

Linking Leaf Functional Traits with Plant Resource Utilization Strategy in an Evergreen Scrub Species *Rhododendron caucasicum* Pall. along Longitudinal Gradient in Georgia (The South Caucasus)

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Abstract

Leaf functional traits widely have been used to understand the environmental controls of resource utilization strategy of plants along the environmental gradients. By using key leaf functional traits, we quantified the relationships between leaf traits and local climate throughout the distributional range of *Rhododendron caucasicum* Pall. in eastern and western Georgian mountains (the South Caucasus). Our results revealed, that all traits showed high levels of intraspecific variability across study locations and confirmed a strong phenotypic differentiation of leaf functional variation along the east-west longitudinal gradient in response to the local climate; out of the explored climatic variables, the moisture factors related to precipitation and number of precipitation and dry days for winter and growth seasons were more strongly related to leaf trait variation than the elevation and air temperature. Among studied leaf traits, the leaf specific area (SLA) showed the highest level of variability indicating the different resource utilization strategies of eastern and western-central *Rh. caucasicum* individuals. High SLA leaves for western-central Caucasian individuals work in relatively resource-rich environments (more humid in terms of precipitation amount and the number of precipitation days in winter) and could be explained by preferential allocation to photosynthesis and growth, while eastern Caucasian samples work in resource-poor environments (less humid in terms of precipitation amount and the number of precipitation days in winter) and the retention of captured resources is a higher priority appearing in a low SLA leaves. However, more evidence from a broader study of the species throughout its distribution range by including additional environmental factors and molecular markers are needed for firmer conclusions of intraspecific variability of *Rh. caucasicum*.

Key Words: *Rhododendron caucasicum*, the South Caucasus, intraspecific leaf trait variation, leaf specific area (SLA), climate growth response, longitudinal moisture gradient

Introduction

A long-standing goal of ecology and forest management is to understand the environmental controls of resource uti-

lization strategy of plants along the environmental gradients (Zhang et al. 2019) and to evaluate the possible impacts that global climate change can have on ecosystems (Gigauri et al. 2013; Garcia et al. 2014; Körner 2021). Plant species

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adapt to climate change by relocating to regions with more suitable environmental conditions (Moritz and Agudo 2013), and this involves a change in resource acquisition and ecological strategies (McGill et al. 2006; Yang et al. 2014).

Leaf functional traits are thought to be highly sensitive to climatic environments (Pratt and Mooney 2013) and may co-vary with climatic variation associated with differences in environmental conditions (González-Rodríguez et al. 2005; Uribe-Salas et al. 2008). Plant functional traits, such as leaf specific area (SLA) and leaf dry matter content (LDMC) correlated with plant relative growth rate and resource capture and utilization (Wright et al. 2004), while leaf area (LA), leaf blade length (BL) and leaf maximal width (LW) mainly reflect light interception and water stress tolerance (Westoby et al. 2002). Therefore, it is important to evaluate the relationships between the climate and plant functional traits for understanding the possible impacts that climate change can have on ecosystems (Wright et al. 2017).

The variation across a climatic gradient is frequently coupled with intraspecific trait variation due to different levels of both, genetic variation and phenotypic plasticity (Pfenningwerth et al. 2017; Ekhvaia et al. 2018; Souza et al. 2018; Aliyeva et al. 2020; Murtazaliev et al. 2020). Phenotypic plasticity is the ability of a genotype to produce different morphological and physiological responses to environmental changes (Karami et al. 2017), and by damping off the effect of these changes to increase plant tolerance to stress (Matesanz and Valladares 2014). Widely distributed species are extensively exposed to different environmental pressures and stressful factors such as variation in precipitation, temperature, and solar radiation with various intensity and unpredictability (Hooper et al. 2012; Garcia et al. 2014). However, an important, unresolved question is how climatic changes will affect plant traits and plant communities as a whole and what impact such changes will have on patterns of forest function (Madani et al. 2018).

Endemic species occupying niches unavailable to others are interesting in connection with intraspecific variation and plasticity of the traits best-reflecting plant ecological strategies, i.e., whether a species is developing a more competitive ecological strategy or adopting a more conservative strategy through adaptation of functional traits to specific

habitat conditions (Westoby et al. 2002; Tonin et al. 2020). The Caucasus region, as one of the 36 global biodiversity hotspots, along with a high degree of vascular plant endemism (more than 25%, Nakhutsrishvili et al. 2015), well known rich topography, and steep climatic gradients can be thus considered as an ideal subject to evaluate the consequences of climate change on natural populations. Here, we elucidated the relationships between climatic factors and functional plant traits in an endemic evergreen shrub species *Rh. caucasicum* Pall. to identify the critical factors affecting the covariation of plant functional traits along climatic gradients in Georgia (the South Caucasus). We selected *Rh. caucasicum* because it is native to the Caucasus and geographically widespread both in the Greater and the Lesser Caucasus in subalpine and alpine zones forming continuous populations along steep altitudinal gradients (Ketskhovali et al. 1971-2011) characterized by different moisture conditions, humid in the western part and continental in the eastern part of the country. Thus, the main purpose of our study was to clarify the relationships between leaf functional traits and climatic variables to detect critical factors affecting the leaf trait variation along a climatic gradient in eastern and western Georgian mountains (the South Caucasus). We aimed to answer: (1) whether the plant functional traits show significant differences with climate changes along four different locations of *Rh. caucasicum* throughout its distributional eastern and western Georgian range in both the Greater and the Lesser Caucasus regions, (2) and which leading climatic factors make these differences in selected plant traits between study locations? Understanding how the target species adjusts its functional plant traits according to the climate gradient can improve our knowledge about the ability of plants to cope with environmental changes, including climate change (Tonin et al. 2020).

