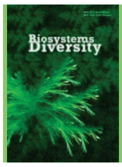

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Seasonal and environmental variability in leaf anatomical traits of *Pistacia lentiscus* among different sites in Northwestern Algeria

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Leaf anatomical traits such as cuticle, epidermis, palisade parenchyma, and total leaf thickness play a critical role in plant adaptation to changing environmental conditions. Mediterranean plants, including sclerophyllous species, have evolved these traits to thrive under these fluctuating conditions. The present study investigates the anatomical variations in *Pistacia lentiscus* L. leaves in response to environmental factors in Northwestern Algeria. Through a comparative analysis across multiple sites and seasons, we evaluated the influence of climatic and edaphic conditions on leaf structural thickness, particularly the cuticle, epidermis, and palisade parenchyma. The results indicate that spatial variability is mainly reflected in changes in cuticle and palisade parenchyma thickness, while seasonal variability primarily affects the epidermis and foliar parenchyma. The highest values were recorded in Bouhriz Forest, the most arid site, during winter, while the highest values in summer were observed in Tessala Mountains, the site with the highest altitude. However, the values in Beni Saf Forest were moderate, followed by the lowest values in M'Sila Forest. These anatomical adjustments highlight the adaptive capacity of *P. lentiscus* to the environmental constraints of the Mediterranean climate. This study underscores the importance of abiotic factors in shaping leaf traits and contributes to a better understanding of the adaptive mechanisms of sclerophyllous species in response to climatic and edaphic variations.

Keywords: adaptation; leaf anatomy; seasonal and spatial variation; soil; Mediterranean climate; Algeria.

Introduction

Mediterranean ecosystems, characterized by arid and semi-arid climates, are highly vulnerable to environmental changes (Miranda et al., 2011). These ecosystems experience two major seasonal stresses: summer drought and winter cold, which shape the composition and structure of their vegetation. In response to these constraints, Mediterranean plant species have developed a wide range of morphological, anatomical, and physiological adaptations (Sardans & Peñuelas, 2013). However, climate change, combined with increasing anthropogenic pressures, threatens to alter these ecological balances, leading to potential shifts in plant distribution and functional traits (Giorgi & Lionello, 2008). Understanding the adaptive strategies of plant species is therefore essential to predicting their responses to environmental stressors and assessing the resilience of Mediterranean ecosystems.

Among these species, *Pistacia lentiscus* L. (Anacardiaceae) is a dioecious evergreen shrub or small tree that thrives in Mediterranean maquis and garrigue ecosystems; it grows on different soil types in the sub-humid and semi-arid regions of Algeria (Alyafi, 1979; Saadoun, 2005). This species is subjected to marked summer drought conditions and significant altitudinal variations in its range (Boudy, 1950). This long-lived species retains its leaves during winter, with individual leaves persisting for up to two years (Ain-Lhout et al., 2004). Despite its ecological importance, *P. lentiscus* remains insufficiently studied in certain regions, particularly in Northwestern Algeria, where detailed anatomical and ecological investigations are lacking. One of the main objectives of this study is to investigate and describe the anatomical traits of *P. lentiscus* leaves in Northwestern Algeria and assess their adaptive potential in response to local environmental conditions.

Leaves, as the primary organs of photosynthesis and transpiration, are directly exposed to environmental stress factors (Nevo et al., 2000; Tian et al., 2016; Haffani et al., 2017). Their anatomical structure exhibits considerable plasticity, allowing plants to optimize resource use and mitigate stress-induced damage (Tian et al., 2016; Haffani et al., 2017). Periodic droughts are characteristic of Mediterranean ecosystems (Correia & Barradas, 1998). Previous research has demonstrated that drought stress intensifies traits such as cuticle thickness, palisade parenchyma development, and overall leaf thickness. (Guerfel et al., 2009; Ait Said et al., 2011; Haffani et al., 2017). Additionally, high irradiation levels may also drive morphological adjustments, such as increased leaf thickness and denser palisade layers, to optimize photosynthetic efficiency while reducing damage from intense light (Filella et al., 1999; Vally et al., 2002; Tiantian et al., 2023). These environmental factors play a crucial role in the observed spatial variability of leaf traits. These responses reflect adaptations to both water stress and high irradiance, which are particularly important factors in a Mediterranean environment. This study focuses on seven key leaf anatomical traits: palisade parenchyma thickness (critical for light capture and photosynthesis), spongy parenchyma thickness (influencing gas exchange), adaxial and abaxial cuticle thickness (regulating water loss), and adaxial and abaxial epidermal thickness (providing structural protection). These traits were selected based on their functional relevance in plant adaptation.

This research aims to assess the influence of environmental factors, including climatic and edaphic variables, on the anatomical variation of *P. lentiscus* leaves across different sites and seasons in North-Western Algeria. By investigating these traits in relation to temperature, precipitation, altitude, and soil-related environmental factors such as soil texture, pH, and carbonate content, this study seeks to enhance our understanding of the complex interactions between plant structure and environmental conditions. Ultimately, this work contributes to a broader understanding of Mediterranean plant adaptations and provides valuable insights into the potential responses of *P. lentiscus* to ongoing climatic and environmental changes.

Materials and methods

The study area is located in Northwest Algeria (Fig. 1). The selection of *P. lentiscus* populations was based on a northwest-southwest transect, encompassing variations in altitude and aridity. Four sites with distinct bioclimatic and edaphic conditions were selected: two coastal sites, M'sila Forest (MF, in Oran province) and Beni Saf Forest (BnF, in Ain Temouchent province), and two continental sites, Tessala Mountains (TMs, in the north of Sidi Bel Abbes province) and Bouhriz Forest (BF, in the south of Sidi Bel Abbes province). The geographical and ecological characteristics of the studied environment are detailed in Table 1.

