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ORIGINAL PAPER

From desert to cloud forest: the non-trivial phenotypic variation of Canary Island pine needles

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Abstract Pine needles reflect some of the most outstanding xeromorphic characteristics of sclerophyllous plants. Detailed descriptive studies about their anatomy and morphology were carried out since the beginning of the past century but the role the different tissues play in adaptation to diverse environments is still little known. In this work we analyzed morphological and anatomical needle traits of seventeen natural populations of *Pinus canariensis* Chr. Sm. Ex DC to identify adaptive traits related to drought resistance and determine their variability throughout a wide range of ecological conditions. A Principal Component Analysis revealed two groups of traits, one related to drought adaptation: maximizing gas exchange, avoiding water loss and storing water and the other to photosynthesis. Results support a predominantly mechanical function of hypodermis and a possible role of transfusion tissue to withstand dry conditions, thanks to its water storing capacity. The inversion of the patterns of the relative area of the dermal and transfusion tissues in the xeric region was justified due to the influence of the extreme dry Saharan wind from the East. A clear adaptation of *Pinus canariensis* needles to contrasted environments is evidenced suggesting distinct phenotypic needle features in the most xeric stands.

Keywords Drought tolerance · Needle anatomy · Needle morphology · *Pinus canariensis*

Introduction

Given that leaves are the most specialized plant organs (Larsen 1927), they are expected to display an optimal adjustment to their habitat conditions (Margaris and Mooney 1981). Pines occupy a wide range of environments commonly associated with harsh conditions: water stressful habitats and nutrient-poor soils. Consequently, pine needles reflect some of the most obvious and outstanding xeromorphic characteristics of sclerophyllous plants (Martínez 1948; Mirov 1967; Farjon and Styles 1997). Although the anatomy of pine needles is well described since the beginning of the past century (reviewed by Mirov 1967) only a few works have dealt with the contribution of the different tissues to adaptation. Particularly the water storing capability of transfusion tissue, an original trait in pine leaves, has not been thoroughly studied, but the resemblance with water-storing parenchyma in succulent plants (Larcher 2003) is worthy of note.

The variation of tissue proportion in conifer needles has been found, for example, among plants growing in chambers with elevated CO_2 and temperature against those growing under ambient conditions (Luomala et al. 2005), among age classes in Douglas-fir (Apple et al. 2002), and between plants exposed to drought during their elongation against well irrigated ones in Canary Island pine (Grill et al. 2004).

Pinus canariensis (Canary Island pine) is probably a privileged species to study adaptive differences in needle morphology. It is an ancient pine, with no competitors of the same genus in its natural habitat and differentiated under a tropical climate and hence the phenotype of its needles is much closer to some subtropical pines (Richardson and

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Rundel 1998) than to the rest of Mediterranean pines (sensu Klaus 1989). The natural distribution area is small, restricted to a volcanic Archipelago near to the African continent, but with remarkable ecological variation due to topography and the variable influence of Trade winds (Climent et al. 2004). While needle anatomy and surface characteristics of this species has been described in detail both for juvenile and mature needles (Jiménez et al. 2000; Zellnig et al. 2001; Stabentheiner et al. 2004), geographical phenotypic variation in needle anatomy and morphology is still unknown. Previous studies have detected significant differentiation among populations in survival (López et al. 2007), ontogeny (Climent et al. 2006), traits related to fire adaptation (Climent et al. 2004) and cone morphology (Gil et al. 2002). Therefore, we expected to find significant differences in needle morphology associated to the environmental variation among populations.

The aim of this study is to identify adaptive traits related to drought resistance in the anatomy and morphology of *P. canariensis* needles by determining the extent of phenotypic variability throughout the natural range of distribution and detecting environmental and/or geographical patterns of variation. Particularly, we hypothesised that the hypodermis and the transfusion tissue play a pivotal role in drought resistance, thus, needles from dry and xeric populations must invest more resources on developing thickly these tissues.

Materials and methods

Plant material

Seventeen natural populations covering most of the species' natural range of distribution were sampled (Fig. 1). Four to seven adult codominant trees per population, separated at least 100 m where chosen at random. Five 3-needle dwarf-shoots were taken from the south half of the crown at the same height (about 2 m) from the ground, one fascicle per branch. All branches were orthotropic, vegetative shoots; shoots with ovulate or pollinate strobili or traces of them were avoided to minimize undesirable variation. All sampled individuals were located at low-density spots within the stands to reduce micro-environmental variation due to different light environments.