Materials and Methods

Material

Rhododendron caucasicum Pall. (Ericaceae) is about 1.5 m tall vegetatively spreading semi-prostrate evergreen shrub. This Caucasian endemic is a dominant and edificatory species of *Rh. caucasicum* communities (in Georgian this species is called 'Deka', and shrubbery – 'Dekiani');

Ketskhoveri 1959). *Rh. caucasicum* occurs almost over the whole Caucasus forming dense thickets with 90-100% cover both on gently (10-15°) and steep (> 40°) north-facing shady and humid slopes with deep and long-term snow cover. It occurs in subalpine and alpine zones between 1900-3000 m a.s.l. (optimal range is 2,300-2,900 m a.s.l.) almost in all gorges of the Caucasus. At the timberline, it forms a dense underwood in the birch, beech, or spruce-beech forests. Some depressed individual specimens of *Rh. caucasicum* can be found even at 3100-3200 m a.s.l. (Ketskhoveri et al. 1971-2011; Dolukhanov 1978; Nakhutsrishvili and Abdaladze 2017). *Rh. caucasicum* is associated with an endotrophic mycorrhiza (ENM), which enables this shrub to successfully colonize poor, acidic soils (Nakhutsrishvili et al. 2006). As a typical chionophilous species for successful wintering, it needs enough long-term snow cover which preserves from photoinhibition and winter drought (Abdaladze et al. 2015). Leaves of *Rh. caucasicum* wintering, coriaceous, glabrous, and dark-green above, very finely ferruginous-tomentose below, narrowly oval or slightly obviate; margins slightly revolute (Ketskhoveri 1971-2011). Plant diversity in *Rh. caucasicum* community is not high, characteristic species are *Vaccinium myrtillus* L., *V. vitis-idaea* L., *Empetrum nigrum* subsp. *caucasicum* (Juz.) Kuvaev, *Daphne glomerata* Lam, *Pyrola minor* L., *P. rotundifolia* L., *Anemonastrum narcissiflora* subsp. *fasciculatum* (L.) Ziman & Fedor., *Calamagrostis arundinacea* (L.) Roth (Nakhutsrishvili et al. 2006). Besides the characteristic species noted, *Juniperus sabina* Sm. and *Salix kazbekensis* A. K. Skvortsov can also be found in the *Rh. caucasicum* communities. However, ecological importance is high and includes protection of steep slopes from erosion, landslides, mud- and debris flows, as well as, regulation of water relations of terrestrial and river ecosystems in lowlands (Nakhutsrishvili and Abdaladze 2017). At the same time, *Rh. caucasicum* plant community is one of the most sensitive to the anthropogenic load including intensive grazing and uncontrolled collection of leaves and stems for medicinal purposes (Akhalkatsi et al. 2006), and climate global change in the Greater and the Lesser Caucasus (Gigauri et al. 2013).

Sites

This study was conducted in four different locations in

Rh. caucasicum throughout its distributional Georgian range in both the Lesser (Ajara, Bakuriani region) and the Greater (Svaneti, Kazbegi region) Caucasus (Fig. 1): (a) mt. Sarbiela (Ajara, western Georgia) is located in the western part of the Lesser Caucasus. Among studied locations, it is the lowest mountain peak (the maximal height is 2471 m a.s.l.) which receives the highest amounts of winter precipitation (about 692 mm; Supplementary Table 1). The mountain holds up the moist air of the sea creating a climate of high humidity that varies between 70-90% resulting in a mild cold winter and long warm/cool summer. The study sites are located on the north-facing shady and humid slopes from 2166 to 2468 m a.s.l. (Supplementary Table 1, Fig. 1) with an annual mean daily maximum air temperature is about 11.84°C ranging from 6.9°C to 12.67°C. The daily mean air temperature during the coldest month (January) is reached 2.28°C ranging from -2°C to 3°C, while the daily mean air temperature during the warmest months (August) is about 20.14°C with the range between 15°C and 21°C. Snow cover is deep and stable and persists for five months from December to May, reaching its maximum in January (about 12.6 snow days per month). The annual precipitation is about 1625 mm with peak values in April-May (about 180 mm per month); (b) mt. Tskhratskaro (Bakuriani region, central Georgia) is located in the east-western part of the Lesser Caucasus. The local climate is



Fig. 1. Sampling sites of the evergreen shrub species *Rh. caucasicum* in Georgia (the South Caucasus) within the range of distribution. Sampling sites: (a) Mt. Sarbiela (Ajara, western Georgia, the Lesser Caucasus), (b) Mt. Tskhratskaro (Bakuriani region, central Georgia, the Lesser Caucasus), (c) Mt. Tetnuli (Svaneti, western Georgia, the Greater Caucasus), (d) Mt. Kazbegi (Kazbegi region, eastern Georgia, the Greater Caucasus).

transitional from humid oceanic to relatively humid continental. The study sites are located on the north-facing deep and shade slopes from 2,254 to 2,769 m a.s.l. (Supplementary Table 1, Fig. 1) with an annual mean daily maximum air temperature of 5.77°C ranging from 3.75°C to 10.08°C. The negative daily mean maximum air temperatures are fixed from December to March (-3.2°C- -0.4°C), the daily mean air temperature during the coldest months (January and February) is reached -5.2°C, while the daily mean air temperature during the warmest months (August) is near 15.8°C. Stable snow cover persists for 155 days reaching its maximum in February (about 10.7 snow days per month). The annual precipitation is about 842 mm with peak values in mid-late spring (about 129 mm in April and 148 mm in May); (c) mt. Tetnaldi (Svaneti, western Georgia) is located in the further western part of the Central Great Caucasus. The local climate is humid with cold and long winter, and chilly, rather humid summer. The study sites are located on north-facing deep and shade slopes from 2070 to 2913 m a.s.l. (Supplementary Table 1, Fig. 1) with an annual mean daily maximum air temperature is about 5.97°C ranging from 3.5°C to 9.17°C. The negative daily mean maximum air temperatures are fixed from December to March (-4.33°C- -1.33°C), the daily mean air temperature during the coldest month (January) is reached -4.33°C, while the daily mean air temperature during the warmest months (July and August) is near 17°C. Snow cover is deep and stable, persists for 8-11 months from September to May reaching its maximum during January-February (from 13.63 to 14.97 days per month). At the relatively lower locations up to 2,555 m a.s.l. the annual precipitation is about 1205 mm with peak values in late spring (about 160 mm in May) and early-middle summer (about 245 mm in June, and 211 mm in July), and at the upper study locations from 2555 m to 2913 m a.s.l. annual precipitation reaches 843 mm with peak values in May (about 134 mm), and June (about 141 mm) (Supplementary Table 1); (d) mt. Kazbegi (Kazbegi region, eastern Georgia) is located in the further eastern part of the Central Great Caucasus. The local climate is moderately humid, winter is relatively dry and cold with the least winter season precipitation (about 187 mm; Supplementary Table 1), and summer is cool and relatively wet with the most growth season precipitation (about 737 mm) among studied locations.