These sites are under a semi-arid climate (Table 1), with varying degrees of aridity based on the De-Martonne Aridity Index (AIDM) (AI = P / (T °C + 10). According to De Martonne (1926), aridity increases when the index value decreases. Among the four sites, BF exhibited the highest aridity (AI = 12.07 mm/°C). BnF showed slightly lower aridity (AI = 12.58 mm/°C). TMs (AI = 13.46 mm/°C) and MF (AI = 14.43 mm/°C) experienced relatively less arid conditions.

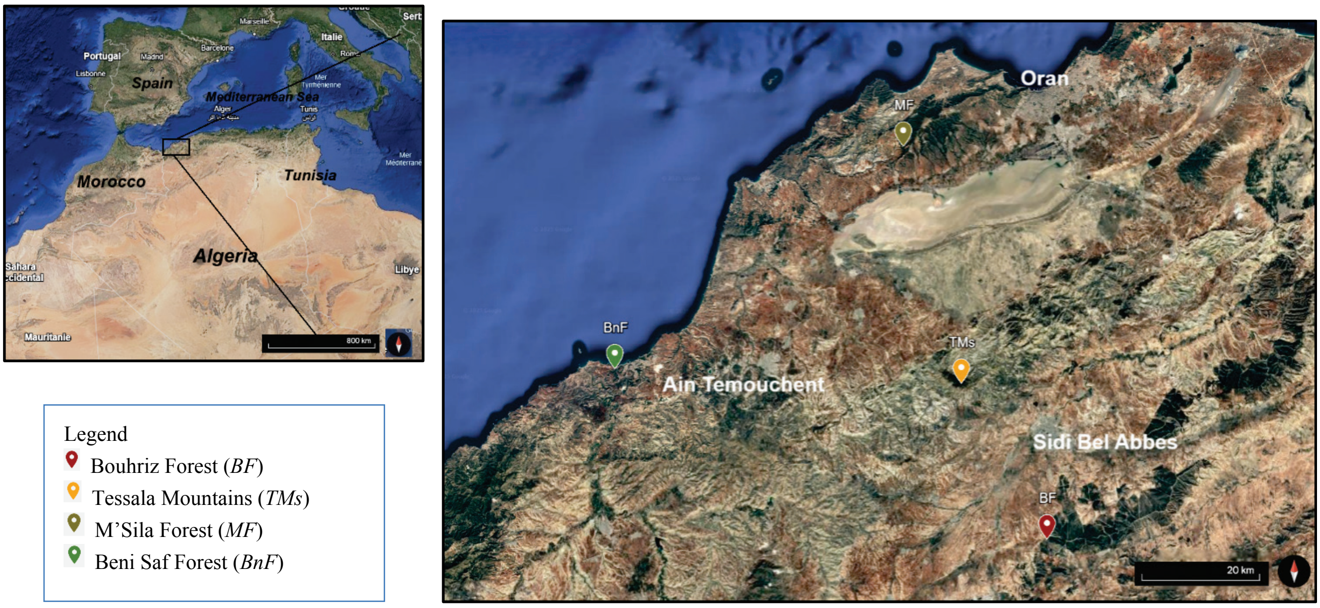

Fig. 1. Location of 4 sampling sites of *Pistacia lentiscus* L. investigated in Northwestern Algeria

Table 1

Geographical and Ecological factors of the *Pistacia lentiscus* L. collection sites

Site	Latitude	Altitude, m	T M, °C	T m, °C	P, mm/y	Emberger Q_2	Bioclimatic stage
Bouhriz Forest *BF*	35°02'42’’North 0°37’ 27’’ West	690	37.42	3.20	336.72	33.54	Inferior Semi-arid to temperate winter
Tessala Mountains *TMs*	35°16'24’’North 0°47’ 5’’ West	952	35.30	2.89	363.93	38.22	Inferior Semi-arid to fresh winter
M’sila Forest *MF*	35°37'30’’North 0°53’ 17’’ West	352	30.00	6.70	400.00	58.70	Superior Semi-arid to mild winter
Beni Saf Forest *BnF*	35°17'36’’North 1°24’ 55’’ West	55	27.75	9.12	354.40	65.24	Superior Semi-arid to hot winter

Note: P – mean annual precipitations (mm); T – mean annual temperature (°C); TM – mean maximum temperature of the warmest month (°C); Tm – mean minimum temperature of the coldest month (°C) (ONM, 2016); emberger Q_2 – the emberger aridity index.

Pistacia lentiscus, commonly known as mastic or lentisk, is an evergreen sclerophyllous shrub. As a thermophilic species, it typically grows between 1 and 5 meters in height. It is distinguished by its paripinnately compound leaves and its strongly acrid resinous odor (Alyafi, 1979).

In order to examine the leaf anatomical traits of these species, mature leaflets were randomly collected from the elderly subjects during the winter season and repeated in the summer of the same year.

At each sampling site, four trees were selected. Then, from the shoots of the current year, three to five mature leaflets at a height of one meter were gathered per individual. Sampling was performed in February and August 2019. A total of 122 leaflet samples from 16 trees were examined. These leaflets were used to study seven leaf anatomical traits.

Samples were cut into half and fixed in situ in FAA (3.7% v/v formaldehyde, 50% ethanol, 5% acetic acid), immediately transported to the laboratory, and stored overnight in the refrigerator at 4 °C.

In the laboratory, the pieces from the middle of the leaflets were dehydrated through a growing series of tert-butanol solutions (50%, 70%, 95%, 100%, and 2x pure tert-butanol, 1 H per bath). These samples were then impregnated in Paraplast at 60 °C for 36 hours (four changes) to form blocks. Cross-sections of 8 µm were cut using a microtome (LECA Biosystems RM2245) and applied to the slides with albumin. After dewaxing in xylene, the sections were rehydrated through a series of ethanol baths with decreasing concentrations (100%, 95%, 70%, 50%), rinsed, and dried overnight in an oven at 37 °C (Mahroug et al., 2006). The prepared cross-sections were stained with Mayer’s hematoxylin-eosin and were dried both in two absolute ethanol baths and then followed by two xylene baths. The slides were permanently mounted with Canada Balsam and were observed under an optical microscope (Axio Scope A1, Carl Zeiss, Germany).

