Acta Physiol Plant (2013) 35:2905–2913
DOI 10.1007/s11738-013-1321-y

ORIGINAL PAPER

Genetic variation of drought-induced cavitation resistance among *Pinus hartwegii* populations from an altitudinal gradient

Cuauhtémoc Sáenz-Romero · Jean-Baptiste Lamy · Esperanza Loya-Rebollar · Andrés Plaza-Aguilar · Régis Burlett · Philippe Lobit · Sylvain Delzon

Received: 28 August 2012 / Revised: 22 May 2013 / Accepted: 23 May 2013 / Published online: 8 June 2013

Abstract Hydraulic failure can cause massive die-back of forest trees during drought. With extreme climatic events set to become more frequent and severe due to climatic change, it is essential to study resistance to water stress-induced cavitation. We investigated the genetic differentiation for cavitation resistance among *Pinus hartwegii* populations, the pine species growing at the treeline in México. Open-pollinated seeds were collected from seven natural populations along an altitudinal gradient (3,150–3,650 masl) from Pico de Tancítaro, Michoacán, western México. Seedlings were raised in a nursery and then established in a randomized complete block design in a common garden experiment. Resistance to cavitation (P_{50}, xylem pressure inducing 50 % loss of hydraulic conductance and S, slope of the vulnerability curve) and specific hydraulic conductivity (k_s), were evaluated on branches of 5-year-old seedlings using the Cavitron technique. Mean P_{50} was -3.42 ± 0.05 MPa, indicating that *Pinus hartwegii* is one of the more vulnerable pine species to cavitation. No significant genetic differentiation was detected between populations for cavitation resistance traits (P_{50} and S), but a significant altitudinal cline was found for S. In contrast, k_s exhibited a significant differentiation among populations and a significant decline with increasing altitude. The lack of genetic differentiation among *P. hartwegii* populations for cavitation resistance is likely to represent a limitation for adapting to the warmer and drier climates that are expected to occur in México under climatic change. Finally, a worldwide comparison within the *Pinus* genus showed that pines growing at the treeline were on average more vulnerable to cavitation than those from lowland. This might reflect an adaptation to dry environmental conditions at low elevation.

Keywords *Pinus hartwegii* · Altitudinal genetic variation · Climatic change · Drought stress · Treeline · Resistance to cavitation

Introduction

There are predictions for Mexico that climatic change will cause (in comparison with the average 1961–1990) an increase in mean annual temperature of 1.5 °C by the decade centered in the year 2030, 2.3 °C by 2060 and 3.7 °C by 2090, while precipitation would decrease 6.7 % by 2030, 9.0 % by 2060 and 18.2 % by 2090 (Sáenz-Romero et al. 2010). Under these conditions, Mexican mountain ranges where conifer forests occur are expected to experience a drier climate (Rehfeldt et al. 2012) with more frequent droughts. There is growing evidence of sudden declines of tree populations linked to climatic change, for example, *Pinus edulis* at low altitudinal limits in south-western USA (Breshears et al. 2005), *Populus tremuloides* in the Rocky Mountains, USA (Rehfeldt et al.

Communicated by P. Sowinski.

C. Sáenz-Romero (✉) · E. Loya-Rebollar · P. Lobit
Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Agropecuarias y Forestales (UMSNH-IIAF), Km 9.5 Carretera Morelia-Zinapécuaro, 58880 Tarímbaro, Michoacán, Mexico
e-mail: csaenzromero@gmail.com

J.-B. Lamy · A. Plaza-Aguilar · R. Burlett · S. Delzon
INRA-Université de Bordeaux, UMR, 1202 BIOGECO, Cestas 33610, France

A. Plaza-Aguilar
Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

2009), *Cedrus atlantica* in the Moyen Atlas mountain range, Morocco (Mátyás 2010), and *Fagus sylvatica* in South-west Hungary (Mátyás et al. 2010). In Catalonia, northeast Spain, the declining species *Fagus sylvatica* is already being replaced by the more drought-tolerant species *Quercus ilex* (Peñuelas et al. 2007). Another recent study revealed global forest die-backs due to drought and heat stress (Allen et al. 2010).