The study sites are located on north-facing deep and shade slopes from 2167 to 2818 m a.s.l. (Supplementary Table 1, Fig. 1) with an annual mean daily maximum air temperature is 5.4°C ranging from 3°C to 7.83°C. The negative daily mean maximum air temperatures are fixed from December to March (-2°C- -7°C), the daily mean air temperature during the coldest month (February) is reached -14.47°C, while the daily mean air temperature during the warmest months (July, August) is near 15°C. Stable snow cover persists for 5-7 months from November to May, reaching its maximum depth of 120 cm in March (about 11.9 snow days per March). The annual precipitation is about 1205 mm with peak values in early summer (about 231 mm in June). All four-study locations are characterized by the presence of poor, acidic soils.

Climatic variables

A climatic data for the last 34 years of hourly weather model simulation for each selected locality were obtained from “Meteoblue” (since 1985) (<https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/>) that have a spatial resolution of approximately 30 km. We used precipitation amount (mm) and the number of precipitation and dry days for each winter and growth season, and an average of mean daily maximum air temperatures (°C) for months of growth season to characterize the climatic heterogeneity of the environments in four different locations of the evergreen shrub species *Rh. caucasicum* in eastern and western Georgian mountains (the South Caucasus) within the range of distribution. The winter season includes climatic data corresponding to November, December, January, February, and March, while the growth season consists of data from May, June, July, and August, respectively (Supplementary Table 1).

Leaf traits

Sampling was done from July to August 2018. Five fully expanded, healthy leaves were harvested from each population presented by six to seven sampling points in each study site. Total, 26 sampling points for four populations of *Rh. caucasicum* were selected among four different locations in Georgia (the South Caucasus) within the range of its distribution (for detailed information see Supplementary Table 1). Collected leaves were pressed in an herbarium,

taken to the laboratory, and scanned. From scanned images leaf blade length, BL (cm), leaf petiole length, PL (cm), blade width, BW (cm) and leaf area, LA (cm²), was calculated using ImageJ (Rueden et al. 2017). Scanned leaves were dried in a dry oven (Vent-Line 180 Prime, Poland) at 80°C for 48 h, and then were weighted to obtain leaf dry mass, LDM (g). To calculate specific leaf area, SLA (cm²/g⁻¹), the ratio between fresh leaf area (LA, cm²) and leaf dry mass (LDM, g⁻¹) were pooled.

Data analysis

Descriptive statistics were produced on original data, listing the minimum (min), maximum (max), mean and standard deviation (SD). The normality and heterogeneity analysis was also performed on original leaf trait data; with the exception of leaf petiole length (PL), not all other studied leaf traits were assumed normal distribution (Supplementary Table 2). So, these parameters were log₁₀ transformed prior to analyses in order to attain approximate normality and homogeneity of residuals. Accordingly, all other analyses

were performed on log₁₀ transformed leaf trait data. One-way analyses of variance (ANOVA) and Tukey’s HSD multiple comparisons were used to analyze differences in studied leaf traits among the four different locations of the target species within the range of distribution. Then principal component analysis (PCA) was used to assess ecological strategy variation of the target species by assessing multivariate shifts of all studied leaf functional traits. As a dimensionality reduction technique, PCA projects a set of composite, uncorrelated variables called principal components (James and McCulloch 1990) subsequently used as dependent variables in partial least square analysis (PLS). Because of high collinearity among the environmental variables, it was not possible to use multiple regressions when looking for relationships between morphology and environmental factors. Instead, projection to latent structures by means of PLS analysis was applied. This multivariate statistical method finds a linear relationship between the matrixes of dependent (Y values) and explanatory (X values) variables and can handle collinear and incomplete data sets (Eriksson et al.

Table 1. One-way ANOVA results for *Rh. caucasicum* populations throughout its distribution range in Georgia, the South Caucasus

	Trait	SS	DF	MS	Proportion of intra- and inter-population variance (%)	F***
Log ₁₀ PL	Between populations	0.875	3	0.292	96.69	27.93***
	Within populations	1.316	126	0.01	3.31	
	Total	2.191	129			
Log ₁₀ BL	Between populations	0.605	3	0.202	98.54	57.93***
	Within populations	0.430	126	0.003	1.46	
	Total	1.036	129			
Log ₁₀ BW	Between populations	0.685	3	0.228	98.27	61.43***
	Within populations	0.468	126	0.004	1.73	
	Total	1.154	129			
Log ₁₀ LA	Between populations	2.373	3	0.791	98.63	69.97***
	Within populations	1.425	126	0.011	1.37	
	Total	3.798	129			
Log ₁₀ LDM	Between populations	2.158	3	0.719	98.47	66.96***
	Within populations	0.011	126	0.011	1.53	
	Total	3.853	129			
Log ₁₀ SLA	Between populations	8.515	3	2.838	98.82	84.15***
	Within populations	4.252	126	0.034	1.18	
	Total	12.767	129			

Asterisks indicate overall significance of F-statistics (***p < 0.001).

SS, sum of squares; DF, degrees of freedom; MS, mean squares; F, F test statistics.

All parameters were log₁₀-transformed to achieve normality. Explanations for leaf traits are given in Table 1.

1999). The environmental data as explanatory variables (X values) in PLS analysis were also transformed using a variant of Bartlett's (1936) square root transformation to make them more normal.

