally distributed. Additionally, this species has been planted extensively as an exotic in southern and western Africa and in western South America (Dvorak *et al.*, 2000).

Selection of the best provenances for intensive plantations is relevant for the Forestry Office of the Native Indian Community of Ixtlán de Juárez, located in the north of Oaxaca State, southern México. This community conducts certified sustainable forest management (Martínez-Guzmán and Colín-Castillo, 2003), in a pine-oak forest were the most productive species among the dominant pines is *P. patula* (Dvorak *et al.*, 2000). Early selection offers a possibility to shorten breeding cycles and to save resources by reducing the number of provenances and families to be tested in the field (Zobel and Talbert, 1992; Viveros-Viveros *et al.*, 2005). However, the efficiency of early selection depends on the strength of age-age correlations (Lambeth *et al.*, 1983; Adams *et al.*, 2001).

Conifer populations may often differentiate genetically along altitudinal gradients in response to differential selection pressures (Rehfeldt, 1991; Rehfeldt *et al.*, 1999). The understanding of such patterning of genetic variation is needed to delineate seed and seedlings transfer guidelines to match genotypes with planting sites that will allow them for best performance under contemporary and future climates (Saenz-Romero *et al.*, 2006).

The objectives of this study were: (1) To determine whether there is genetic differentiation for plant height among *P. patula* populations along an altitudinal gradient; (2) To determine whether there is genotype x environment interaction; and (3) To generate information that could be used later to calculate age-age correlations.

MATERIALS AND METHODS

Wind-pollinated cones were collected from 11 randomly selected trees from each of 13 *Pinus patula* natural stands distributed along an altitudinal transect from 2400 to 3000 m of altitude, in the forests of the Native Indian Community of Ixtlán de Juárez, state of Oaxaca, México. Sampled stands were separated by an altitudinal interval of approximately 50 m. Hereafter, the group of trees on which cones were collected in each stand, is named populations while the geographic location of the population is called provenance (Table 1). Trees within populations were selected randomly in order to have a good representation of the genetic variability of each population, and to avoid bias toward trees with superior phenotypes (regarding commercial traits). Seeds were manually extracted from the cones of each tree and then bulked by provenance. Seeds were stored at 4 °C until they were ready for germination.

Table 1. Geographical and altitudinal localization of 13 *Pinus patula* Schiede & Cham. populations sampled along an altitudinal transect at Ixtlán de Juárez, state of Oaxaca, México.

Site	Latitude N	Longitude W	Altitude (m)
1	17°22.270'	96°26.987'	3000
2	17°23.154'	96°27.402'	2950
3	17°23.185'	96°27.839'	2900
4	17°21.793'	96°27.399'	2850
5	17°21.795'	96°27.562'	2800
6	17°23.004'	96°28.577'	2750
7	17°21.749'	96°27.880'	2700
8	17°23.162'	96°28.938'	2650
9	17°23.059'	96°28.861'	2600
10	17°22.902'	96°28.902'	2550
11	17°22.808'	96°29.067'	2500
12	17°22.679'	96°28.986'	2450
13	17°22.353'	96°28.971'	2400

In order to determine whether there is genotype x environment interaction, a provenance test was established at two locations: (a) The forest nursery of the Native Indian Community of Ixtlán de Juárez (17° 19' 50" LN, 96° 29' 14" LW, 2030 masl, annual average temperature of 16.8 °C, and annual average precipitation of 964 mm), to which we will hereafter refer as "Ixtlán"; and (b) The research facilities of the Laurentian Forestry Centre of Natural Resources of Canada, Quebec City, Quebec, Canada (46° 47' LN, 71° 17' LW, 27 masl, annual average temperature of 5.0 °C, and annual average precipitation of 1173 mm), to which we will refer as "Quebec".

At both locations seedlings were grown following locally standard procedures to promote a healthy development. In Quebec, seedlings stayed in the greenhouse longer than usual in comparison to local species, to prevent damage by Spring cold temperatures. Culture of the seedlings was done according to the following schedule in each location:

For the Quebec test

1) Seeds were washed with running tap water for 48 h, stratified for 17 d at 4 °C and sowed on 11-12 April, 2007, in 320 cm^3 containers filled with a mix of Farfarf®'s peat-moss and Holiday®'s vermiculite (168 L of peat-moss per 8.6 kg of vermiculite). Seeds were covered with a 0.5 cm layer of inert silicate, to avoid washing out of the seed by irrigation.