Pinus hartwegii Lindl. is a pine species from the temperate-cold zones of Mexico and Central America. It grows at one of the world's highest treelines between altitudes of 3,000 and 4,000 m, where it constitutes the upper altitudinal limit of tree vegetation (Lauer 1978; Perry 1991). The extreme altitudinal distribution of *P. hartwegii* makes it highly vulnerable to global warming, since its exclusive habitat could be reduced, because their suitable climatic habitat will occur at even higher altitudes, where there are less land surface and none when it is reached the summit (Gomez-Mendoza and Arriaga 2007; Viveros-Viveros et al. 2009). There are predictions that by the end of the current century, suitable climates for the conifer forests in the Trans-Mexican Volcanic Belt in Mexico could be reduced by 92 % (Rehfeldt et al. 2012).

Resistance to cavitation is a good estimator of a species tolerance to drought in vascular plants (Brodribb and Cochard 2009; Brodribb et al. 2010). Previous studies have reported a high variability of P_{50} (a proxy of cavitation resistance, corresponding to the xylem pressure inducing 50 % loss of hydraulic conductance) among conifer species, ranging from −3 to −11 MPa (Delzon et al. 2010; Pittermann et al. 2010). However, although recent studies have investigated the intra-specific variability of drought-induced cavitation resistance in conifer species (*Abies alba* and *Abies pinsapo*, Peguero-Pina et al. 2011; *Pinus sylvestris*, Martínez-Vilalta et al. 2009; *Pseudotsuga menziesii*, Dalla-Salda et al. 2011), only a few have quantified the genetic variations among populations (see Lamy et al. 2011; see also Wortemann et al. 2011 for angiosperm tree). Our concern is that a low intra-specific genetic variation for drought-induced cavitation resistance might reduce the ability of *P. hartwegii* populations to rapidly adapt to the increasingly arid environments expected under climate change. There are indications that many forest tree species have a very narrow (<1 MPa) hydraulic safety margins against injurious levels of drought stress and, therefore, they potentially will face long-term reductions in productivity and survival if temperature and aridity increase as predicted due to climatic change (Choat et al. 2012). The study of the patterning of genetic differentiation among conifer populations along altitudinal gradients offers the opportunity to understand the matching between genotypes and environmental conditions and so foreseen management options to deal with the potential effects of climatic change (Sáenz-Romero et al. 2006; Rehfeldt and Jaquish 2010).

In this work, we have assessed the hydraulic safety (drought-induced cavitation resistance) and efficiency (hydraulic conductivity) of *Pinus hartwegii* Lindl., for which we found only one previous study obtained from a single individual growing in a botanical garden (Jansen et al. 2012). We quantify the magnitude of genetic differentiation among *Pinus hartwegii* populations originated from different altitudes and growing in a common garden test. We also compared several pine species growing at low and high altitudes in the north hemisphere to determine whether there is a general relation between altitude and drought-induced cavitation resistance, and how *Pinus hartwegii* compares with other pine species.

Materials and methods

Sample collection

Open-pollinated seeds were collected from seven natural populations along an altitudinal gradient, from 3,150 m (19°25.967′N, 102°16.972′W) to 3,650 m (19°25.179′N, 102°18.589′W), from Pico de Tancítaro, Michoacán, western Mexico (same provenances as Viveros-Viveros et al. 2009; we made a correction of +150 m of provenance altitude). Trees represented by these samples are termed populations while the location of a population is called the provenance. Although the distance between the most distant populations collected is of only 1.95 km, the slope is very steep, creating a pronounced environmental gradient: the provenance of the lowest altitude has a mean annual temperature of 11.9 °C and an annual precipitation of 1,187 mm, whereas the highest provenance averaged 9.5 °C and 1,295 mm, respectively (estimation using a spline climatic model; see Sáenz-Romero et al. 2010). That made a decrease in mean annual temperature of 2.4 °C and increase in mean annual precipitation of 9 % at the highest altitude as compared with the lowest. Such an environmental cline has been sufficient to promote genetic differentiation among *P. hartwegii* populations for quantitative traits such as seedling growth, expression of grass stage and frost damage resistance (see Viveros-Viveros et al. 2009). When collecting cones at the lowest altitudinal populations, we avoided trees that had morphological aspects intermediate between *P. hartwegii* and *P. montezumae* to prevent the inclusion of putative hybrids. It is documented that natural hybridization occurs between those two species at their areas of sympatric distribution