The statistical package SPSS ver. 21.0 (<https://www-01.ibm.com/>) was used for ANOVA and multiple comparisons, while PCA and PLS analysis were performed by software PAST (Hammer et al. 2001) (<http://palaeo-electronica.org/2001-1/past/issue1-01.htm>) and XLSTAT ver. 2014.5.03 (<https://www.xlstat.com/>).

Results

Leaf trait variability

All leaf traits showed high levels of intraspecific leaf trait variation across populations; the one-way ANOVA F-statistics together with Tukey's HSD multiple comparisons showed that means of all studied leaf traits are significantly different between populations ($p \leq 0.000$, Table 1, Fig. 2). SLA showed the highest level of variability among the

measured traits, and PL was the least variable, while variations in BL, BW, LA, and LDM were moderate (Fig. 2). Among studied traits, the BL, BW, LA, LDM, and SLA followed a general trend; they were decreased in size from the west to the east, and populations from the Lesser Caucasus (Ajara, Bakuriani region) are predominantly much larger than from the Greater Caucasus (Svaneti, Kazbegi region) (mean values for each trait at each population are given in Fig. 2). Additionally, Tukey's multiple comparisons between pairs of populations showed statistically highly reliable differences ($p < 0.001$) in means (Fig. 2), and except for leaf petiole (PL) clearly differentiated eastern (Kazbegi region) and western-central Georgian populations (Ajara, Bakuriani region, Svaneti) from each other. The breakdown of total variance into inter- and intra-population variance showed that for all trait values inter-population variance was much higher compared to intra-population variance, in all cases exceeding 95%, and was highest for SLA (98.82%, Table 1).

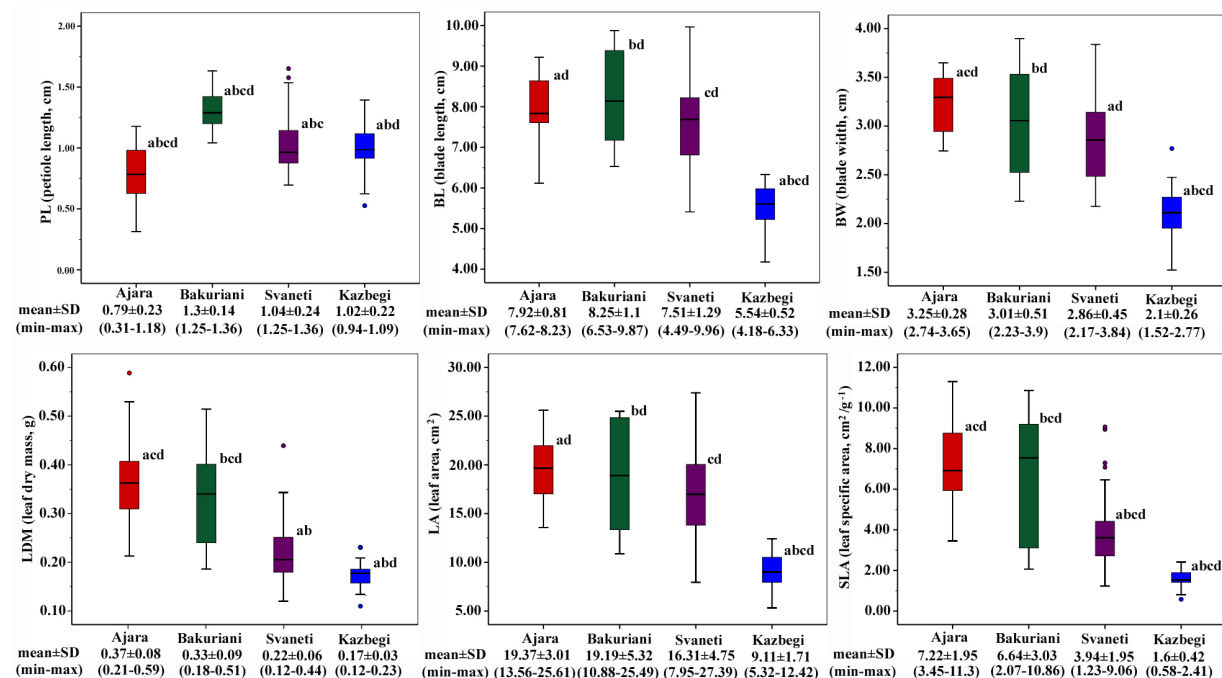


Fig. 2. Boxplots with descriptive statistics including mean, standard deviation (SD), and minimum-maximum values for each six functional leaf traits of *Rh. caucasicum* populations in four different study locations in Georgia (the South Caucasus). The means multiple comparisons between each pair of populations were done with Tukey's HSD test; the same letters next to each boxplot indicate significant differences among the means between pairs of populations at $p \leq 0.001$.

Comparison of leaf traits variability among study locations

The results of PCA (Table 2, Fig. 3) revealed that the first two principal components (PCs) cumulatively accounted for 88.14% of total leaf trait variation. However, a comparison of eigenvalues expected under a random model (red “broken stick” curve in Fig. 3a) showed that only the first principal component (PC1) above this curve could be considered a significant component (Jackson 1993) accounting for 82.08% of total leaf trait variation (Table 2). As the most important component, it was positively correlated with BL, BW, LA, LDM, and SLA. Among them, SLA showed the highest eigenvalue (0.78; Table 2) and had the greatest influence on the differentiation of populations. PC1 clearly differentiated eastern and western Georgian *Rh. caucasicum* samples comparing the relatively dry Kazbegi region (the eastern Great Caucasus) in terms of the lowest amounts of winter precipitation (not more than 187 mm per winter season; Supplementary Table 1) to humid Ajara (the western Lesser Caucasus) characterized by the highest amounts of winter precipitation (about 692 mm per winter season; Supplementary Table 1). Individuals from the Kazbegi region had smaller values of all traits, correlated with the first axis and grouped on the left side of the

Table 2. Leaf trait loadings, eigenvalues, and the percentage of leaf trait variation explained by the first two principal components (PCs)