Pistacia lentiscus anatomical leaf traits were measured as leaf thickness (LTh, µm), adaxial cuticle thickness (ADC, µm), abaxial cuticle thickness (ABC, µm), adaxial epidermis thickness (ADE, µm), abaxial epidermis thickness (ABE, µm), palisade parenchyma thickness (PPT, µm), and spongy parenchyma thickness (SPT, µm). The software Motic Images Plus v. 2.0 ML, (Motic China Group Co., Ltd) was used for measuring the anatomical traits (Fig. 2).

Twelve environmental factors were selected for the four sites, including four climatic indicators, altitude (AL), and seven soil indicators. Climatic data were obtained from the National Office of Meteorology (ONM, 2016). Two main climatic parameters were collected: precipitations and temperature. The pluviothermal quotient was determined ($Q_2 = 2000\ P / M^2–m^2$) according to Emberger (1952), where P is the mean annual precipitations (mm); M – mean maximum temperature of the warmest month (°C); and m – mean minimum temperature of the coldest month (°C) (Table 1).

Soil samples were collected in February 2019. At each site, four rhizospheric soil samples of *P. lentiscus* were taken at a depth of 0–

30 cm. In the laboratory, soil samples were air-dried and sieved using a 2 mm-diameter mesh screen. Physico-chemical analyses of the soil were performed. Soil moisture (SM) was measured using the gravimetric method. The determination of mineral fractions was carried out using the Robinson pipette method after the destruction of organic matter with hydrogen peroxide (Gee & Or, 2002). Soil pH was measured using a suspension of soil water at a ratio of 1:2.5 (w/v) with a pH meter (Hanna Instruments, Netherlands). Electrical conductivity (EC, mS/cm) was determined using a conductivity meter (Hanna Instruments, The Netherlands). Soil organic carbon (SOC) was measured using the Walkley and Black titrimetric method (Nelson & Sommers, 1996) to calculate soil organic matter content (SOM). Total limestone (CaT) was determined by Bernard's calcimeter method using HCl acid, and the active limestone determination (CaA) was done by the Drouineau-Galet method (1942).

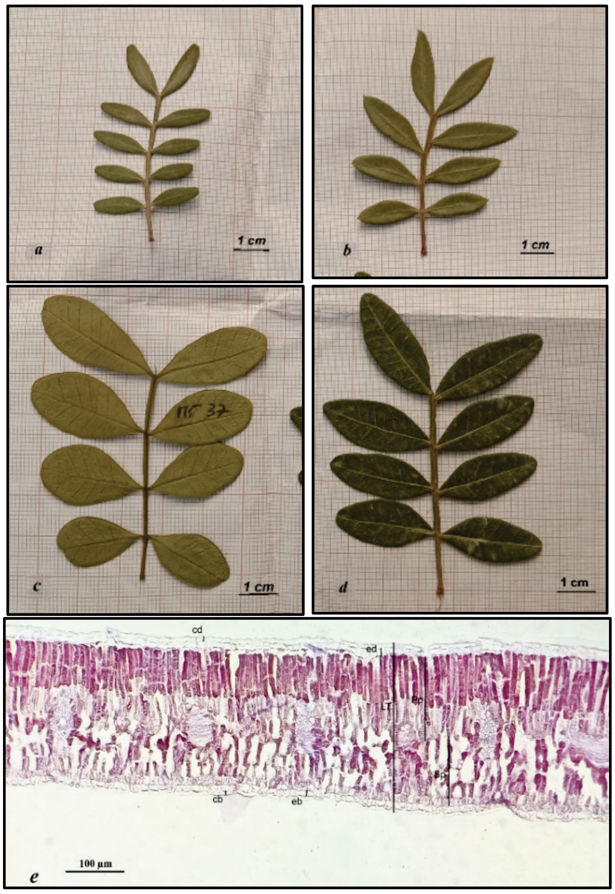

Fig. 2. Cross-section of *Pistacia lentiscus* leaf and leaves from four study sites: *a* – leaf from Bouhriz Forest site; *b* – leaf from Tessala Mountains site; *c* – leaf from M'Sila Forest site; *d* – leaf from Beni Saf Forest site; *e* – cross-section of *P. lentiscus* leaf showing measured anatomical traits: *cd* – adaxial cuticle (ADC); *cb* – abaxial cuticle (ABC); *ed* – adaxial epidermis (ADE); *eb* – abaxial epidermis (ABE); *pp* – palisade parenchyma (PPT); *sp* – spongy parenchyma (SPT); *lt* – leaf thickness (LTh)

A comprehensive statistical analysis was conducted to investigate the variations in seven measured parameters among the four populations of *P. lentiscus* across two seasons (winter and summer). The analysis included descriptive statistics, normality tests, one-way ANOVAs followed by Tukey's HSD when variances were equal, or Dunnett's T3 test otherwise (to compare habitat effects), two-way ANOVAs (with η^2, Partial eta squared: 0.0099 (small effect size), 0.0588 (medium), and 0.1379 (large) (Fritz et al., 2012)), correlation tests (Spearman), and principal component analysis (PCA). We retained statistically significant levels at $P < 0.05$. Additionally, correlation analyses and PCA provided insights into the relationships between the measured parameters and the environmental variables (Q_2, TM, Tm, P, altitude, edaphic parameters). Analyses were performed with SPSS 22.0. Correlation analysis was carried out using the corrplot package (Taiyun & Viliam, 2024). Overall, the statistical findings contribute to a better understanding of the factors influencing the leaf anatomical traits in four populations.

