

Leaf phenotypic variation in natural populations of *Quillaja saponaria* and its relationship with climatic variation

Variación fenotípica foliar en poblaciones naturales de *Quillaja saponaria* y su relación con la variación climática

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ABSTRACT

Quillaja saponaria is a sclerophyllus evergreen tree species distributed from 30° to 38° S in Central Chile. In this wide distribution it is expected that the species exhibits phenotypic plasticity in the morphology of leaves associated to climate variation; however, the information on this topic is still scarce. We studied leaf phenotypic variation and its relationship with temperature, precipitation, and aridity in 85 stands of *Q. saponaria* throughout the natural distribution of the species. The results show that the basal diameter of petiole, the number of secondary veins, and the basal diameter of the principal vein increased with precipitation and decreased with aridity, while the length, basal diameter and the relative length of the petiole decreased with temperature. This confirms that phenotypic variation for foliar characters in *Q. saponaria* is related to climatic variables indicating an adapting capability of the species to the wide range of environmental conditions in which grows.

Keywords: Phenotypic variation, morphological traits, leaf plasticity, quillay.

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RESUMEN

Quillaja saponaria es una especie esclerófila siempreverde que se distribuye desde los 30° a los 38° S en Chile central. En este amplio ámbito de distribución se espera que la especie exhiba plasticidad fenotípica en su morfología foliar asociada a la variación climática; sin embargo, la información sobre este tema es escasa. Se estudió la variación morfológica de la hoja en 85 rodales de *Q. saponaria* a lo largo de la distribución natural de la especie y su relación con la temperatura, precipitación y aridez. Los resultados muestran que el diámetro basal del pecíolo, el número de venas secundarias y el diámetro basal de la vena principal aumentan con la temperatura y disminuyen con la aridez. Por otra parte, la longitud, diámetro basal y longitud relativa del pecíolo disminuyen con el aumento en temperatura. Esto confirma que la variación fenotípica de los caracteres foliares de *Q. saponaria* está relacionada con las variables climáticas indicando una capacidad de adaptación de la especie a las condiciones climáticas contrastantes en las que se desarrolla.

Palabras clave: Caracteres morfológicos, plasticidad de la hoja, quillay, variación fenotípica.

INTRODUCTION

Quillaja saponaria Mol. (quillay) is a sclerophyllus evergreen native tree species with a widespread range across Chile. The species is an important source of industrial saponins (Pelah *et al.* 2002) and its extracts have been used in beverages, food, and in photography (Martín and Briones 1999), threatening the species due to habitat destruction and overexploitation (Benoit 1989, Squeo *et al.* 2001). The species distribution ranges from the arid northern part of the country (30° S) to the humid climates in the south (38° S). The altitudinal range is from 15 m in the coastal mountains to 1750 m in the Andes (Rodríguez *et al.* 2006, Cruz *et al.* 2013). In this extensive range of environments, it is expected that the species exhibit a wide range of leaf morphological variation according to the climate.

Evidence supports that leaf morphology variation has adaptive significance linked with climate (Peppe *et al.* 2011), especially with latitude, altitude, temperature, and rainfall (Ackerly *et al.* 2002, Byars *et al.* 2007, Mediavilla *et al.* 2012). However, the physiological ecology of *Q. saponaria* along a latitudinal gradient has received little attention. As leaves are considered important for plant production, changes of leaf morphology need to be further elucidated. In this study, we analyzed variation in leaf characteristics within and among *Q. saponaria* trees in natural populations adapted to different ecological conditions and investigated their relationship with precipitation, temperature, and aridity of provenance origin.