	PC axis	
	PC1	PC2
Eigenvalue	0.16	0.02
Explained (%)	82.08	6.13
Cumulative (%)	82.08	88.14
Traits Eigenvectors*		
Log ₁₀ PL	0	0.99
Log ₁₀ BL	0.2	0.06
Log ₁₀ BW	0.21	-0.02
Log ₁₀ LA	0.4	0.04
Log ₁₀ LDM	0.37	-0.05
Log ₁₀ SLA	0.78	-0.01

*Loadings ≥ 0.2 in magnitude are shown in bold font. All leaf trait data were log₁₀-transformed to achieve normality. Explanations for leaf traits are given in Table 1.

graph, whilst samples from Ajara with the higher values were grouped on the right side of PC1 (Figs. 2, 3b). At the same time, Svanetian samples (the western Great Caucasus) were scattered along the first axis in both directions; samples from lower elevations (below 2500 m a.s.l. the amounts of winter precipitation are reached 395 mm; Supplementary Table 1) were closer to Ajarian individuals, while samples above 2600 m a.s.l. (the amounts of winter precipitation are lower and reach 204 mm; Supplementary Table 1) were grouped with individuals from the Kazbegi region (Fig. 3b). PC2 explained 6.13% of the total leaf trait variation and was positively correlated with PL (Table 2). However, the random model did not confirm the significance of the second component (PC2, Fig. 3a) and it did not provide any clear pattern in the distribution of *Rh. caucasicum* populations across study locations (Fig. 3b).

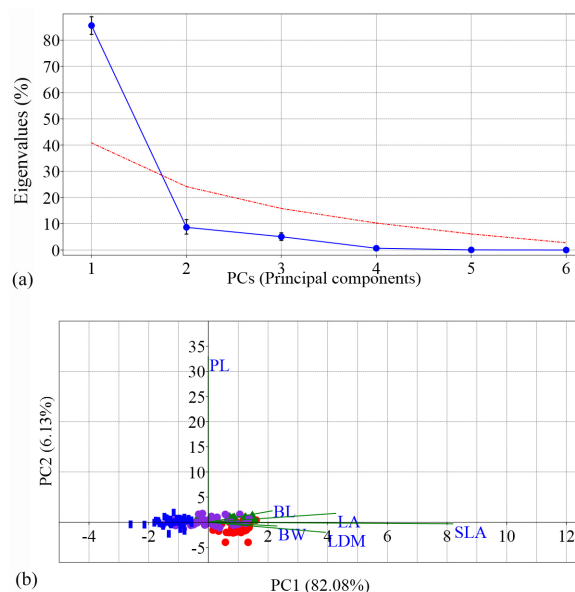


Fig. 3. (a) The “Scree plot” (simple plot of eigenvalues) expected under a random model (red “broken stick” curve) indicating the number of significant principal components, (b) Principal component analysis (PCA) plot showing the separation of studied leaf traits of measured *Rh. caucasicum* populations in four different study locations in Georgia (the South Caucasus) within the range of distribution. Red circles indicate samples from Ajara (western Georgia, the Lesser Caucasus), green triangles – Bakuriani region (central Georgia, the Lesser Caucasus), violet circles – Svaneti (western Georgia, the Great Caucasus), blue bars – Kazbegi region (eastern Georgia, the Great Caucasus). All leaf trait data were log₁₀-transformed to achieve normality.

Influence of the climate on the variability of the leaf traits

The scores of PC1 as the most significant and influenced component were used in the PLS analysis. Accordingly, in a PLS model, the leaf trait variability extracted from PC1 was mainly explained by PLS component 1 (57.2%) and to a lesser content by PLS component 2 (2.7 %). Both PLS components were calculated with Q^2 (cum), R^2Y (cum) and R^2X (cum) parameters of 0.57, 0.65, and 0.44 for the first component (PLS component 1), and 0.58, 0.7, and 0.8 for the second component (PLS component 2), respectively. The quality of components assessed through cross-validation showed that only PLS component 1 ($Q^2=0.57$) were significant (Q^2 limit > 0.0975 , corresponding to $p < 0.05$; Eriksson et al. 1999). The leaf trait variability was positively correlated with winter precipitation (mm) ($r=0.85$), winter precipitation days ($r=0.91$), growth season dry days ($r=0.9$), and average of mean daily maximum air temperatures ($^{\circ}C$) for growth season ($r=0.42$); and negatively correlated with latitude ($r=-0.66$), longitude ($r=-0.88$), altitude (m a.s.l.) ($r=-0.4$), winter dry days ($r=-0.9$), growth season precipitation (mm) ($r=-0.76$) and growth season precipitation days ($r=-0.96$) (Fig. 4a). The VIPs (Variable Importance for the Projection) for each explanatory variable of PLS component 1 showed that winter precipitation days, winter dry days, longitude, winter, and growth season precipitation contributed the most to the first PLS model ($VIPs > 1$; Fig. 4b). In fact, SLA and other leaf traits related to PC1 followed a general trend; their values increased longitudinally from the east to the west with increasing winter precipitation and winter precipitation days, and on the contrary, leaf values decreased with the increase of growth season precipitation and number of winter dry days. The goodness of fit statistics showed that the leaf trait variability explained by PC1 was accurately predicted by the PLS model ($r^2=0.7$; $SD \pm 0.22$).