Results

Some of the studied edaphic factors also varied obviously among the four *P. lentiscus* populations. Soil texture was silty clay for Bouhriz, clay loam for Tessala, silty clay loam for Beni Saf, and loam for M'sila (Ranking of the soil texture classification used in SPSS: 1 – loam, 2 – silty clay loam, 3 – clay loam, 4 – silty clay). Soil pH was lowest in Tessala (4.67–5.60) and highest in Bouhriz (5.28–6.55). Electrical conductivity (EC) varied among the sites, with the lowest values recorded in Bouhriz (0.01–0.14 mS/cm) and the highest in Tessala (0.17–0.36 mS/cm). The calcium carbonate content also showed marked differences. Total limestone (CaT) ranged widely, with the highest maximum value observed in Bouhriz (56.00%) and the lowest in Tessala (0.00–41.33%). Active limestone (CaA) was particularly high in Bouhriz (up to 71.00%), whereas the lowest values were recorded in Tessala (0.00–53.00%). Soil organic matter (SOM) and soil organic carbon (SOC) also displayed notable variation. The highest SOM values were found in Tessala (up to 5.04%), while Beni Saf exhibited the lowest (0.08–5.15%). Similarly, SOC ranged from 0.04% in Beni Saf to 2.52% in Tessala, suggesting variations in soil fertility across sites.

This heterogeneity in soil properties reflects the environmental variability between the studied habitats, which may influence the anatomical traits and adaptation strategies of *P. lentiscus*.

The anatomical traits of *P. lentiscus* leaves showed variability among Bouhriz Forest (BF), Tessala Mountains (TMs), M'Sila Forest (MF), and Beni Saf Forest (BnF) in the two seasons. The measurements for ADC, ABC, ADE, ABE, PPT, SPT, and LT ranged from 3.3 to 20.3 µm, 3.0 to 14.8 µm, 5.7 to 24.7 µm, 6.9 to 23.0 µm, 51.7 to 202.3 µm, 70.4 to 296.9 µm, and 112.4 to 460.9 µm, respectively (Table 2).

Significant differences ($P < 0.05$) were observed between sampling sites for most leaf parameters in both winter and summer.

In winter, statistical analyses (Table 3) revealed significant differences between sites for four anatomical traits (i.e., ADC, ABC, PPT, and LT; $P < 0.001$), except for ADE, ABE, and SPT. The highest averages for ADC (11.1 ± 1.8 µm), PPT (161.9 ± 24.7 µm), and LT (381.1 ± 58.5 µm) were recorded in Bouhriz Forest, while Tessala Mountains had the highest ABC thickness (10.2 ± 2.6 µm). The lowest average for ADC (7.5 ± 1.9 µm), ABC (7.0 ± 1.6 µm), PPT (88.1 ± 29.3 µm), LT (273.9 ± 53.0 µm), and all other traits were found in M'Sila Forest. Dunnett's T3 post-hoc test revealed statistically significant differences between two population groups for ABC and between three distinct groups for PPT. For ADC and LT, Tukey's test showed two homogeneous groups (Table 2; Fig. 3).

In summer, the ANOVA (Table 3) showed significant differences between the four populations of *P. lentiscus* for ADC, ABC, ABE, and LTh ($P < 0.001$), as well as for PPT and SPT ($P < 0.01$). The Bouhriz forest had the highest average for ADC (14.3 ± 3.4 µm) and ABC (10.2 ± 2.4 µm), followed by Tessala Mountains, then Beni Saf Forest, while M'Sila Forest had the lowest average. For PPT and LTh, Tessala Mountains had the highest average (112.8 ± 20.9 µm and 346.0 ± 31.6 µm, respectively), followed by Bouhriz Forest, Beni Saf Forest, and M'Sila Forest. With regard to SPT, Tessala Mountains also recorded the highest mean thickness (177.9 ± 22.7 µm), followed by Bouhriz Forest (163.8 ± 46.7 µm), M'Sila Forest (136.4 ± 18.7 µm), and Beni Saf Forest (133.5 ± 58.9 µm).

In contrast to winter, the anatomical traits ABE ($P < 0.001$) and SPT $P < 0.01$) showed significant differences between sites in the summer season. These differences highlight the influence of seasonality on morphological diversity within populations. Regarding ABE, Tessala Mountains had the highest average (15.3 ± 2.7 µm), followed by M'Sila Forest, Beni Saf Forest, and Bouhriz Forest. Dunnett's T3 post-hoc test indicated three homogeneous groups for ADC, ABC, and LT, while two groups were identified for SPT. Tukey's post-hoc test revealed two groups for PPT and ABE (Table 2; Fig. 3).

Table 2
Anatomical traits of *Pistacia lentiscus* leaves from different sampling sites in North-Western Algeria in winter and summer (mean ± SE, min–max, μm)