(Matos and Schaal 2000), and within the lowest altitudinal population in this study we observed trees with needle lengths and cone sizes intermediate between what is typical for those two species.

Seedlings were raised in a nursery (380 cm^3 rigid containers with commercial Creciroot® substrate), and then established in a randomized complete block design in a common garden provenance test when seedlings were 19 months old. Common garden conditions consisted of two rectangular wooden-structure raised beds, 12.3 m long × 1.5 m wide × 0.6 m high each; the wooden-structures were filled with a 20-cm layer of extrusive volcanic coarse stones for improving drainage (particle size: 28.4–37.3 mm), and then a 40 cm of a 4:1 mixture of local Andosol pine-oak forest top-soil, and commercial Creciroot® substrate. Seedlings were placed in plots of five seedlings per row, spaced 0.3 m apart within plots and 0.3 m apart between plots. The first and the last plots of each wooden-structure were flanked by a row of randomly selected seedlings to control for edge effect. The test was covered by a 35 % shade net. The test was located at a Universidad Michoacana de San Nicolás de Hidalgo facility, at Morelia, Michoacán (101°14′59″ Long W, 19°41′20″ Lat N, 1955 masl, mean annual temperature 17.0 °C, average annual precipitation 881 mm).

In the seven studied populations, drought-induced resistance to cavitation was evaluated in branches of 5-year-old seedlings. In each population, between three and seven individuals (4.6 in average) were measured in October/November 2010, depending on the size of the available seedlings per provenance. Where possible, two branches were sampled per seedling and no significant variability was found within individual. Branches were cut in the early morning to avoid high temperatures and all needles were immediately removed to prevent desiccation. The samples were then wrapped in wet paper towels, placed in black bags, and immediately posted to France. Vulnerability to drought-induced cavitation was determined at the high-throughput phenotyping platform for hydraulic traits (CavitPlace, University of Bordeaux, Talence, France; http://sylvain-delzon.com/caviplace). The samples were kept wet and cool (3 °C) until cavitation resistance was measured within 3 weeks after collection. Prior to measurement, all branches were cut under water to a standard length of 27 cm, and bark was removed with a razor blade. Although seedlings were 5 years old, they had an average height of only 58 cm (Loya-Rebollar et al. 2013) due to the very slow growth rate that characterizes this species; thus, we consider that competition could not be a confounding factor in this experiment, given the spacing between seedlings and their short stature.

Measurement of resistance to cavitation

Xylem cavitation was assessed with the CAVITRON, a centrifuge technique following the procedure described by Cochard (2002) and Cochard et al. (2005). Centrifugal force was used to establish negative pressure in the xylem and to provoke water stress-induced cavitation, using a custom-built honeycomb rotor (Precis 2000, Bordeaux, France) mounted on a high-speed centrifuge (Sorvall RC5, USA). Xylem pressure (P_i) was first set to a reference pressure (−0.5 MPa) and hydraulic conductivity (k_i) was determined by measuring the flux through the sample. The centrifugation speed was then set to a higher value for 3 min to expose the sample at a more negative pressure. Conductance was measured four times for each pressure step, and the average was used to compute the percent loss of xylem conductance (PLC in%) at that pressure. PLC was determined at each pressure step (see Delzon et al. 2010 for details). The procedure was repeated for at least eight pressure steps with a −0.5 MPa step increment until PLC reached at least 90 %. Rotor velocity was monitored with a 10 rpm resolution electronic tachymeter and xylem pressure was adjusted to about −0.02 MPa. We used Cavisoft software (version 2.0, BIOGECO, University of Bordeaux) for conductance measurements and computation of all vulnerability curves (VC).