Discussion and Conclusion

Overall, *Rh. caucasicum* showed high intraspecific variability in leaf functional traits across the climatic gradient due to the environmental heterogeneity existent in the Caucasus mainly promoted by its well-known complex top-

ography and steep climatic gradients (Nakhutsrishvili and Abdaladze 2017). For all trait values, the input of inter-population variation exceeded 95% (Table 1) pointing to an adaptation of each population to the respective site conditions and reflecting resource acquisition would presumably serve as a buffer against climate global change

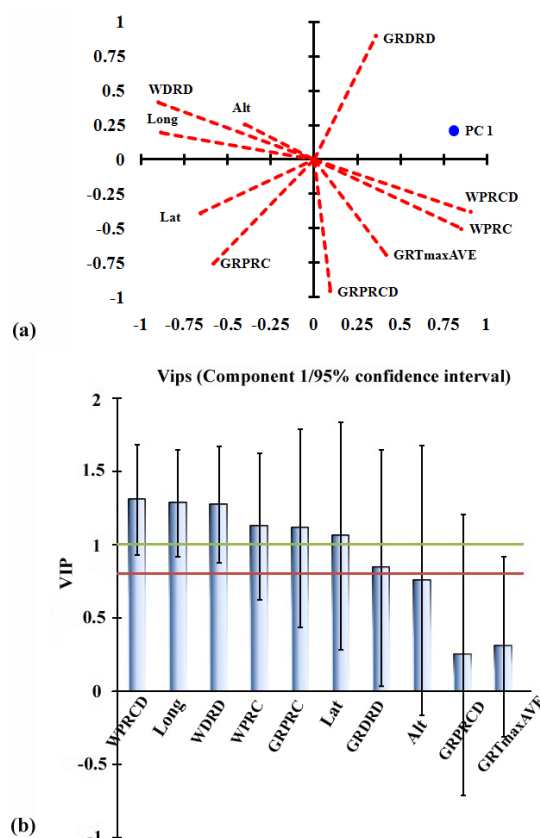


Fig. 4. (a) Results of PLS analysis, illustrate the correlations of the variables with two first axes associated with the PLS component 1 and PLS component 2 in the PLS model, (b)The VIPs (Variable Importance for the Projection) for each explanatory variable of PLS component 1 in the PLS model, illustrate ecological factors contributed the most to the observed leaf trait variability of *Rh. caucasicum* individuals in four different study locations in Georgia (the South Caucasus) within the range of distribution with different moisture conditions and temperatures. All ecological variables were square-root transformed to achieve normality and used as explanatory variables (X values) in the first PLS model (Lat, latitude; Long, longitude; Alt, altitude (m a.s.l.); WPRC, winter precipitation (mm); WPRCD, winter precipitation days; WDRD, winter dry days; GRPRC, growth season precipitation (mm); GRPRCD, growth season precipitation days; GRDRD, growth season dry days; GRTmaxAVE, an average of mean daily maximum air temperatures ($^{\circ}C$) for growth season in May, June, July, and August). PC1- a synthetic variable extracted from PCA - as the most significant and influenced principal component was used as a dependent variable (Y values) in the PLS model.

(Wellstein et al. 2013), which is considered to be particularly pronounced in mountainous regions (Körner 2021). Both, PCA and ANOVA analysis revealed that among studied traits SLA showed the highest level of trait variability and had the greatest influence on the differentiation of studied populations confirming the notion that SLA is the best indicator of plants growth and abundance in a community (Poorter et al. 2009; Fajardo and Piper 2011; Baruch et al. 2017; Meng et al. 2017). Significant differences among studied traits especially in SLA might be indicating the different strategies of the eastern and western-central *Rh. caucasicum* individuals. Populations with low SLA are thought to have relatively higher construction costs and had a slower conservation strategy (Villar et al. 2005) than the samples with a high SLA with preferential allocation to photosynthesis and growth (Wright et al. 2004). Our results correspond to this notion. Particularly, high SLA leaves for the western-central Georgian (Ajara, Bakuriani region, and Svaneti) populations work in some relatively resource-rich environments (more humid in terms of precipitation and the number of precipitation days in winter, and with less number of wet days during the growth season; Supplementary Table 1, Figs. 3b, 4) while in some resource-poor environments such as Kazbegi region (the eastern Great Caucasus) (less humid with the lowest precipitation and the number of precipitation days in winter, and in contrary more humid during the growth season with the peak values of precipitation and the number of wet days) the retention of captured resources is a higher priority exhibited by the low SLA.

Such pronounced east-west longitudinal clinal pattern of leaf functional variation confirms a strong phenotypic differentiation among *Rh. caucasicum* populations in response to the climatic gradient. The influence of altitude and latitude on interspecific leaf trait variation is well known: alpine plants tend to have smaller leaves with lower SLA values compared to lowland plants, which indicates a more conservative resource use in the harsh conditions of highlands (Wright et al. 2004; Pérez-Harguindeguy et al. 2013; Körner 2021). However, according to our results, for *Rh. caucasicum* the moisture factors along a longitudinal gradient were more strongly related to leaf intraspecific trait variation than the elevation and air temperature, as we found more moisture variables with higher correlations to

leaf traits in the PLS model (VIPs > 1, Fig. 4b). Some applicable ecological variables related to moisture, such as precipitation and the number of precipitation and dry days for winter and growth seasons were shown to change significantly along a longitudinal gradient. All of them showed an extensive array of values along with the east-west distribution, particularly, values for the number of winter precipitation days (from 51.6 to 83.9) and winter precipitation amount (from 187 mm to 692 mm) increased longitudinally in this direction, and on contrary, the number of dry days in winter together with precipitation amount during the growth season decreased from the east to the west from 99.4 to 67.6, and from 737 mm to 489 mm, respectively (Supplementary Table 1). It seems that the reduction of leaf traits, especially in SLA throughout the west-east distribution of *Rh. caucasicum* populations in the mountains of Georgia (the South Caucasus) might be considered as a mechanism of adaptation to sites with decreasing moisture conditions (Liu et al. 2017; Wright et al. 2017). Like other evergreen shrub species (for instance, common juniper; Pellizzari et al. 2014) higher snow accumulation would increase the insulation with warmer soil temperature promoting microbial activity, and shrub vegetative growth would benefit from a resulting increase in nutrient supply as well. As was shown by Nakhutsrishvili et al. (2006), the association of *Rh. caucasicum* with the ericoid mycorrhizal fungi (ENM) allows this shrub to colonize pure, acidic soils and successful vegetative growth and establishment of shrub seedlings. However, such a mechanism could be explained either as a phenotypic plastic response (Hoff and Rambal 2003; Uribe-Salas et al. 2008; Melo and Boeger 2016; Aliyeva et al. 2020; Murtazaliev et al. 2020) to the diverse environments, or a result from adaptive genetic differentiation among populations (Pyakurel and Wang 2013; Ekhvaia et al. 2018; Rodríguez-Gómez et al. 2018). Our data could not discern the amount of variation due to phenotypic response or genetic differentiation; the question needs coverage of a broader geographic range, including additional environmental factors and molecular markers in further studies for a more accurate examination of phenotypic plasticity and genetic adaptation of target traits.