Sites		ADC	ABC	ADE	ABE	PPT	SPT	LTh
BF	W	11.09 ± 0.49^{a}	9.24 ± 0.36^{a}	12.57 ± 1.09	13.99 ± 1.09	161.96 ± 6.61^{a}	170.80 ± 8.27	381.08 ± 15.63^{a}
	n = 14	8.10–14.90	8.10–12.40	5.70–17.60	9.00–23.00	114.60–201.10	88.90–207.60	242.70–458.30
	Su	14.32 ± 0.89^{a}	10.19 ± 0.63^{ab}	14.50 ± 0.98	11.04 ± 0.42^{b}	101.39 ± 4.02^{ab}	163.80 ± 12.06^{ab}	315.11 ± 13.38^{ab}
	n = 15	9.50–20.30	5.40–13.30	9.50–22.10	9.00–15.40	81.00–126.30	102.80–259.50	244.30–433.10
TMs	W	10.68 ± 0.67^{a}	10.22 ± 0.64^{a}	13.26 ± 0.68	12.46 ± 0.54	130.36 ± 10.15^{ab}	166.36 ± 8.43	343.46 ± 16.27^{a}
	n = 16	7.30–16.20	5.70–14.80	8.20–17.00	8.20–16.40	73.30–202.30	92.20–231.90	202.40–425.90
	Su	12.87 ± 0.38^{ab}	10.14 ± 0.55^{a}	14.24 ± 0.51	15.32 ± 0.72^{a}	112.76 ± 5.58^{a}	177.86 ± 0.06^{a}	346.00 ± 8.45^{a}
	n = 14	9.40–15.40	6–13.90	11.50–18.00	10.90–21.10	69.10–145.00	136.10–202.70	264.50–379.30
MF	W	7.52 ± 0.51^{b}	6.98 ± 0.42^{b}	11.16 ± 0.53	11.12 ± 0.79	88.10 ± 7.82^{c}	147.63 ± 7.69	273.94 ± 14.15^{b}
	n = 14	5.40–12.10	4.90–9.80	7.90–13.70	6.90–14.90	51.70–141.90	103.60–223.70	214.50–374.70
	Su	5.50 ± 0.36^{c}	4.54 ± 0.25^{c}	14.88 ± 0.81	12.69 ± 0.48^{b}	86.86 ± 4.61^{b}	136.36 ± 4.53^{b}	264.30 ± 7.31^{c}
	n = 17	3.30–8.10	3–6.70	11.30–24.70	9.20–16.50	61.10–124.50	102.70–164.90	215.20–314.50
BnF	W	9.88 ± 0.56^{a}	8.62 ± 0.52^{ab}	11.52 ± 0.75	11.56 ± 0.58	130.08 ± 6.84^{b}	162.98 ± 8.42	337.88 ± 13.43^{a}
	n = 17	5.60–13.50	5.60–12.10	8.10–21.10	8.10–16.90	59.70–194.10	89.00–222.40	235.90–412.40
	Su	10.48 ± 0.79^{b}	7.94 ± 0.51^{b}	13.02 ± 0.51	11.09 ± 0.41^{b}	95.68 ± 5.96^{ab}	133.46 ± 15.20^{ab}	271.11 ± 21.73^{bc}
	n = 15	5.60–13.50	5.40–12.20	9.80–18.90	8.20–13.50	54.90–148.30	70.40–296.90	112.40–460.90

Notes: ^{a}, ^{b}, ^{c} – separation of population groups by Tukey's test and Dunnett's T3 post hoc test (one-way ANOVA; P <0.05); different letters indicate significant differences between sites of each season (W – winter; Su – summer); ADC – adaxial cuticle thickness; ABC – abaxial cuticle thickness; ADE – adaxial epidermis thickness; ABE – abaxial epidermis thickness; PPT – palisade parenchyma thickness; SPT – spongy parenchyma thickness; LTh – leaf thickness; BF – Bouhriz Forest; TMs – Tessala Mountain; MF – M'Sila Forest; BnF – Beni Saf Forest.

Table 3
ANOVA results of leaf anatomical traits of *Pistacia lentiscus* L. in North-Western Algeria

Sources of variation	Df		ADC	ABC	ADE	ABE	PPT	SPT	LTh
One-Way ANOVA									
Site (winter)	3	P	0.000	0.001	0.220	0.057	0.000	0.261	0.000
Site (summer)	3	P	0.000	0.000	0.326	0.000	0.006	0.008	0.000
Two-Way ANOVA									
Site	3	P	0.000	0.000	0.220	0.001	0.000	0.003	0.000
		η^2	0.51	0.45	0.04	0.14	0.29	0.11	0.26
Season	1	P	0.022	0.118	0.000	0.609	0.000	0.172	0.001
		η^2	0.05	0.02	0.11	0.00	0.24	0.02	0.09
Site*Season	3	P	0.000	0.010	0.308	0.000	0.000	0.182	0.025
		η^2	0.16	0.09	0.03	0.16	0.15	0.04	0.08

Notes: P-values in the bold indicate significant effects (P < 0.05); effect size of factors η^2, Partial eta squared: 0.0099 (small effect size), 0.0588 (medium), and 0.1379 (large) (Fritz et al., 2012); sites per season (one-way ANOVA) and sites x seasons (two-way ANOVA).

The two-way ANOVA (Table 3) showed a significant main effect of the season (P < 0.05) for PPT, ADE, LTh, and ADC, indicating overall differences in these parameters between winter and summer (Fig. 3). However, the effect size for the season was generally smaller than that for habitat.

The one-way ANOVA (Table 4) showed significant differences between winter and summer for PPT, ADE, and LTh (P < 0.001). In contrast, the other traits ADC, ABC, ABE, and SPT showed no significant differences. However, the two-way ANOVA (Table 3, Season) showed a difference for ADC (P = 0.022), but it was less pronounced than for PPT, ADE, and LTh. This underlines the fact that the climatic characteristics of the seasons vary from site to site, leading to different impacts.

Figure 3 demonstrates that anatomical trait variations differed between winter and summer across all study sites. Traits such as ADC, ABC, ABE, SPT, and LTh had higher summer averages at some sites and lower at others, while ADE consistently showed higher summer averages at all sites. In contrast, PPT consistently had higher winter averages across all sites.

A significant interaction between the habitat and the season was found for several parameters (Table 3): for ADC, ABE, and PPT (P < 0.001), as well as for ABC and LTh (P < 0.05). This indicates that the effect of season on leaf parameters may vary depending on the specific habitat. The results of the two-way ANOVA (Table 3) showed that the site factor had a highly significant effect on ADC (P < 0.001; $\eta^2 = 0.51$) and ABC (P < 0.001, $\eta^2 = 0.45$), as well as PPT (P < 0.001, $\eta^2 = 0.29$), LTh (P < 0.001, $\eta^2 = 0.26$), and ABE (P ≤ 0.001, $\eta^2 = 0.14$); therefore, the differences between sites explain a large proportion of the variance in these variables. For the season factor, the differences are highly significant for PPT ($\eta^2 = 0.24$) and ADE ($\eta^2 = 0.11$), suggesting that these variables are particularly sensitive to seasonal variation, although the effect of season is generally less pronounced than that of site.