Morphological and anatomical measurements

The needles were fixed in a mixture of distilled water, ethanol and glycerine until taken to the laboratory. Needle length (NL) was measured with a caliper. Cross-sections of the central portion of three needles per tree were obtained with a sliding microtome, stained and mounted following a standard method (Johansen 1940). Sections were observed through an optical microscope and digitalized. Needle width (NW) and the areas corresponding to the following tissues were measured using Scion Image software (Scion corporation, Frederick, MD, USA): total cross section (CROSS), epidermis together with hypodermis (HYP), mesophyll (MES), sum of resin canals lumen (RES), endodermis (END), transfusion tissue (TRANS), xylem (XYL) and phloem (PHLO) (Fig. 2).

Stomata counts were performed on five needles from each sample tree, one from each dwarf-shoot. Stomatal densities were determined using a dissecting microscope. All counts were conducted in two adjacent portions near the middle of the needle. The stomatal density (number of stomata per surface needle area, SD) and the number of stomata rows (SR) were counted at the middle needle portion on the convex (or "adaxial") side, twice in contiguous portions of each needle.

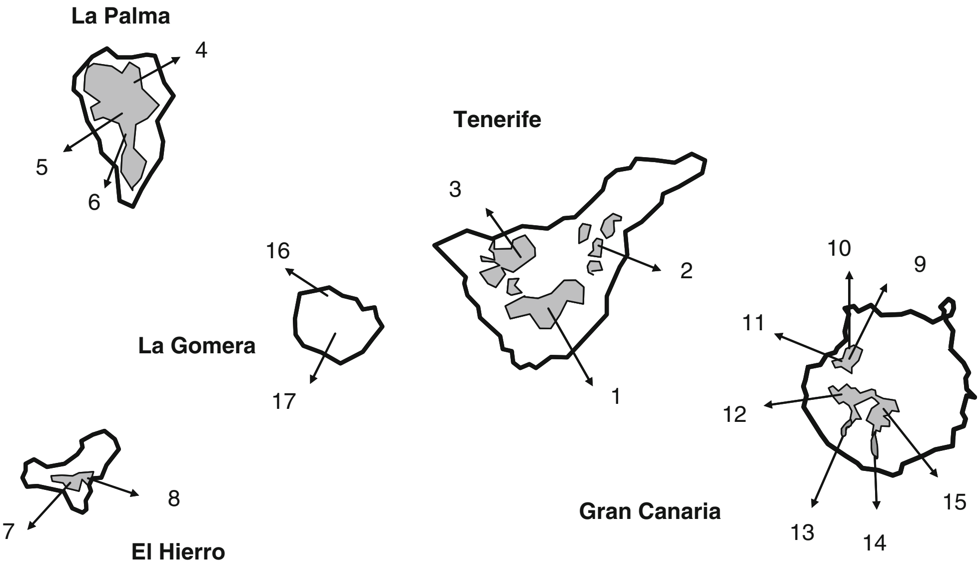

Fig. 1 Present natural distribution area (*grey spots*) of *Pinus canariensis* and location of the sampled populations in the Canary Islands

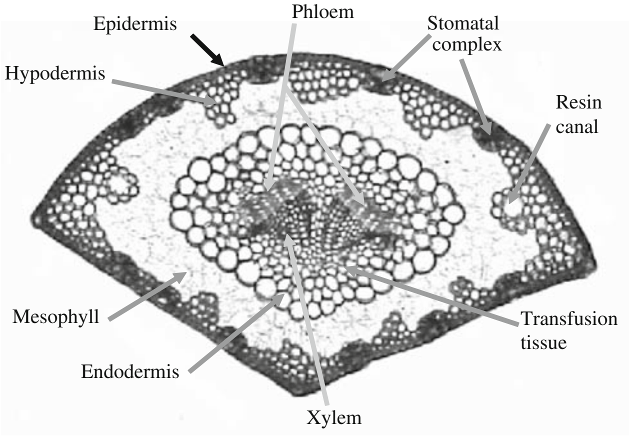

Fig. 2 General view of an adult needle of *P. canariensis* and measured traits

Several relations between the measured variables were obtained: needle slenderness (NL/NW), the area of xylem/stomatal density (XYL/SD) representing the relation between the conducting and the transpiration systems, and the area of xylem/area of mesophyll (XYL/MES) representing the relation between water conduction and photosynthesising parenchyma. Averages per tree of all measurements were computed and used in further analysis.