The percent loss of xylem conductance as a function of xylem pressure (MPa) represents the sample's vulnerability curve (VC). A sigmoid function (Pammenter and Van der Willigen 1998) was fitted to the VC from each sample using the following equation:

$$\mathrm{PLC} = \frac{100}{\left[1 + \exp\left(\frac{S}{25} * (P - P_{50})\right)\right]}, \tag{1}$$

where P_{50} (MPa) is the xylem pressure inducing 50 % loss of conductance and S (% MPa^{-1}) is the slope of the vulnerability curve at the inflexion point. The xylem-specific hydraulic conductivity (k_s, $\mathrm{m}^2\ \mathrm{MPa}^{-1}\ \mathrm{s}^{-1}$) was calculated by dividing the maximum hydraulic conductivity measured at low speed by the sapwood area of the sample.

Statistical analysis

Genetic differentiation among populations was tested by an analysis of variance (ANOVA), using the Procedure GLM of SAS (SAS Institute Inc. 2004). Measurements of more than one branch of the same individual were averaged prior to analysis. The statistical model used was

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij} \tag{2}$$

where Y_{ij} = value of the ijth observation, μ = general mean, τ_j = effect of the ith population, and ε_{ij} = experimental error. Population was considered as random effect.

Variance components were estimated using the Procedure VARCOMP with the method of restricted maximum likelihood (REML) of SAS (SAS Institute Inc. 2004).

To determine the altitudinal pattern of genetic variation, if any, the relationship between the mean values of assessed characteristics and the elevation of the sites was modeled by population, using the Procedure REG of SAS, (SAS Institute Inc. 2004) with the following model:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \varepsilon_{ij}, \tag{3}$$

where Y_{ij} is population mean of P_{50}, k_s, or S; β_0 the intercept, β_1 the regression parameter, X_i the altitude (m) of ith provenance, and ε_{ij} is error.

Results and discussion

Cavitation resistance at treeline

For each population, vulnerability curves showed a similar sigmoid shape (see Fig. 1 as an example). This similarity allows us to robustly estimate P_{50} and S using the Pammenter model (Pammenter and Van der Willigen 1998). More negative P_{50} (xylem pressure inducing 50 % loss of conductance) values indicate higher resistance to cavitation, while the slope of the vulnerability curve, S, indicates how fast cavitation progresses at P_{50}. The overall average value for P_{50} was −3.42 ± 0.047 MPa (±standard error, SE) and the average value for S was 121 ± 9 % MPa^{-1}. The estimated P_{50} average value is very close to the single available reported value ($P_{50} = -3.43 \pm 0.18$; Jansen et al. 2012).

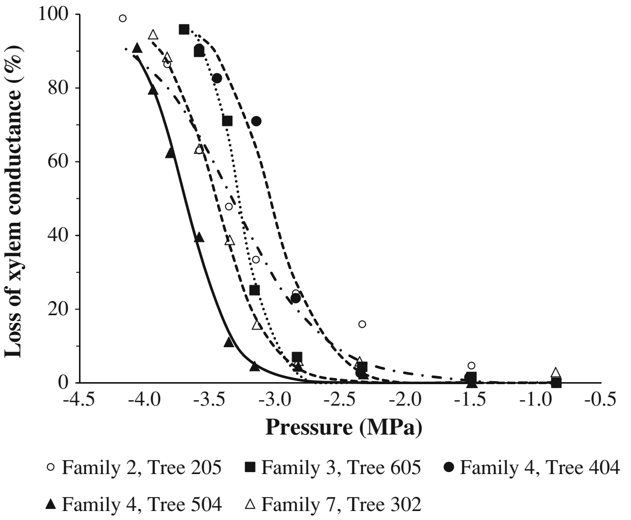

Fig. 1 Examples of vulnerability curves as loss of xylem conductance (PLC, in %, see Eq. 1) against pressure (in MPa), of five seedlings from a single *Pinus hartwegii* population (3,250 m of altitude)