MATERIALS AND METHODS

Study stands

A total of 438 trees of *Q. saponaria* were sampled during summer between 2012 and 2013 from 85 stands with at least 30 trees per hectare each and located in northern, central, and central-southern regions of Chile (Fig. 1). Selected trees were growing in open areas and isolated from other trees. Climatic data for each of the 85 stands were extracted from the WORLDCLIM dataset (Hijmans *et al.* 2005). Annual precipitation and temperature records were used to create the De Martonne aridity index as $P/T + 10$ (De Martonne 1926), where P is precipitation in mm, and T the temperature in °C. After that, the spatial distribution of 85 *Q. saponaria* stands was built in QGIS software (QGIS Development Team 2018) using Jenks Natural Breaks optimization (Jenks 1967). This method calculates groupings of data values based on natural groups in the data distribution. Four distinct groups of populations, from Group 1 with dry habitat, to Group 4 with wet habitat, were recognized in different geographical and ecological conditions according to the De Martonne index (Fig. 1).

Assessment of leaf morphological traits

In each of the 85 stands we selected between three to 20 mature trees growing under similar conditions in the field. Trees in a stand were separated 50 m from each other. One branch located in the north cardinal compass direction (length from 20 to 40 cm) was taken from upper third of the canopy. The leaves in each branch were practically of