In conclusion, this study demonstrated a large phenotypic leaf trait variation (especially in SLA) and a clear influence of moisture gradient throughout the range of the

Rh. caucasicum distribution in eastern and western Georgian mountains (the South Caucasus) and encourage using plant functional traits at the intraspecific level across species as a tool to understand the future ecological transformations of vegetation in changing environments. The remarkable longitudinal west-east decrease of *Rh. caucasicum* leaf traits related to leaf economics (especially in SLA) confirmed that phenotypic variance plays a crucial role in plants adapting to environmental change (more snow winter vs. less snow winter) and resulted in different resource utilization strategies (recourse capture vs. conservation) along the environmental gradient. However, the conclusions need more evidence from a broader study of the species throughout its distribution range and including additional environmental factors and molecular markers in a further study to discern and quantify heritable genetic vs. plastic sources of variation in response to environmental changes.

References

- Abdaladze O, Nakhutsrishvili G, Batsatsashvili K, Gigauri K, Jolokhava T, Mikeladze G. 2015. Sensitive Alpine Plant Communities to the Global Environmental Changes (Kazbegi Region, the Central Great Caucasus). *Am J Environ Prot* 4: 93-100.
- Akhalkatsi M, Abdaladze O, Nakhutsrishvili G, Smith WK. 2006. Facilitation of Seedling Microsites by *Rhododendron caucasicum* Extends the *Betula litwinowii* Alpine Treeline, Caucasus Mountains, Republic of Georgia. *Arct Antarct Alp Res* 38: 481-488.
- Aliyeva GN, Mammadova ZA, Ojaghi JM, Pourbabaei H. 2020. Inter- and intrapopulation variations in leaf morphological and functional traits of *Quercus petraea* ssp. *iberica* under ecological factors in Azerbaijan. *Plant Fungal Res* 3: 61-68.
- Bartlett MS. 1936. The Square Root Transformation in Analysis of Variance. *Suppl J R Stat Soc* 3: 68-78.
- Baruch Z, Christmas MJ, Breed MF, Guerin GR, Caddy-Retalic S, McDonald J, Jardine DI, Leitch E, Gellie N, Hill K, McCallum K, Lowe AJ. 2017. Leaf trait associations with environmental variation in the wide-ranging shrub *Dodonaea viscosa* subsp. *angustissima* (Sapindaceae). *Austral Ecol* 42: 553-561.
- Dolukhanov AG. 1978. The Timberline and the Subalpine Belt in the Caucasus Mountains, USSR. *Arct Alp Res* 10: 409-422.
- Ekhvaia J, Simeone MC, Silakadze N, Abdaladze O. 2018. Morphological diversity and phylogeography of the Georgian durmast oak (*Q. petraea* subsp. *iberica*) and related Caucasian oak species in Georgia (South Caucasus). *Tree Genet Genomes* 14: 17.
- Eriksson L, Johansson E, Kettapeh-Wold S, Wold S. 1999. Introduction to Multi and Megavariate Data Analysis Using Projection Methods (PCA & PLS). Umetrics, Umeå.
- Fajardo A, Piper FI. 2011. Intraspecific trait variation and co-variation in a widespread tree species (*Nothofagus pumilio*) in southern Chile. *New Phytol* 189: 259-271.
- García RA, Cabeza M, Rahbek C, Araújo MB. 2014. Multiple dimensions of climate change and their implications for biodiversity. *Science* 344: 1247-1251.
- Gigauri K, Akhalkatsi M, Nakhutsrishvili G, Abdaladze O. 2013. Monitoring of vascular plant diversity in a changing climate in the alpine zone of the Central Greater Caucasus. *Turk J Bot* 37: 1104-1114.
- González-Rodríguez A, Oyama K. 2005. Leaf morphometric variation in *Quercus affinis* and *Q. laurina* (Fagaceae), two hybridizing Mexican red oaks. *Bot J Linn Soc* 147: 427-435.
- Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electron* 4: 1-9.
- Hoff C, Rambal S. 2003. An examination of the interaction between climate, soil and leaf area index in a *Quercus ilex* ecosystem. *Ann For Sci* 60: 153-161.
- Hooper DU, Adair EC, Cardinale BJ, Byrnes JE, Hungate BA, Matulich KL, Gonzalez A, Duffy JE, Gamfeldt L, O'Connor MI. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486: 105-108.
- Jackson DA. 1993. Stopping Rules in Principal Components Analysis: A Comparison of Heuristical and Statistical Approaches. *Ecology* 74: 2204-2214.
- James FC, McCulloch CE. 1990. Multivariate Analysis in Ecology and Systematics: Panacea or Pandora's Box? *Annu Rev Ecol Syst* 21: 129-166.
- Karami L, Ghaderi N, Javadi T. 2017. Morphological and physiological responses of grapevine (*Vitis vinifera* L.) to drought stress and dust pollution. *Folia Horti* 29: 231-240.
- Ketskhoveli N, Kharadze A, Gagnidze R. 1971-2011. [Flora of Georgia (Sakartvelos Flora). 16 Vols]. Metsniereba, Tbilisi (in Georgian).
- Ketskhoveli N. 1959. [Georgian Plant Cover]. Metsniereba, Tbilisi (in Georgian).
- Körner C. 2021. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. 3rd ed. Springer International Publishing, Cham.
- Liu M, Wang Z, Li S, Lü X, Wang X, Han X. 2017. Changes in specific leaf area of dominant plants in temperate grasslands along a 2500-km transect in northern China. *Sci Rep* 7: 10780.
- Madani N, Kimball JS, Ballantyne AP, Affleck DLR, van Bodegom PM, Reich PB, Kattge J, Sala A, Nazeri M, Jones MO, Zhao M, Running SW. 2018. Future global productivity will be affected by plant trait response to climate. *Sci Rep* 8: 2870.
- Matesanz S, Valladares F. 2014. Ecological and evolutionary re-