Both P_{50} and S values in *Pinus hartwegii* fall within the range found on other treeline pine species. Similar P_{50} values were found for *Pinus cembra* (−3.02 ± 0.17 MPa), *Pinus albicaulis* (−3.19 ± 0.1 MPa) and *Pinus mugo* (−3.75 ± 0.17 MPa) (Delzon et al. 2010). *Pinus albicaulis* is found in mountain ranges in western USA and Canada, predominantly within the Rocky Mountains (Bower and Aitken 2008), and *Pinus cembra* and *Pinus mugo* are distributed through the Alps and Carpathian mountains, Europe (Critchfield and Little 1966; Christensen 1987).

Comparing P_{50} values between previously studied pine species, grouped according to their altitudinal position (treeline or low altitude), we found that treeline pine species were on average more vulnerable to drought-induced cavitation (Table 1). This worldwide comparison showed that P_{50} is significantly less negative (Wilcoxon test: $P = 0.0346$; Kruskal–Wallis test: $P = 0.0352$) for treeline pine species ($P_{50} = -3.39$ MPa) than for middle-lowland pine species ($P_{50} = -4.04$ Mpa, Table 1); treeline species are, therefore, more vulnerable to drought-induced cavitation than those at lower altitudes.

Like all pine studies previously characterized, *Pinus hartwegii* appears to be vulnerable to cavitation compared to species from other conifer families. In conifers, reported values for P_{50} range from 2.91 Mpa for *Metasequoia glyptostroboides*, to between −9 and −10 Mpa *Juniperus osteosperma* and *J. scopulorum*, which distribute in semiarid regions and even −11.32 Mpa for *Cupressus glabra* (Delzon et al. 2010). Generally, species from dry environment are more resistant to cavitation (Maherali et al. 2004). Concerning the slope of the cavitation curve, Delzon et al. (2010) consider that slope values larger than 50 % Mpa^{-1} indicate a very fast rate of embolism. When comparing *P. hartwegii* to other pine species, its P_{50} is within the range of other treeline species but its S value is the lowest among all treeline species, suggesting that this species has small tracheids (see "Results and discussion" below).

Genetic differentiation among populations

Cavitation resistance

The cavitation resistance traits (P_{50} and S) did not show significant differences among populations ($P = 0.3038$ and $P = 0.2445$, respectively; Table 2), and there was no detectable altitudinal trend among P_{50} population means in relation to the altitude of the provenance ($r^2 = 0.001$, $P = 0.9541$; Fig. 2a). However, a significant negative correlation was found between S and altitude ($r^2 = 0.816$, $P = 0.0053$, Fig. 2b), with higher S values in populations from low altitudes. The data available for conifers have focused largely on differences between species rather than within species (Delzon et al. 2010). However, the genetic

Table 1 Comparative values (means and standard errors) for cavitation resistance (P_{50}) and slope of the vulnerability curve for several pine species, grouped for range of altitudinal distribution and sorted by average P_{50} values