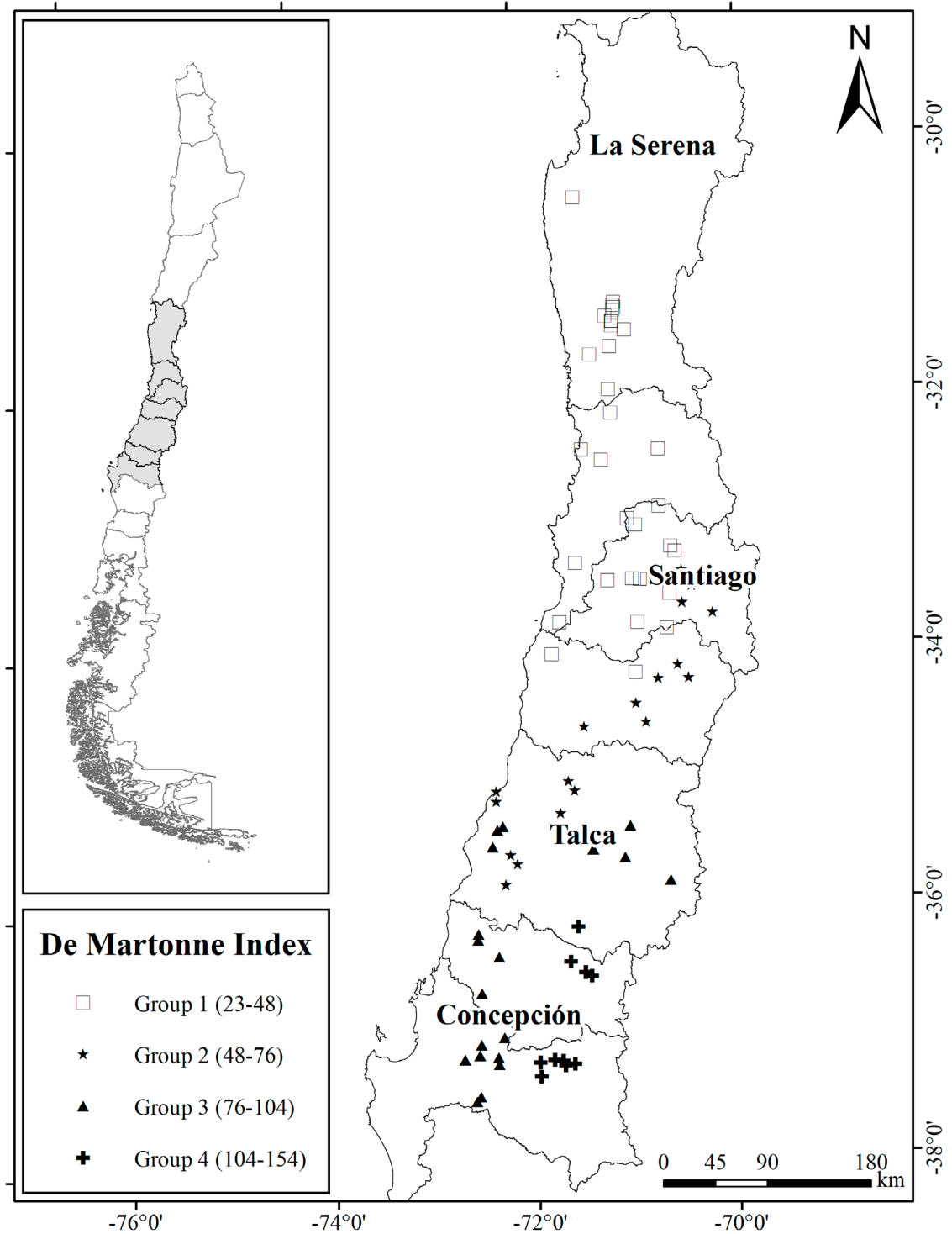


Figure 1. Spatial distribution for the 85 stands of *Q. saponaria* in four ecological groups according to the De Martonne index and using Jenks Natural Breaks optimization. Group 1 represents a dry habitat while Group 4 represents a wet habitat.

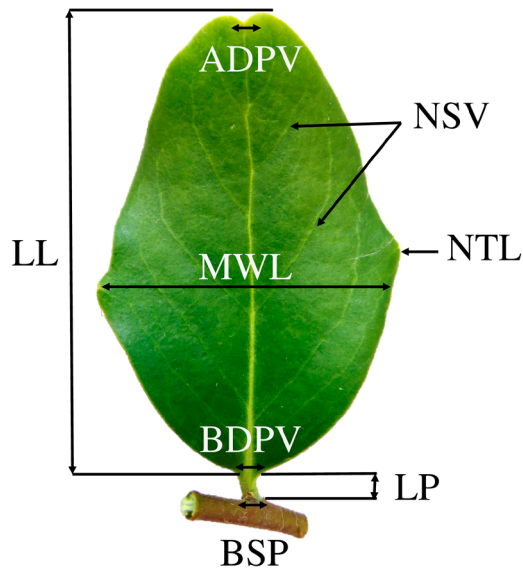


Figure 2. Leaf morphological traits measured in *Q. saponaria*. LL= Lamina length (mm); MWL= Maximal width of lamina (mm); LP= Length of petiole (mm); NSV= Number of secondary veins; BDPV= Basal diameter of the principal vein (mm); ADPV= Apical diameter of the principal vein (mm); NTL= Number of teeth per lamina; BSP= Basal diameter of petiole (mm).

the same age and only new leaves were collected each year. Samples were field-collected and herborized under a paper press for further laboratory analysis. Ten leaf characters related to the lamina, petiole, veins, and teeth were assessed on each leaf (Fig. 2, Table 1) (Kremer *et al.* 2002, Bruschi *et al.* 2003, Ponton *et al.* 2004). We counted the total leaves on each branch and leaf characteristics were determined for \approx 30 leaves of each branch.

Data analysis

Normality of distribution of characters was assessed for all variables. An analysis of variance (ANOVA) was performed following the model:

$$Y_{ijk} = \mu + G_i + T(G)_{ij} + e_{ijk}$$

Table 1. List and description of the morphological characters examined.

Variable abbreviation	Description
Primary variables	
LL (mm)	Lamina length
MWL (mm)	Maximal width of lamina
LP (mm)	Length of petiole
NSV (no)	Number of secondary veins
BDPV (mm)	Basal diameter of the principal vein
ADPV (mm)	Apical diameter of the principal vein
NTL (no)	Number of teeth per lamina
BSP (mm)	Basal diameter of petiole
Calculated variables	
RLP = LP/(LL+LP) (%)	Relative length of the petiole
RWL = MWL/LL (%)	Relative width of the lamina

Table 2. F-value and significance level for the analyzed traits. Significance level (** $P < 0.01$, * $P < 0.05$, ns = non-significant). In parenthesis the percentage (%) of the total variance that was accounted for each level of the hierarchy.