In this study, significant correlations were observed between the leaf anatomical traits (Fig. 4a, 4b). However, the correlations between some traits were stronger in summer than in winter, such as between ADC and ABC (r = 0.90). In winter, PPT was highly significantly correlated with LTh, SPT, and ADC. Additionally, the LTH showed strong correlations with SPT, ADC, and ABC in both winter and summer. This indicates that the interrelations among the leaf anatomical traits of the four *P. lentiscus* populations studied were differently affected by environmental factors in both winter and summer.

Table 4
The one-way ANOVA for leaf anatomical traits of *Pistacia lentiscus* L. in North-Western Algeria between two seasons, winter and summer

Sources of variation	Df	ADC	ABC	ADE	ABE	PPT	SPT	LTh
Season	1	0.234	0.122	0.000	0.667	0.000	0.143	0.002

Note: P-values in the bold indicate significant effects (P < 0.05).

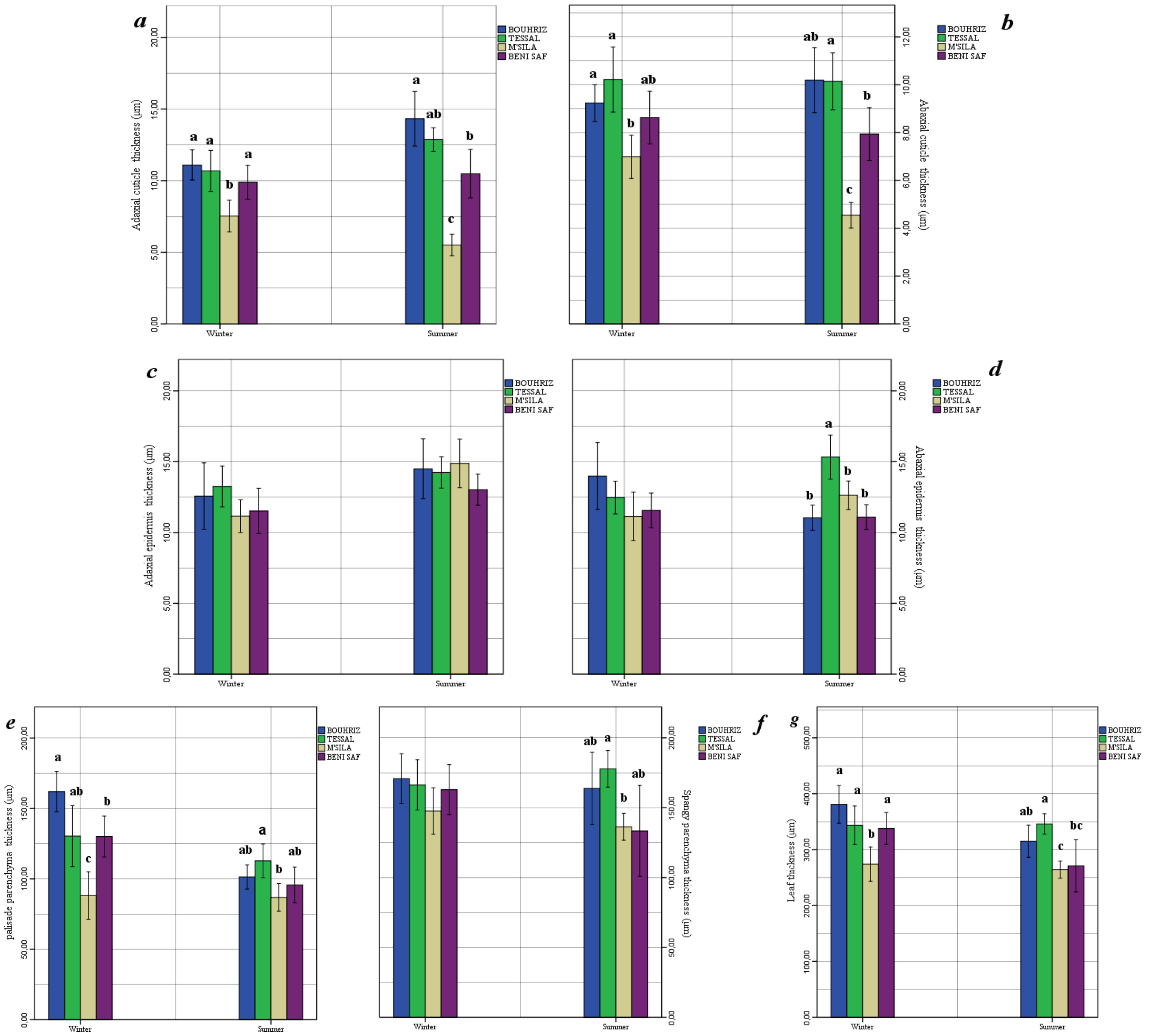

Fig. 3. Differences in leaf anatomical traits among the four populations of *Pistacia lentiscus* L. in winter and summer: data are presented as mean ± SE; different letters indicate significant differences between sites (P <0.05, ANOVA)