2) Seedlings were arranged in a randomized complete block design with 18 blocks, where each provenance was represented by a three-seedling row plot. One border row per block as well as two extra blocks containing surplus

seedlings were placed as protection rows for reducing the border effects on the experimental material.

3) Seedlings were grown during four months in a greenhouse; temperature of 24 °C during the day and 18 to 20 °C during the night; cycles of 16 h of light and 8 h of darkness; watering and fertilization was applied as needed.

4) Four months old seedlings were transferred to an outdoor shadehouse in August 2007, to promote bud setting, maintaining the initial experimental design.

For the Ixtlán test

1) Seeds were sowed on 21-22 November, 2007, in 380 cm^3 containers filled with a mixture peat-moss, vermiculite and agrolite (50:25:25).

2) Seedlings were arranged in a randomized complete block design with three blocks, and within a block each provenance was represented by 49 seedlings-square plot. Containers with surplus seedlings were placed around the experimental layout to serve as protection rows of reducing border effects.

3) Seedlings were grown for six months inside a shadehouse at the nursery; watering and fertilization was applied as needed.

Although the experimental design for both test sites are notoriously different in number of seedlings per plot (47 seedlings at Ixtlán and 3 at Quebec), note that such difference is partially compensated by an opposite relation of number of blocks (3 blocks at Ixtlán and 18 at Quebec). Thus, the total initial number of seedlings that represented each provenance in each test site was: 147 at Ixtlán and 54 seedlings at Quebec. We consider these sample sizes large enough to have a robust estimation of the variance among provenances within test sites, particularly considering that environmental conditions at Quebec were more controlled than at Ixtlán.

Seedling total height was measured at both locations when seedlings were 6-months-old (October 2007 at Quebec, May 2008 at Ixtlán).

In order to test the significance of variation between locations, among populations, and the interaction population x location (that can be interpreted as genotype x environment interaction), an analysis of variance was conducted using PROC GLM of SAS (SAS Institute, 1999), with the following full model:

$$Y_{ijkl} = \mu + \lambda_i + p_j + b_k + u_{ij} + v_{ijk} + e_{ijkl}...(\text{Eqn. 1})$$

Where Y_{ijkl} = observation on the lth seedling (Ixtlán: l = 1, 2, ..., 49; Quebec: l = 1, 2, or 3) of the kth block (Ixtlán: k = 1, 2 or 3; Quebec: k = 1, 2, ..., 24) of the jth population (j = 1, ..., 13) of the ith location (i = 1 or 2); μ = overall mean; λ_i = fixed effect of the ith location (i = 1 or 2); p_j = random effect of the jth population, assuming that p_i is an observation from a normal distribution with mean zero and variance σ^2_p; b_k = random effect of kth block nested in the ith location, assuming that $b_k \sim N(0, \sigma^2_k)$; u_{ij} = random effect due to the interaction of location i with population j, assuming that $u_{ij} \sim N(0, \sigma^2_u)$; v_{ijk} = random effect due to the interaction of population j with block k at location i, assuming that $v_{ijl} \sim N(0, \sigma^2_v)$; and e_{ijkl} = random error term associated with the lth seedling of the provenance j in the location i and measured in the block k, assuming that $e_{ijkl} \sim N(0, \sigma^2_e)$.

The relative magnitude of the genotype x environment interaction was evaluated as type B genetic correlation ($r_{B;}$ Burdon, 1977) submitting standardized data to analysis of variance using Eqn. 1 (Isik *et al.*, 2000) with the following formula:

$$r_B = \sigma^2_p / \sigma^2_p + \sigma^2_u.............(\text{Eqn. 2})$$

In order to test the significance of variation among populations for each location, data collected on each location were also submitted separately to analysis of variance using the following reduced model:

$$Y_{jkl} = \mu + p_j + b_k + v_{jk} + e_{jkl}........(\text{Eqn. 3})$$

Where Y_{jkl} = observation on the lth seedling of the kth block of the jth population; μ = overall mean; p_j = random effect of the jth population, assuming that p_i is an observation from a normal distribution with mean zero and variance σ^2_p; b_k = random effect of kth block, assuming that $b_k \sim N(0, \sigma^2_k)$; v_{jk} = random effect due to the interaction of population j with block k, assuming that $v_{ijl} \sim N(0, \sigma^2_v)$; and e_{jkl} = random error term associated with the lth seedling of the provenance j measured in the block k, assuming that $e_{jkl} \sim N(0, \sigma^2_e)$.