Leaf trait	Variance component	
	Between Groups	Within group
LL	1.77ns (2.6)	1.38* (6.0)
MWL	0.76ns (2.4)	0.85ns (5.8)
LP	8.66** (9.5)	0.74ns (0.0)
NSV	14.9** (10.3)	1.85** (2.3)
BDPV	9.78** (5.8)	1.71** (0.0)
ADPV	0.14ns (0.0)	2.22** (0.0)
NTL	4.70** (10.7)	0.91ns (0.0)
BSP	4.13** (2.6)	1.09ns (2.0)
RPL	4.56** (8.6)	0.80ns (0.8)
RWL	2.01ns (0.0)	1.61** (0.0)

For acronyms, refer to Table 1.

where, Y_{ijk} is the observed leaf morphological trait, μ is the overall mean, G_i is the fixed effect of i^{th} Jenks group (i.e., 1 to 4), $T(G)_{ij}$ is the random effect of the j^{th} tree nested within the i^{th} Jenks group, and e_{ijk} is the experimental random error. Patterns of variation of each morphological trait were evaluated by comparing percent of total variance of the different m levels. Associations between leaf morphological traits and climatic variables were analyzed using the Pearson's correlation coefficient. In addition, a Principal Component Analysis (PCA) was carried with the aim to explore the relationship between leaf characters with the De Martonne aridity index.

RESULTS

Provenance differences in leaf morphology

Differences in leaf morphology attributed to the group effect were significant for 60 % of the morphological traits under study while differences due to trees within-groups were significant for half of the traits (Table 2). Group 4 (i.e., a wet site with the highest De Martonne index) exhibited the highest LP, NSV, BDPV, BSP, and RLP, while Group 1 (a dry site with the lowest De Martonne index), exhibited the opposite tendency in NSV and RLP (Table 3). With the exception of ADPV and RWL, total variance was higher in the

among-group variation for most traits (i.e., variation at the group level), while the total variance due to within-group variation was higher only in LL and MWL.

Association between climate and leaf morphology

There was a positive but low correlation between BSP, NSV, and BDVP with De Martonne's index and MAP, while LP, BSP and RPL were negatively and poorly correlated with MAT (Fig. 3). The remnant leaf morphological traits exhibited no significant relationship with the climatic data (data not shown). The PCA indicates that the 87.4 % of the data variability is explained by PC1 and PC2 (Fig. 4). NTL is more associated to dry climates with lower values of De Martonne index. At the right of biplot is observed a group of leaf characters negatively correlated with NTL. In this group LL, LP, BSP, BDPV, MWL, and NSV, are associated with wetter and colder environments.

DISCUSSION

The abiotic factors along environmental gradients affect leaf morphological properties (Hultine and Marshall 2000). In our study, several leaf morphological traits differed significantly among groups of populations of *Q. saponaria* and among trees within the same group, clearly demonstrating the high phenotypic diversity of the species (Cruz et al. 2013). We found that leaf morphological plasticity of *Q. saponaria* was related to the climatic variation in the species' distribution range. The results of the correlation analyses between climate and leaf morphology showed that BSP, NSV, and BDPV increased with increasing precipitation and decreasing aridity, whereas LP, BSP, and RPL decreased with increasing temperature. This suggests that populations of *Q. saponaria* at the dry edge of the species' distribution could have a resource-conservative stress tolerance strategy based on a restricted water transport driven by shorter petioles and thinner veins. In addition, leaf teeth; which are negatively correlated with temperature (Royer and Wilf 2006, Peppe et al. 2011), and are sites of increased transpiration in the leaf (Wolfe 1993), were not significantly correlated with MAT. However, trees in dry habitats had slightly higher NTL than trees in wet habitats, corroborating those trees in dry habitats have higher rates of transpiration (Royer and Wilf 2006) and water losses (Xu et al. 2009), which are driven by the higher transpiratory demands experienced in those sites. The PCA corroborates that bigger leaves are related with the south locations of the species,