Characterisation of climate types

The studied populations were classified into four ecological categories (climate types): wet, mild, dry and xeric (Table 1). We considered soil and climate data extrapolated from sampled plots throughout the distribution area of the species (Blanco et al. 1989), plant communities associated with each population (Climent et al. 2002) and wide-sense aspect (North or windward/South or leeward) within each island as a major climate effect to consider the influence of the humid Trade Winds from the north–east and the desiccating Saharan winds from the west. This last factor is essential to explain differences in water availability. The present ecological types are closely related to the five climate types described for the species in Climent et al. (2002).

Data analysis

Statistical analyses were conducted on the individual-tree mean values for each trait. The analyses were performed with Statistica (StatSoft, Tulsa, OK). The effects of the climate type and the population on the morphological and anatomical variables were tested by a general linear model equation considering populations nested within climate types.

Relationships between morphological and anatomical variables and environmental parameters were checked using correlation coefficients and principal component analysis (PCA). Once PC were obtained a Varimax rotation was applied to analyse the relationship among anatomical and morphological traits.

Results

Needle morphology

Population was the main source of variation for morphological traits explaining more than the 42% of the deviation (Table 2). The most extreme mean values of needle length were found in Gran Canaria, minimum of 12 cm in Tirajana and maximum of 24 cm in Galdar. In general, needle length and needle slenderness decreased with increasing dryness but width was non-significantly affected by the climate type (Table 3), showing the dry and xeric regions the highest coefficients of variation, 21.78 and 24.65% respectively. No relationship was found between needle length and width.

Needle anatomy

Needle anatomy differed significantly both among climate types and populations except for the percentage of mesophyll which was only significantly different among populations within the same climate type (Table 2). Needles of trees at the mesic sites had the highest percentage of hypodermis plus epidermis and also the highest percentage of endodermis. The number of cells constituting the hypodermis varied from 1 to 5 with prominent intrusions into the mesophyll that increased the area of the dermal tissue, except in the areas below the stomata where it disappeared. Dermal tissues and endodermis showed a downward tendency as dryness increased except for the populations within the xeric type (Fig. 3a, b). Here the pattern is reversed and the xeric type stood between the mean values of the wet and mild types. The percentage of cross-sectional area occupied by the vascular bundle also decreased with xericity by 41%. On the other hand, the percentage of the stele (vascular bundle and transfusion tissue) plus endodermis followed an opposite pattern to the dermal tissues (Fig. 3a) as a result of the large differences found in the percentage of transfusion tissue (Fig. 3b). The dry populations presented more than twice as much percentage of transfusion tissue as wet and mild populations and 60% more than xeric ones. Although the number of resin canals appeared to be rather constant for the populations studied, two or three, the area percentage occupied varied between 0.63 in the dry populations and 0.98 in the xeric ones.

Table 1 Sampled provenances, climatic characterization and climate types

Population	Location	Elevation	Pa (mm)	*T* (°C)	Tr (°C)	Dp (months)	Aspect	Climate type
1. Granadilla	Tenerife	1,500	415.1	13.4	18.3	5.6	S-SE	Mild
2. Güimar	Tenerife	1,500	1214.9	13.8	19.9	3.96	E	Wet
3. Icod	Tenerife	1,100	939.9	12.7	14.4	3.6	N	Wet
4. Barlovento	La Palma	1,500	945.3	15.2	21.4	5.06	N-NE	Wet
5. Bejenado	La Palma	1,600	719.9	14	18	5.12	SE	Mild
6. Paso	La Palma	1,100	920	14.1	18	4.78	S-SW	Wet
7. S. Salvador	El Hierro	1,200	485.1	16.1	15.7	6.48	E	Dry
8. Playas	El Hierro	900	580	15.6	15.7	5.7	S	Dry
9. Galdar	Gran Canaria	1,400	618.7	15.6	21.2	4.86	NE	Mild
10. Tamadaba	Gran Canaria	1,250	598.8	15.5	20.8	4.92	NW	Mild
11. Tirma	Gran Canaria	1,000	379.6	18	20.6	6.93	SW	Dry
12. Pajonales	Gran Canaria	850	439.7	16	22.3	6.43	SW	Dry
13. Tauro	Gran Canaria	1,200	401	17	20	7.0	SE	Xeric
14. Arguineguín	Gran Canaria	300	144	20.5	12.7	9.5	S	Xeric
15. Tirajana	Gran Canaria	950	394.7	14.6	22.4	6.89	SE-E	Xeric
16. Garabato	La Gomera	500	415.2	19.1	11.9	7.28	NW	Dry
17. Imada	La Gomera	1,180	520	16.7	11.9	6.44	E	Dry