Altitudinal range	Species	Mean		Standard error		*n*	References
		P_{50}	Slope	P_{50}	Slope		
High or treeline	*Pinus wallichiana*	−2.83	147	0.112	23.8	5	Jansen et al. (2012)
High or treeline	*Pinus cembra*	−3.02	159	0.170	18.6	3	Delzon et al. (2010)
High or treeline	*Pinus albicaulis*	−3.19	189	0.101	11.6	4	Delzon et al. (2010)
High or treeline	*Pinus hartwegii*	−3.42	121	0.047	9.0	32	This study
High or treeline	*Pinus mugo*	−3.75	169	0.066	17.9	3	Delzon et al. (2010)
High or treeline	*Pinus uncinata*	−4.18	127	0.169	8.7	4	Delzon et al. (2010)
	Mean	−3.39	135				
Mid or low elev.	*Pinus sylvestris*	−3.20	129	0.021	8.7	5	Delzon et al. (2010)
Mid or low elev.	*Pinus caribea*	−3.24					Pittermann et al. (2006a, b)
Mid or low elev.	*Pinus flexilis*	−3.71	100	0.180	10.7	4	Delzon et al. (2010)
Mid or low elev.	*Pinus pinaster*	−3.73	69	0.070	0.5	2	Lamy et al. (2011)
Mid or low elev.	*Pinus nigra*	−3.8					Hacke et al. 2004
Mid or low elev.	*Pinus taeda*	−3.8					Hacke et al. (2004)
Mid or low elev.	*Pinus ponderosa*	−3.86	152	0.051	18.4	3	Delzon et al. (2010)
Mid or low elev.	*Pinus contorta*	−3.90	168	0.180	22.4	5	Delzon et al. (2010)
Mid or low elev.	*Pinus edulis*	−4.03	102	0.061	15.1	5	Delzon et al. (2010)
Mid or low elev.	*Pinus pinea*	−4.34	89	0.161	6.4	3	Delzon et al. (2010)
Mid or low elev.	*Pinus radiata*	−4.38	67	0.143	5.4	5	Jansen et al. (2012)
Mid or low elev.	*Pinus halepensis*	−4.67	78	0.050	8.4	3	Delzon et al. (2010)
Mid or low elev.	*Pinus monophylla*	−5.82					Pittermann et al. (2006b); Hacke et al. (2004)
	Mean	−4.04	109				

Table 2 Analysis of variance of xylem cavitation resistance traits (P_{50}, xylem pressure inducing 50 % loss of conductance and S, slope of the vulnerability curve at the inflexion point) and xylem transport efficiency (k_s, xylem-specific hydraulic conductivity), for 5-year-old seedlings originated from 7 *Pinus hartwegii* populations collected along an altitudinal gradient

Trait	Population			Error
	CV	RV	*P*	RV
P_{50}	2.3	9.1	0.3038	90.9
S	12.4	8.6	0.2445	91.4
k_s	48.5	47.4	0.0039	52.6

CV Coefficient of variation (in %) for the between population variance component, *RV* Ratio (in %) of variance component of each random effect to total variance estimated, *P* significance value

differentiation among populations for traits such as P_{50} has being actively studied in recent years. One study explored cavitation resistance between populations of *Pinus pinaster*, including populations along environmental gradients from warm and dry sites in Tamrabta, Southern Morocco, to cooler and wetter sites in Mimizan, South-western France. The results indicated no significant differentiation among populations for cavitation resistance (P_{50}), and suggest that canalization or uniform selection has shaped the phenotypic variability of the trait (Lamy et al. 2011). A second extensive study of cavitation resistance among 17 populations of *Fagus sylvatica* growing in provenance tests revealed a remarkably constant cavitation resistance across populations (Wortemann et al. 2011). In other words, the evidence from other studies suggests that genetic architecture could narrow trait variability to preserve functional phenotypes.

Given the low coefficient of variation (2.3 % for the between population variance component, Table 2) of cavitation resistance, our results support the hypothesis that P_{50} is a canalized trait or under uniform selection. The extremely low variation is surprising, considering that: a) the population sampling fully represents the thermal amplitude of this species, at least within in the studied region, and b) the number of *P. hartwegii* populations studied (7) is 30 % larger than the average number (4.8) of populations examined in other studies of cavitation resistance in diverse forest species provenance tests: *Ambrosia dumosa*, *Hymenoclea salsola* (Mencuccini and Comstock 1997), *Artemisia tridentata* (Kolb and Sperry 1999), *Fagus*