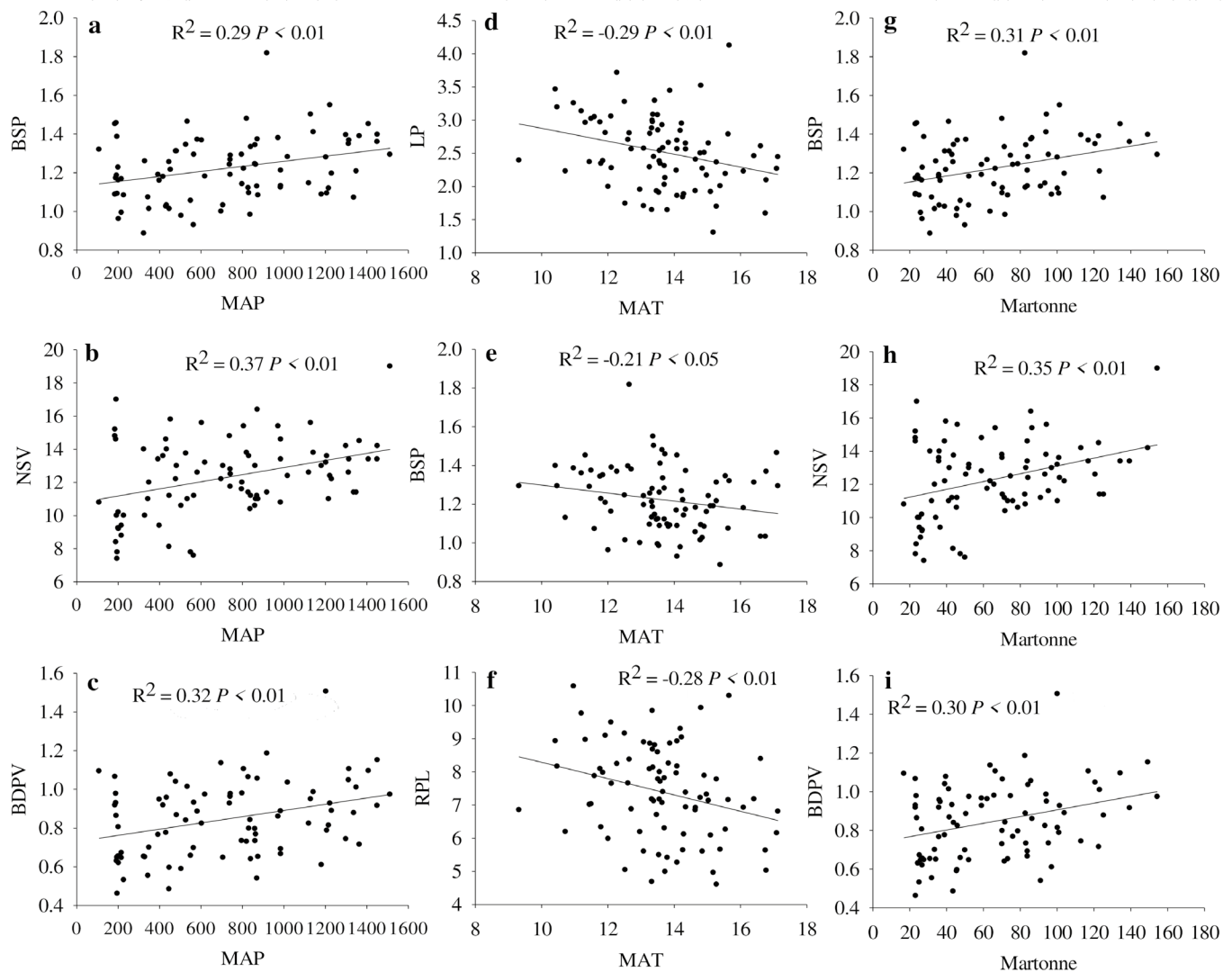


Figure 3. Pearson coefficient of correlations between leaf traits and the climatic variables mean annual precipitation (MAP, left panels a, b, c), mean annual temperature (MAT, center panels d, e, f), and the De Martonne index (right panels g, h, i). LP= Length of petiole; NSV= Number of secondary veins; BDVP= Basal diameter of the principal vein; BSP= Basal diameter of petiole; RPL= Relative length of the petiole; RWL= Relative width of the lamina.

meanwhile smaller and serrated leaves are common at northern locations where it is necessary to use water more efficiently. Ramirez *et al.* (2020) and Ogaya and Peñueñas (2007) found that the sclerophyllous species *Quercus lobata* Nee and *Quercus ilex* L. exhibited a high plasticity of leaf morphology to climate variation as an adaptation to cope with environmental stress imposed in Mediterranean climates. In general, populations located at drier sites exhibited lower leaf areas.

The positive correlations among leaf traits such as BSP, NSV, and BDVP with MAP may indicate the adapting capability of *Q. saponaria* to gas exchange. Leaf traits such as vein density influence leaf water transport (Buckley *et*