Pa annual precipitation, *T* mean annual temperature, *Tr* annual temperature range, *Dp* drought period

Table 2 Percentage of the explained variation and significance values from the GLM to determine the sources of variation for the anatomical and morphological traits

Source	NL	NW	HYP	END	MES	TRANS	XYL	PHLO	RES	SD	SR
Climate type	2.40**	NS	48.34***	35.14***	NS	67.50***	50.12***	56.26***	11.98**	14.34***	10.24***
Population	42.26***	48.67***	26.79***	19.82***	22.02**	12.82***	17.88***	10.03*	21.31*	23.52***	34.28***

F values with asterisks are significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Table 3 Mean values ± SE of length, width and slenderness of needles in the four climate types

Climate type	Length (cm)	Width (mm)	Slenderness
Wet	19.7 ± 0.6	1.60 ± 0.03	123.8 ± 3.7
Mild	19.4 ± 0.6	1.53 ± 0.02	128.2 ± 4.3
Dry	18.3 ± 0.6	1.57 ± 0.02	116.4 ± 3.9
Xeric	16.1 ± 0.8	1.60 ± 0.03	100.7 ± 5.4

Traits related to stomata were highly correlated ($r = 0.78$) and increased significantly with xericity but they seemed to be less influenced by the climate type and more dependent on the population. The variation explained by the climate type for the stomatal density and number of stomata rows was around 12% whereas population effect accounted for 23.5 and 34.3% respectively. On the contrary, the ratios percentage of xylem/stomatal density and percentage of xylem/percentage of mesophyll decreased with xericity. The variation was such that the values in the wet and mild climates were 1.6 higher than in the xeric one.

Correlations among traits

The first three principal components (eigenvalue > 1) explained 67.39% of the total variance of anatomical and morphological traits. The PC1 had high positive factor loads for traits related to stomata (SD, SR) and transfusion tissue and negative for endodermis and phloem. The PC2 was mostly related to needle length, percentage of hypodermis, mesophyll and xylem and the PC3 with needle width. However, PC1 and PC2 had also considerable factor loads for some other traits (Fig. 4) and consequently their interpretation was neither trivial nor with a clear ecological meaning.

The percentage of TRANS was positively correlated with stomatal traits ($r = 0.66$ with SD and $r = 0.59$ with SR) and negatively with HYP ($r = -0.63$), END ($r = -0.78$) and PHLO ($r = -0.56$), NL was correlated with the area of vascular bundles ($r = 0.69$ and $r = 0.70$ with XYL and PHLO respectively), MES ($r = 0.51$) and SD ($r = -0.50$) while the percentage of HYP was positively correlated with

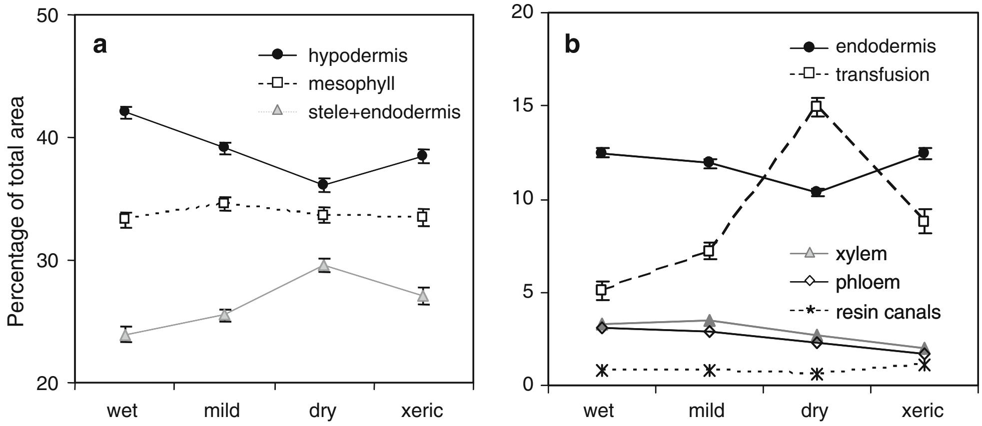

Fig. 3 Relative area (in percentage) occupied by **a** hypodermis, mesophyll and stele+endodermis and **b** endodermis, transfusion tissue, xylem, phloem and resin canals in the four climate types

the percentage of END ($r = 0.61$) and RES ($r = 0.58$) and negatively with the percentage of MES ($r = -0.60$).