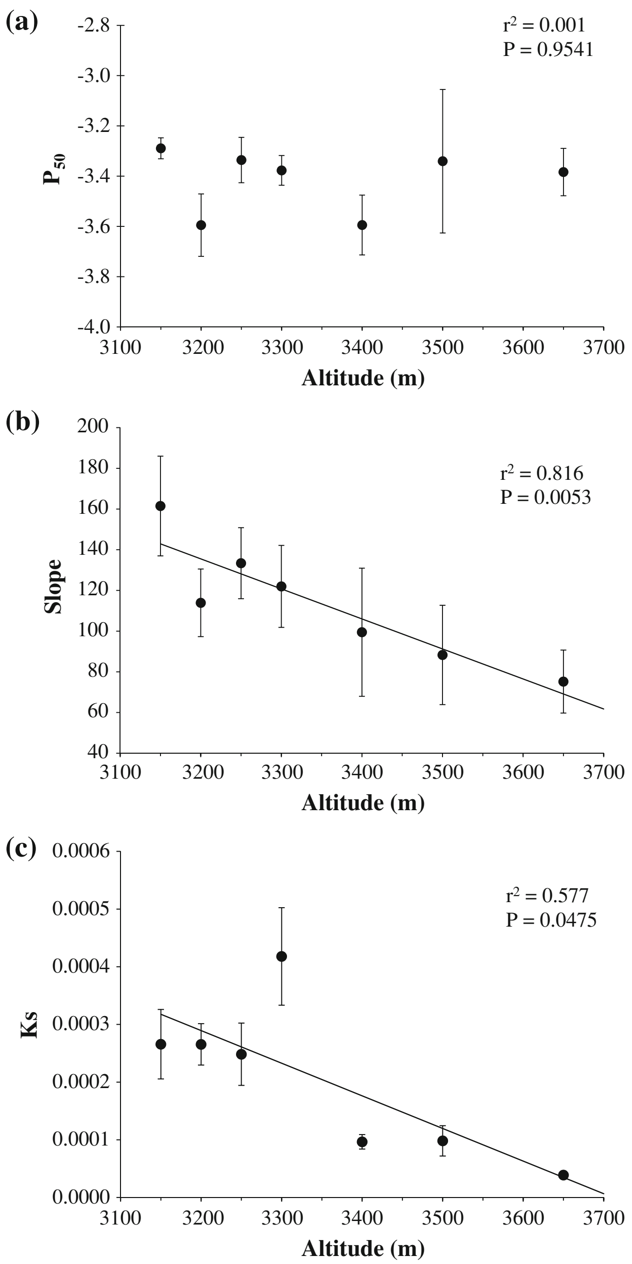

Fig. 2 Evolution of hydraulic safety (cavitation resistance) and efficiency (hydraulic conductance) according to altitude of population origin: **a** P_{50} in MPa, xylem pressure inducing 50 % loss of hydraulic conductance; **b** *S* in % MPa^{-1}, slope of the vulnerability *curve* at the inflexion point, and **c** k_s in m^2 MPa^{-1} s^{-1}, xylem-specific hydraulic conductivity. *Vertical bars* represent standard errors

sylvatica (Wortemann et al. 2011), *Olea europea* (Ennajeh et al. 2008), *Pinus contorta* var. *latifolia* (Wang et al. 2003), *P. pinaster* (Corcuera et al. 2011; Lamy et al. 2011), *Populus trichocarpa* (Sparks and Black 1999), *Pseudotsuga menziessi* (Kavanagh et al. 1999), and *Quercus wislizenii* (Matzner et al. 2001). Despite our relatively large sample size compared with other studies, the average number of individuals measured for each population remains the largest limitation of the work and our inferences need to be confirmed by larger sample sizes.