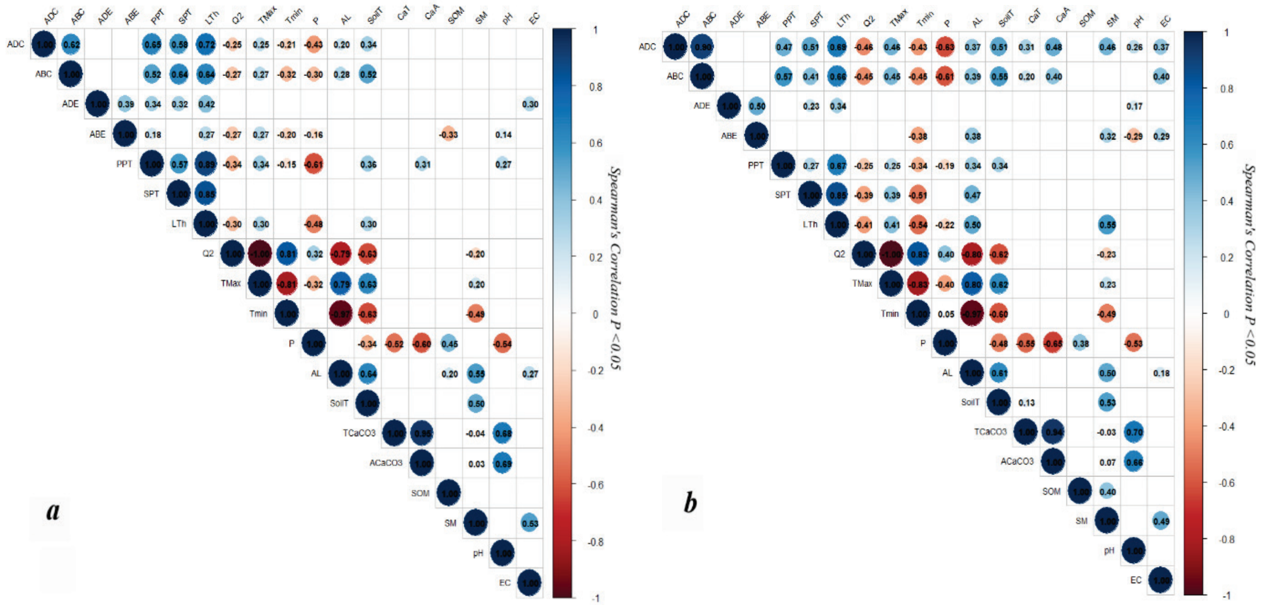

Fig. 4. Spearman's correlation coefficients (P < 0.5) of leaf anatomical traits and environmental factors across two seasons (winter – *a*, summer – *b*) in North-Western Algeria: ADC – adaxial cuticle thickness; ABC – abaxial cuticle thickness; ADE – adaxial epidermis thickness; ABE – abaxial epidermis thickness; PPT – palisade parenchyma thickness; SPT – spongy parenchyma thickness; LTh – leaf thickness; Q2 – emberger Q2; TMax – maximum temperature; Tmin – minimum temperature; P – precipitation; AL – altitude; SoilT – soil texture; pH – potential of hydrogen; SM – soil moisture; EC – electrical conductivity; CaT – total limestone; CaA – active limestone; SOC – soil organic carbon

Significant correlations ($P < 0.05$) were observed between leaf traits and environmental factors, exhibiting disparate correlation patterns in both winter and summer (Fig. 4). The interrelations observed for the majority of parameters were more pronounced in the summer season. Specifically, the ADC and ABC demonstrated a strong positive correlation with TM, AL, and the soil-related environmental parameters (i.e., SoilT, CaA, SM, and EC) and a negative correlation with mean annual precipitations (P), Q2, and Tm. Additionally, LTh and SPT showed significant correlations with AL, Tm, TM, and Q2, whereas PPT was significantly correlated with AL and soil texture as well as negatively correlated with Tm (Fig. 4b).

In winter, measured anatomical traits showed weak correlations with environmental factors, except for PPT and LTh, which were strongly correlated with mean annual precipitations (Fig. 4a). Additionally, ADC was negatively correlated with mean annual precipitations, while ABC was positively correlated with soil texture.

The four populations of *P. lentiscus* studied for the seven leaf anatomical traits in both seasons were the subject of a principal component analysis (PCA) (Fig. 5). The PCA analysis of the winter data (Fig. 5a, 5c) revealed two principal components (PCs), PC1 accounting for 30.8% of the total variance and PC2 accounting for 15.8%, resulting in a cumulative variance of 46.6%. PC1 was significantly related to LTh, PPT, ADC, ABC, and SPT for anatomical traits and to Q2, AL, SoilT, and mean annual precipitations (P) for environmental factors; it represents adaptive anatomical traits and the influencing environmental factors. In contrast, PC2 was positively correlated with CaA, CaT, and pH but inversely correlated with mean annual precipitations; it represents the factors that less influenced anatomical traits. The PCs clearly showed four populations occupying different dials of the PC space, which separated the populations into four clusters: BF, TMs, MF, and BnF, with the BF site showing higher LTH, PPT, and ADC, while MF recorded the lowest anatomical traits (Fig. 5c). ANOVA confirmed significant differences between the populations from different sites.

The summer results were primarily interpreted through the first two principal components (Fig. 5b, 5d), explaining a total variance of 51.4% (33.8% for PC1 and 17.6% for PC2). PC1 showed significant correlations with LTh, ADC, ABC, SPT, and PPT, as well as environmental factors such as Q2, AL, and SoilT. PC2 was significantly related to ADC for anatomical traits and to CaA, CaT, and pH while showing an inverse relation to mean annual precipitations. Thus, PC1 reflects adaptive anatomical traits responding to challenging environmental factors, whereas PC2 represents soil characteristics that were more influential in summer, particularly on ADC. The scatter plot and biographic analysis indicated a clear separation of the populations into four distinct clusters (BF, TMs, MF, and BnF) based on the two principal components (Fig. 5d). The BF site exhibited higher values for ADC and ABC, while TMs showed elevated values for LTh, SPT, PPT, and ABE. The MF site reported the lowest values for all anatomical traits. ANOVA results confirmed significant differences between populations from different sites.

Comparing the two seasons, summer accounts for slightly more variance (51.4%) than winter (46.6%), indicating a greater environmental influence on leaf traits in summer. In both seasons, PC1 relates to anatomical traits associated with environmental factors, but soil factors such as CaA, CaT, and pH are more significant in the summer. The populations show a clearer separation in summer, with site BF showing consistently higher ADC and ABC values, indicating a better adaptation to summer conditions. In contrast, the MF site shows lower trait values in both seasons, especially in summer.