- sponses of Mediterranean plants to global change. *Environ Exp Bot* 103: 53-67.
- McGill BJ, Enquist BJ, Weiher E, Westoby M. 2006. Rebuilding community ecology from functional traits. *Trends Ecol Evol* 21: 178-185.
- Melo JCF, Boeger MRT. 2016. Leaf traits and plastic potential of plant species in a light-edaphic gradient from *Restinga* in southern Brazil. *Acta Biol Colomb* 21: 51-62.
- Meng H, Wei X, Franklin SB, Wu H, Jiang M. 2017. Geographical variation and the role of climate in leaf traits of a relict tree species across its distribution in China. *Plant Biol (Stuttg)* 19: 552-561.
- Moritz C, Agudo R. 2013. The future of species under climate change: resilience or decline? *Science* 341: 504-508.
- Murtazaliev R, Anatov D, Ekhvaia J, Guseinova Z, Batsatsashvili K. 2020. Intraspecific variability of some functional traits of *Trigonocaryum involucratum* (Steven) Medw., a Caucasus endemic plant. *Bot Serbica* 44: 129-136.
- Nakhutsrishvili G, Abdaladze O, Akhalkatsi M. 2006. Biotope types of the treeline of the Central Greater Caucasus. In: *Nature Conservation* (Gafta D, Akeroyd J, eds). Springer, New York, pp 211-225.
- Nakhutsrishvili G, Abdaladze O. 2017. Vegetation of the Central Great Caucasus along W-E and N-S transects. In: *Plant Diversity in the Central Great Caucasus: A Quantitative Assessment*. Geobotany Studies (Nakhutsrishvili G, Abdaladze O, Batsatsashvili K, Spehn E, Körner C, eds). Springer, Cham, pp 11-16.
- Nakhutsrishvili G, Zazanashvili N, Batsatsashvili K, Montalvo Mancheno CS. 2015. Colchic and Hyrcanian forests of the Caucasus: similarities, differences and conservation status. *Flora Mediterr* 25(Special Issue): 185-192.
- Pellizzari E, Pividori M, Carrer M. 2014. Winter precipitation effect in a mid-latitude temperature-limited environment: the case of common juniper at high elevation in the Alps. *Environ Res Lett* 9: 104021.
- Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC. 2013. New handbook for standardised measurement of plant functional traits worldwide. *Aust J Bot* 61: 167-234.
- Pfennigwerth AA, Bailey JK, Schweitzer JA. 2017. Trait variation along elevation gradients in a dominant woody shrub is population-specific and driven by plasticity. *AoB Plants* 9: plx027.
- Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol* 182: 565-588.
- Pratt JD, Mooney KA. 2013. Clinal adaptation and adaptive plasticity in *Artemisia californica*: implications for the response of a foundation species to predicted climate change. *Glob Chang Biol* 19: 2454-2466.
- Pyakurel A, Wang JR. 2013. Leaf morphological variation among paper birch (*Betula papyrifera* Marsh.) genotypes across Canada. *Open J Ecol* 3: 284-295.
- Rodríguez-Gómez F, Oyama K, Ochoa-Orozco M, Mendoza-Cuenca L, Gaytán-Legaria R, González-Rodríguez A. 2018. Phylogeography and climate-associated morphological variation in the endemic white oak *Quercus deserticola* (Fagaceae) along the Trans-Mexican Volcanic Belt. *Botany* 96: 121-133.
- Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. 2017. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18: 529.
- Souza ML, Duarte AA, Lovato MB, Fagundes M, Valladares F, Lemos-Filho JP. 2018. Climatic factors shaping intraspecific leaf trait variation of a neotropical tree along a rainfall gradient. *PLoS One* 13: e0208512.
- Tonin R, Gerdol R, Wellstein C. 2020. Intraspecific functional differences of subalpine plant species growing in low-altitude microrefugia and high-altitude habitats. *Plant Ecol* 221: 155-166.
- Uribe-Salas D, Sáenz-Romero C, González-Rodríguez A, Téllez-Valdéz O, Oyama K. 2008. Foliar morphological variation in the white oak *Quercus rugosa* Née (Fagaceae) along a latitudinal gradient in Mexico: Potential implications for management and conservation. *For Ecol Manag* 256: 2121-2126.
- Villar R, Marañón T, Quero JL, Panadero P, Arenas F, Lambers H. 2005. Variation in relative growth rate of 20 *Aegilops* species (Poaceae) in the field: The importance of net assimilation rate or specific leaf area depends on the time scale. *Plant Soil* 272: 11-27.
- Wellstein C, Chelli S, Campetella G, Bartha S, Galìè M, Spada F, Canullo R. 2013. Intraspecific phenotypic variability of plant functional traits in contrasting mountain grasslands habitats. *Biodivers Conserv* 22: 2353-2374.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. 2002. Plant Ecological Strategies: Some Leading Dimensions of Variation between Species. *Annu Rev Ecol Syst* 33: 125-159.
- Wright IJ, Dong N, Maire V, Prentice IC, Westoby M, Díaz S, Gallagher RV, Jacobs BF, Kooyman R, Law EA, Leishman MR, Niinemets Ü, Reich PB, Sack L, Villar R, Wang H, Wilf P. 2017. Global climatic drivers of leaf size. *Science* 357: 917-921.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf econom-

- ics spectrum. *Nature* 428: 821-827.
- Yang J, Zhang G, Ci X, Swenson NG, Cao M, Sha L, Li J, Baskin CC, Slik JF, Lin L. 2014. Functional and phylogenetic assembly in a Chinese tropical tree community across size classes, spatial scales and habitats. *Funct Ecol* 28: 520-529.
- Zhang X, He X, Gao J, Wang L. 2019. Latitudinal and climate effects on key plant traits in Chinese forest ecosystems. *Glob Ecol Conserv* 17: e00527.