al. 2015). In general, the higher the number and diameter of veins, the higher the leaf hydraulic conductance, the stomatal conductance and gas exchange (Sack and Scoffoni 2013, de Boer *et al.* 2016). We observed a trend to increase BSP, NSV, and BDVP as MAP increases, but trees in Group 1 had lower values for these traits in relation to trees in Group 4, suggesting they have reduced construction cost in carbon and nutrient investment (Sack and Scoffoni 2013), but at the expenses of living under constant restrictions for stomatal conductance (Sack and Frole 2006). Similarly, the negative correlations between LP and BSP with temperature may also indicate variations in leaf hydraulic conductance of the groups under study. When temperature increases *Q. saponaria* trees tend to

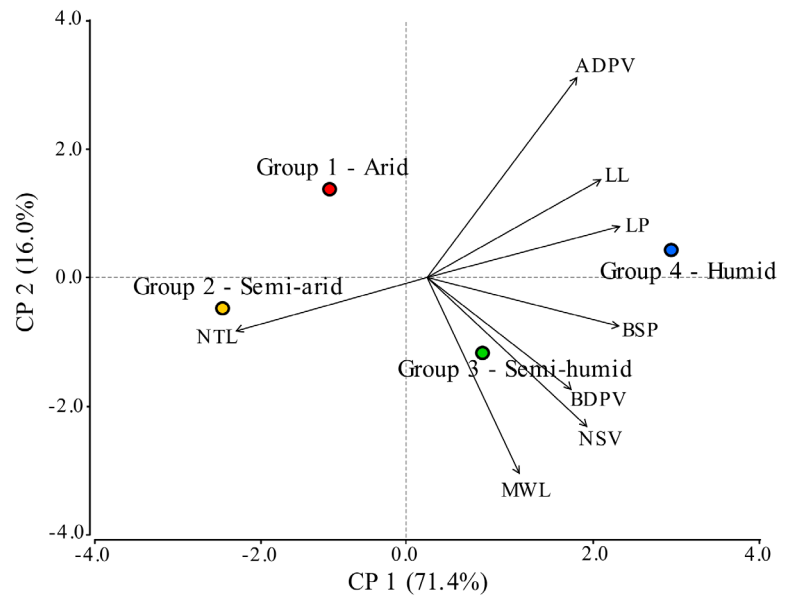


Figure 4. Principal Component Analysis for the relationship among leaf characters and the De Martonne aridity index. Colored circles correspond to the Groups 1 to 4 according to the De Martonne index. LL= Lamina length (mm); MWL= Maximal width of lamina (mm); LP= Length of petiole (mm); NSV= Number of secondary veins; BDPV= Basal diameter of the principal vein (mm); ADPV= Apical diameter of the principal vein (mm); NTL= Number of teeth per lamina; BSP= Basal diameter of petiole (mm).