Climate variables were mainly correlated with anatomical traits within the stele. Positive relationships were found between mean annual precipitation and XYL ($r = 0.30$) and PHLO ($r = 0.65$) and negative with TRANS ($r = -0.55$) while the opposite occurred with the drought period length ($r = -0.69$, $r = -0.79$ and $r = 0.52$). Mean annual temperature was also correlated with TRANS ($r = 0.52$) and with END ($r = -0.56$) and SR ($r = 0.46$).

Discussion

The phenotypic variation encountered among natural populations of *P. canariensis* suggests a complex adaptive role of the different adult needle tissues facing diverse ecological conditions. In contrast to our expectations, the relative area of the hypodermis decreased with xericity while the relative area of the transfusion tissue followed an opposite pattern.

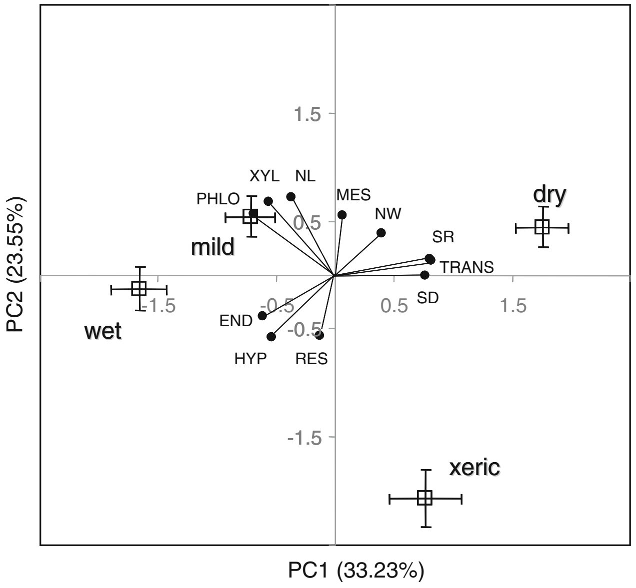

Fig. 4 Principal Components Analysis. Percentage of explained variation and projection of the original variables and the climate types on the factor plane. *Bars* correspond to the standard errors of the mean values of all individual trees in each climate type

We have distinguished three different types of traits in accordance with their variation among climate types. First, those traits with little or non-significant variation across environments: needle width and relative area of the mesophyll. Secondly, those which decreased as drought became more intense, needle length and relative area of the vascular bundle, or increased with xericity, relative area of the resin canals and stomata traits. Thirdly, traits which in the xeric type did not follow the general pattern: relative area of the hypodermis plus epidermis, relative area of the endodermis and relative area of the transfusion tissue (Fig. 3).

Since needle morphology and anatomy influence two key physiological processes: photosynthesis and transpiration, the amount and position of the different tissues composing the needle reflect adaptations to diverse environmental conditions (Pachepsky et al. 1995). This is coherent with the results of the PCA. The first PC, mostly positively correlated with stomata traits and transfusion tissue and negatively with relative area of the vascular bundles could be interpreted as a component of drought adaptation maximizing gas exchange with a minimum loss of water and water storing capacity, the second PC could be linked with the photosynthetic function, mesophyll as the photosynthetic tissue and the conductive structures to provide and adequate transport. On the other hand resin canals, hypodermis and endodermis have protective and structural functions and do not take part actively in photosynthesis but they take up a space and require resources that limit the area of photosynthetic and vascular tissues.

The reduction in needle length in response to environmental stress is almost a general pattern in pines, although there are a few exceptions (Richardson and Rundel 1998). The smaller surface area and fewer stomata per needle related to drought tolerant populations may serve to conserve water (Cregg 1994). Furthermore, great nutrient availability increases foliage dimensions (Will 2005), and the formation of longer needles could entail negative energetic balances due to the high production costs of secondary walls, lignin and cuticle in locations where resource availability is scarce and needle development must complete in the short favourable spring period.