Contribution to total variance of each variance component was estimated using Proc VARCOMP of SAS, with the REML Method (SAS Institute, 1999). To

test for altitudinal patterns of genetic variation among populations, least square means by population were regressed on provenance altitude using PROC REG of SAS (SAS Institute, 1999).

RESULTS AND DISCUSSION

Differences between locations

Overall, seedling average height across provenances was three times larger at Quebec (407.9 $\pm$ 7.0 mm) than at Ixtlán (134.7 $\pm$ 5.7 mm) (Figure 1), a difference that was significant (P < 0.0001; Table 2). It seems that the growing conditions in the greenhouse located at Quebec favoured growth in seedling height as compared with outdoor climate at the forest nursery in Ixtlán, where the average monthly temperatures were around 15 °C between November and February. Also, the type of substrate, amount of light and water, temperature and fertilization regime which varied between locations could have positively influenced seedling growth in the Quebec greenhouse.

Differences among populations

Significant differences among populations were detected in the combined (both sites, Eqn. 1) datasets analysis (P = 0.025; Table 2). Similar results were obtained when data from each location were analyzed separately (Eqn. 3), that is P = 0.0325 and P < 0.0001 for Ixtlán and Quebec, respectively (Table 3). Populations explained 5.8 % of the total phenotypic variation when data of both locations were combined (Table 2), and 5.6 and 5.3 % for Ixtlán and Quebec, respectively when an analysis by location was conducted (Table 3).

The population x location interaction was not significant (P = 0.426; Table 2). The best population at Ixtlán was also the best at Quebec, and some of the worst populations at Ixtlán were also the worst at Quebec (Figure 1). Variation due to blocks was significant in the combined analysis (P < 0.0001; Table 2), and was significant for the test at Quebec (P < 0.0001) but not for the test at Ixtlán (Table 3).

The multiple means comparison test (Least Significant Difference, P ≤ 0.05; Table 4) and the plotting of population means against provenance altitude, revealed that the population originated at 2650 m of altitude was the one of best growth for seedling height, in both the combined analysis (Figure 2a) and on the analysis by site

(Figures 2b and 2c). The superiority of this population was more evident at the Quebec test (Figure 2c).

Table 2. Percentage of contribution to total variance (%) and significance level (P) after an analysis of variance combining results from the two localities (Ixtlán and Quebec), of 6-month-old seedlings from 13 *Pinus patula* populations.

Source of variation	df	%	P
Localities	1	------	< 0.0001
Populations	12	5.76	0.0250
Block (Location)	25	7.74	< 0.0001
Population x Location	12	0.51	0.4264
Population x Block (Location)	300	23.30	< 0.0001
Error	2281	62.69	

df = degrees of freedom; P = probability.

Regression analysis of overall population means against altitude of the provenances indicates that, although weak, there is a trend where populations originated at mid-altitude have a slightly higher growth potential (expressed as larger total seedling height) than those populations originated from both altitudinal extremes (upper and lower). However, such trend was not found significant (quadratic model, R^2 = 0.159; P = 0.421; Figure 2a). The pattern was not significant either (Ixtlán: R^2 = 0.132; P = 0.492; Figure 1b; Quebec: R^2 = 0.127; P = 0.508; Figure 2c) in the single site analysis.

The lack of significant genotype x environment interaction, and the fact that the population originated at 2650 m of altitude was by far the best population at the two locations tested, is an indication that genotypes were phenotypically stable (Karlsson *et al.*, 2001; Sánchez-Vargas *et al.*, 2004; Koo *et al.*, 2007). This is confirmed by the high value of type B genetic correlation (r_B = 0.93). High values of type B genetic correlations have been used as indicators of low genetic x environment interaction, as it was found when breeding regions for *Pinus brutia* (Isik *et al.*, 2000), were compared where the type B genetic correlation across sites was considered relatively high.