Specific hydraulic conductivity

In contrast with the absence of genetic differentiation or an altitudinal trend for P_{50}, the decrease in hydraulic conductivity (k_s) with increasing altitude (Fig. 2c) could be due to the presence of small tracheids in plants originated from high altitude. This pattern has been observed in cold environments in response to a high frequency of freeze–thaw cycles (Davis et al. 1999; Pittermann and Sperry 2006). However, confirming such inferences would require an assessment of the variation of tracheid morphology among populations along the altitudinal gradient. So far, we do not have any information of lumen area for *P. hartwegii*, and so the relationship between tracheid width, k_s and conduit conductance cannot be directly established. The lack of trade-off between transport safety (cavitation resistance) and efficiency (hydraulic conductivity) has also been reported in tracheid-bearing species as the conduit size did not affect cavitation resistance (Sperry et al. 2006). Finally, the decline in *S* with increasing altitude observed in our study might also be explained by the narrower tracheid size at high altitude, reducing the rate of hydraulic conductance loss when establishing a vulnerability curve.

Implications for management

The lack of genetic differentiation among populations for cavitation resistance represents a potential limited ability of *Pinus hartwegii* populations to adapt to the warmer and drier climates predicted in climate change scenarios for México (Sáenz-Romero et al. 2010). However, further investigations are needed to estimate the magnitude of phenotypic plasticity of cavitation resistance, using for instance several common garden tests located in contrasting environments.

Populations of other high altitude pines that conform a timberline and have similar cavitation resistance values to *Pinus hartwegii* have started to show a severe decline, for example, *Pinus albicaulis* (Bower and Aitken 2008; Bower and Aitken 2011). Also *Pinus hartwegii* populations have already decreased their growth rate in response to climatic change that has occurred in recent years (Ricker et al. 2007). The fact that *P. hartwegii* conforms the timberline in high altitude mountains in México limits options for conducting an assisted migration to higher altitudes, because sometimes the current distribution is at the summit of the mountains already, as in the case at Pico de Tancítaro, state of Michoacán (Viveros-Viveros et al. 2009). Where the mountain does have higher elevation (like Popocatépetl and Iztaccíhuatl volcanoes), the soils are typically poor above the timberline, due to the lack of organic material and abundance of sand and stones of volcanic origin (Lauer 1978), making establishment of pine saplings more difficult.

Conclusions

Our results are in agreement with recent studies on altitudinal gradients showing significant natural clines for several leaf functional traits, but weak effects of genetic variation measured in common garden tests, suggesting a strong effect of the environment on functional traits (Bresson et al. 2009, 2011; Premoli and Brewer 2007). We showed here that *Pinus hartwegii* is a vulnerable species to cavitation relative to the conifer cavitation resistance spectrum. This finding supports the preliminary generalization that pine species growing at the treeline apparently have a less resistance xylem to cavitation compared to those growing in lowlands, as demonstrated by our literature survey.

The lack of differences between populations for cavitation resistance traits is congruent with previous studies supporting the hypothesis that uniform selection or canalization has shaped the variability among populations in *Pinus* species. However, there were significant differences among populations for the xylem-specific hydraulic conductivity (k_s), and a clear altitudinal trend with low altitude populations has larger k_s values than high altitude populations. This might be due to an adaptation to cold temperature at high elevation, whereby tracheid diameter is reduced to protect against freezing-induced embolism (Pittermann and Sperry 2003). Further investigations of xylem anatomy are needed to test this hypothesis.

Author contribution C.S.R.and S.D. designed the experiment. C.S.R., S.D. and J.B.L. made the statistical analysis and wrote the manuscript. E.L.R. provided maintenance, measurements, and statistical analysis of the common garden tests. A.P.A, J.B.L. and R.B. set up the methodology for vulnerability curves and processed the samples in the Cavitron. P.L. helped to improve the discussion.

Acknowledgments Funding was provided to CSR by the joint research funds between the Mexican Council of Science and Technology (CONACyT), and the State of Michoacán (CONACyT-Michoacán, Grant 2009-127128), and the Mexican National Forestry Commission (CONACyT-CONAFOR, Grant 2005- C02-14783); and funds from the Coordination for Scientific Research of the Universidad Michoacana de San Nicolás de Hidalgo (CIC-UMSNH). This study was also supported by the Institut Scientifique de Recherche Agronomique (INRA) innovative project (2010) to SD and JBL. Beccy Wilbore helped to improve the English wording and three anonymous reviewers helped to improve substantially the manuscript.

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