Xylem and phloem have a primary conductive function. In *P. canariensis*, their relative area decreased in dry and xeric environments, what can be interpreted to be a consequence of the limited supply of water and nutrients. On the contrary, both stomatal density and number of stomata rows increased with xericity. Stomatal traits are controversial in respect to drought stress; there are evidences of increasing stomatal density (and a reduction of stomata size) as a response to drought in some plants, interpreted as an adaptive response through the quicker onset of stomatal regulation (Dunlap and Stettler 2001; Pearce et al. 2006) but other works reflect opposite trends (Glass et al. 2005; Gratani et al. 2006). It is possible that a high stomatal density is advantageous for Canary Island pines growing in harsh environments, by facilitating a higher leaf gas interchange in short favourable periods and consequently maximizing biomass production under environmental constraints (Wang et al. 1995; Al Afas et al. 2007). Both shorter needles and a higher stomatal density agree with the results found in other Mediterranean pines in natural populations: *P. brutia* (Dangasuk and Panetsos 2004), *P. pinaster* (Wahid et al. 2006) and in provenance trials of *P. halepensis* (Calamassi 1986) and *P. brutia* (Calamassi et al. 1988).

Lignified dermal tissues in conifer needles have two main functions, firstly they prevent from water loss and secondly they confer mechanical resistance (stiffness) keeping the needle erect and hence optimizing energy capture when radiation is parallel to leaves (Esau 1977). The pattern encountered in *P. canariensis* fits better with the second function since longer needles, occurring in more fertile environments, had a higher percentage of hypodermis. Cuticular traits and stomata structure of juvenile and adult needles of *P. canariensis* showed adaptive strategies to avoid light and water stress (Stabentheiner et al. 2004) thus it is possible that the xerophytic adaptation is rather due to a singular stomata structure (Zellnig et al. 2001) and different surface properties than to hypodermal thickness. As Larsen (1927) suggested in the study of the epidermis of eight different moisture-demanding conifers, differences among susceptibility to water loss could be a matter of quality rather than quantity of dermal tissues. The number of hypodermal layers in species occurring in arid habitats varies from one in *P. nelsonii* to more than three in *P. monophylla*. This range is even wider in tropical habitats: *P. caribaea* presents a hypodermis with up to five layers while in *P. ayacahuite* is uniform and unilayered (Farjon and Styles 1997). In our study we have observed between one and five hypodermal layers.

Opposite to the pattern of the dermal tissues, the relative area of the transfusion tissue was almost three times higher in the dry than in the wet climate type. The capacity of the transfusion tissue to store water (Gambles and Dengler 1982), the concentration of solutes from the transpiration stream and the retrieval from it of selected solutes that are returned to the phloem or forwarded through the endodermis to the mesophyll (Canny 1993) could be an asset for leaves which experience periods of water stress.

Why, then, the inversion of the pattern of relative area of the hypodermis and transfusion tissue in the populations within the xeric region? We suggest that the influence of the extreme dry Saharan wind could cause higher rates of water loss via cuticular transpiration than plant could assume and inhibit needle elongation (Smith and Ennos 2003) due to high temperatures, wind speed and abrasion by soil particles in suspension (Cleugh et al. 1998). Populations within the xeric region, responsible for the breaking-off of the general tendency of percentage of dermal and transfusion tissues, were located to the windward of the Saharan wind (Dunion and Velden 2004). The direct exposure to desiccating winds could induce the formation of thicker defensive structures to restrain water loss and subsequently a reduction of the proportion of the transfusion tissue since they are negatively correlated.

In conclusion, needle phenotypic traits evaluated in *P. canariensis* showed significant differences both among climate types and populations within the same climate type that suggest some ecotypic differentiation rather than the clinal variation observed in natural populations of other Mediterranean pines (Dangasuk and Panetsos 2004; Wahid et al. 2006). Moreover, the adaptive interpretation of each particular trait is nontrivial. Differing with the general idea of hypodermis as the main trait related to sclerophylly, the results are rather coherent with a more complex function of this tissue, which variation did not follow a specific environmental gradient. On the other hand, our data supported a relevant role of the transfusion tissue facing drought in this species. Similar analyses performed on multisite provenance trials are necessary to separate genetic and environmental factors and subsequently to separate the environmental effect (phenotypic plasticity) from genetic differentiation among populations. Physiological measurements in natural conditions on leaf hydraulic conductance and its rehydration kinetics could be used to significantly bolster claims about the water storage and drought tolerance efficacy of various anatomical traits that fall along the PCA axes.

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