The weak tendency for seedlings from mid-altitudinal populations to be taller, is similar to the one found for *Pinus pseudostrobus* in common garden tests conducted at two contrasting locations (Morelia, Michoacán, México and Moscow, Idaho, USA) (Saénz-Romero *et al.,* Com. pers.[1]), where mid-altitudinal population produced taller

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[1] **Sáenz-Romero C, G E Rehfeldt, J C Soto-Correa, S Aguilar-Aguilar,V Zamarripa-Morales, J López-Upton (2005)** Variación genética altitudinal entre poblaciones de *Pinus pseudostrobus*. Análisis preliminar de una prueba en dos localidades en casas de sombra. Memorias del VII Congreso Mexicano de Recursos Naturales. Soc. Mex. de Recursos Naturales. Chihuahua, Chih., 26-28 octubre 2005. pp:384-349.

Table 3. Percentage of contribution to total variance (%) and significance level (P) after an analysis of variance by each locality, of 6-month-old seedlings from 13 *Pinus patula* populations.

Source of variation	Ixtlan			Quebec		
	df	%	P	df	%	P
Populations	12	5.57	0.0325	12	5.29	< 0.0001
Block	2	0.55	0.2884	23	5.73	< 0.0001
Population x Block	24	13.40	< 0.0001	276	6.68	0.0230
Error	1683	80.48		598	82.31	

Df = degrees of freedom; P = probability.

Table 4. Least square means (mm) by population, after a combined analysis and by analysis preach location. Letters indicate grouping after Tukey test (α = 0.05). Means are sorted in descending order after the combined analysis (Tukey, 0.05)

| Altitude	Population	Combined		Ixtlán		Quebec	
		Mean	Tukey	Mean	Tukey	Mean	Tukey
2650	8	303	a	156	a	450	a
2450	12	284	ab	146	ab	421	ab
2600	9	276	ab	143	ab	410	abc
2700	7	275	ab	142	ab	409	bc
2800	5	274	ab	146	ab	402	bc
2750	6	272	ab	130	ab	414	bc
2500	11	271	ab	136	ab	406	bc
2900	3	270	ab	137	ab	404	bc
3000	1	269	ab	130	ab	408	bc
2950	2	267	ab	128	ab	406	bc
2850	4	257	b	133	ab	380	bc
2550	10	256	b	105	ab	407	bc
2400	13	252	b	120	b	385	c
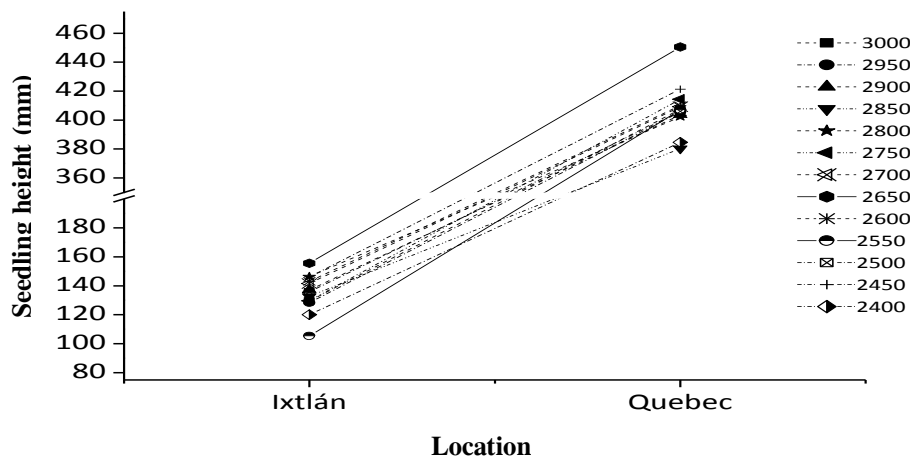
Figure 1. Least square means of height (mm) of *Pinus patula* seedlings, by location and by population of 6-month-old seedlings from tests at Ixtlán de Juárez, Oaxaca, México and at Quebec, Canada.

seedlings although such trend was not statistically significant. Other species have shown this pattern more clearly: populations from mid-altitude showed the best growth at 6 years-old seedlings of *Pinus brutia* (Isik and Kara, 1997); *P. oocarpa* populations from middle-low altitudes exhibited the best basal diameter at 6 months

(Sáenz-Romero *et al.*, 2004) and the largest seedling height at 2.5 years of age (Sáenz-Romero *et al.*, 2006).