have shorter and thinner petioles, suggesting a decrease in leaf hydraulic conductance (Sack and Frole 2006), but this needs to be corroborated with *in situ* quantification of leaf hydraulic conductance (Carrasco-Urra et al. 2019). Trees in Group 1 had lower LP and BSP in comparison to Group 4, corroborating the stressful environment in which trees of Group 1 grew. The lack of correlation between temperature and precipitation with important functional traits such as leaf area (LL and MWL in our study), may be attributed to the levels of genetic diversity and population structure of *Q. saponaria* (Ramos et al. 2010, Letelier et al. 2015, Abarca 2019) which could have improved the buffering capability against environmental constraints. However, this hypothesis needs further research.

Our results must be interpreted carefully because MAP, MAT, and the very simple aridity index (i.e., the De Martonne aridity index), only gives global climatic information at the groups considered with no details on variation at the microsite level. Changes in leaf morphological and physiological attributes must be analyzed by considering soil moisture, aspect, forest cover (Beerling et al. 1996), air temperature and humidity (Panek and Waring 1995, Codarin et al. 2006), and atmospheric CO₂ concentrations (Marshall and Monserud 1996). Additionally, further work on the morphological leaf plasticity of *Q. saponaria* should include leaf position and specific leaf area as indicators of adaptation to light and water.

CONCLUSION

We found a lack of phenotypic variation and no association of leaf area with climate in the natural populations of *Q. saponaria* under study. However, the significant relationships between petioles and veins with climate variables suggests that shorter petioles and thinner veins are adaptations of populations located at dry sites to face with a restricted water transport and higher rates of transpiration.

AUTHOR'S CONTRIBUTION

CM, EM, and BA, designed the study; PP, IG, SW, and NT collected data and reviewed literature; SE performed analyses and wrote the early and final versions of the manuscript; PG participated in final correction of manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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Table 3. Mean \pm standard error for leaf morphological traits of *Q. saponaria* samples according to groups defined by Jenks. Different letters indicate statistical differences among groups ($P < 0.05$).

Leaf trait	Groups			
	Group 1	Group 2	Group 3	Group 4
LL	32.0 \pm 0.5a	30.8 \pm 0.8a	32.1 \pm 0.4a	32.7 \pm 0.6a
MWL	19.4 \pm 0.5a	19.3 \pm 0.6a	20.3 \pm 0.3a	19.6 \pm 0.4a
LP	2.4 \pm 0.0bc	2.2 \pm 0.1c	2.6 \pm 0.0b	3.0 \pm 0.0a
NSV	11.3 \pm 0.2c	12.2 \pm 0.6bc	12.9 \pm 0.2ab	13.7 \pm 0.3a
BDPV	0.78 \pm 0.03b	0.87 \pm 0.04b	0.87 \pm 0.02b	0.97 \pm 0.04a
ADPV	0.31 \pm 0.02a	0.30 \pm 0.02a	0.30 \pm 0.02a	0.31 \pm 0.02a
NTL	5.8 \pm 0.2ab	6.5 \pm 0.3a	5.6 \pm 0.7ab	4.9 \pm 0.2b
BSP	1.20 \pm 0.03ab	1.17 \pm 0.04b	1.28 \pm 0.02ab	1.32 \pm 0.04a
RLP	7.3 \pm 0.2b	6.8 \pm 0.3b	7.5 \pm 0.1ab	8.4 \pm 0.2a
RWL	61.1 \pm 1.3a	62.9 \pm 2.1a	64.7 \pm 1.1a	61.1 \pm 1.8a

For acronyms, refer to Table 1.

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