There are indications that *P. patula* populations from lower altitudes grow more than populations from higher altitudes; however, those results come from studies where
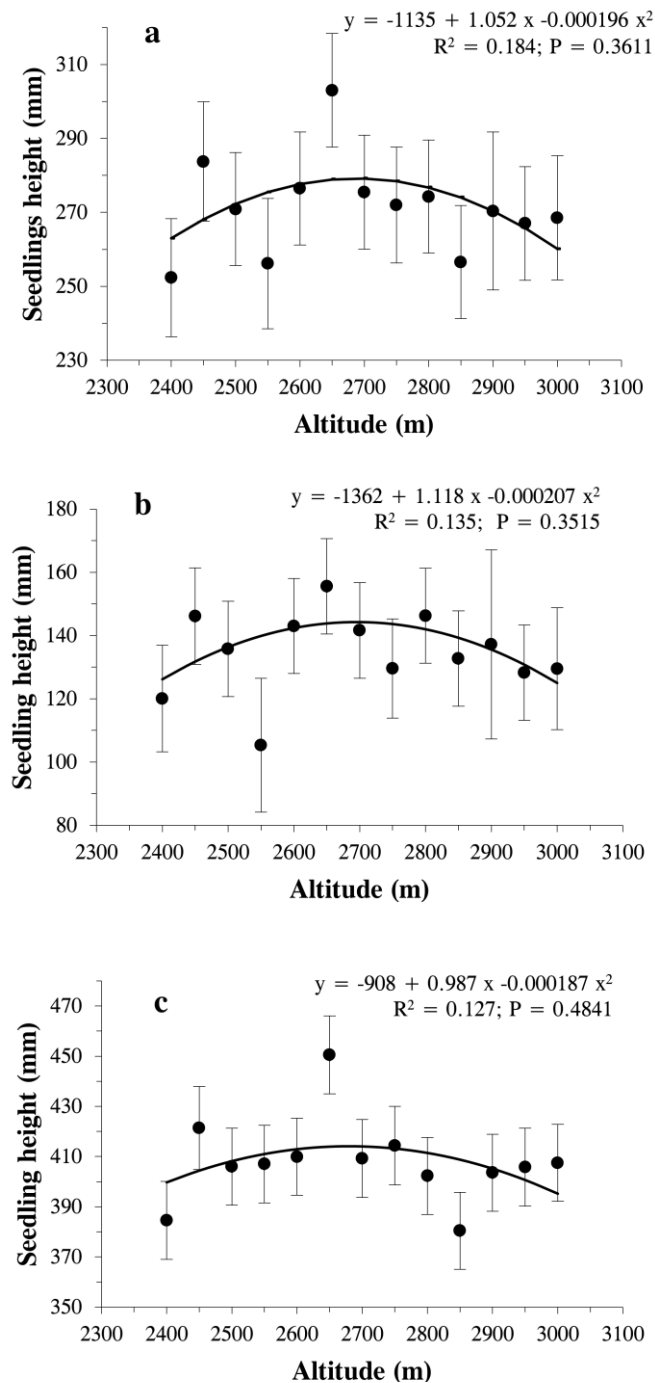
Figure 2. Least square means of height (mm) of *pinus patula* seedlings, by population of 6-month-old seedlings plotted against provenance elevation (masl). (a) Overall means from combined analysis, (b) Means from test at Ixtlán de Juárez, Oaxaca, México, (c) Means from test at Quebec, Canada. Vertical bars indicate confidence intervals (95 %).

provenances of different altitudes came also from different latitudes (Salazar-García *et al.*, 1999; Dvorak *et*

al., 2000), thus making difficult to establish clearly how much of the genetic variation among populations is associated with the altitudinal origin, because it would be needed to study several provenances from different altitudes and similar latitudes. An ongoing field test of the same set of *P. patula* populations as those used in the present study, was recently established on two contrasting altitudes in the forest of Ixtlán de Juárez, Oaxaca. Hence, in a few years it will be possible to confirm whether population stability is maintained over time, and if the altitudinal pattern that was reported here would be expressed at later ages.

CONCLUSIONS

There was significant genetic differentiation among populations of 6-months-old seedlings for total height. However there was not a significant genotype x environment interaction ($r_B = 0.93$). The population sampled at 2650 m of altitude (middle) had the largest value of total seedling height.

A weak altitudinal pattern was found where populations originating from middle altitudes exhibited the highest growth potential and populations from the upper and lower altitudinal extremes exhibited lower growth potential.

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