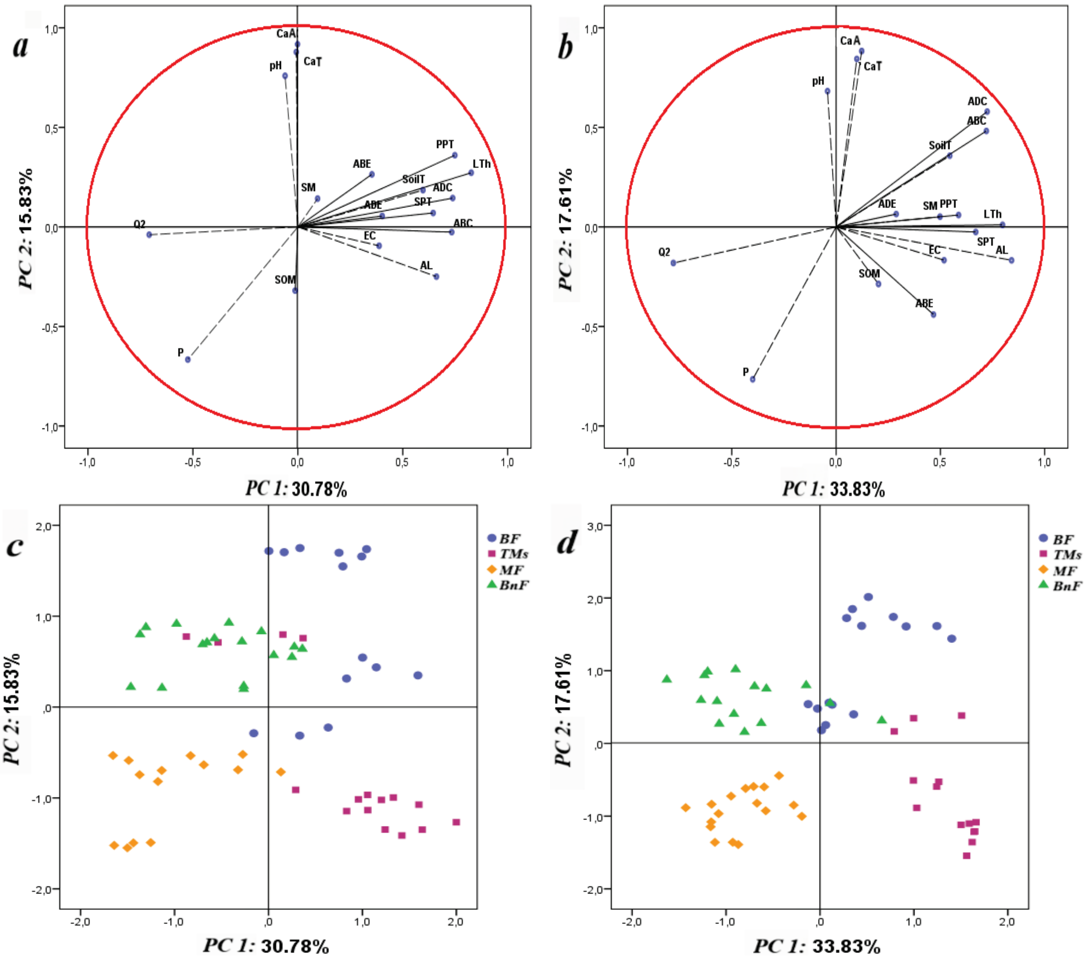

Fig. 5. Principal component analysis (PCA) graph, PCA winter (*a*, *c*) and PCA summer (*b*, *d*): BF – Bouhriz Forest; TMs – Tessala Mountains; MF – M'Sila Forest; BnF – Beni Saf Forest; ADC – adaxial cuticle thickness; ABC – abaxial cuticle thickness; ADE – adaxial epidermis thickness; ABE – abaxial epidermis thickness; PPT – palisade parenchyma thickness; SPT – spongy parenchyma thickness; LTh – leaf thickness; Q2 – emberger Q2; P – precipitation; AL – altitude; SoilT – soil texture; pH – potential of hydrogen; SM – soil moisture; EC – electrical conductivity; CaT – total limestone; CaA – active limestone; SOC – soil organic carbon

Discussion

Anatomical traits are among the principal indicators of plant adaptation to environmental conditions (De Mico & Aronne, 2012). The observed differences in leaf anatomical traits among the Bouhriz Forest (AI = 12.07 mm/°C; AL – 690 m), Tessala Mountains (AI = 13.46 mm/°C; AL – 952 m), Beni Saf Forest (AI = 12.58 mm/°C; AL – 55 m), and M'Sila Forest (AI = 14.43 mm/°C; AL – 352 m) sites can be attributed to the effect of aridity and altitude gradients on *P. lentiscus* and different levels of drought stress (Filella et al., 1999; Zandalinas et al., 2018; Doghbage et al., 2023; Iozia et al., 2023). The highest values were recorded in Bouhriz Forest, the most arid site, during winter, while the highest values in summer were observed in Tessala Mountains, the site with the highest altitude. However, the values in Beni Saf Forest were moderate, followed by the lowest values in M'Sila Forest. Previous studies have indicated that cuticle, epidermis, palisade parenchyma, and leaf thickness increase with the degree of aridity, enhancing water retention and providing greater protection for inner tissues in highly arid environments (Nevo et al., 2000; Ait Said et al., 2011; Haffani et al., 2017).

Our results showed significant variability in leaf traits among *P. lentiscus* populations in both winter and summer. The seasonal differences in trait expression, where only four anatomical traits (ADC, ABC, PPT, and LTh) vary in winter compared to six out of seven (ADC, ABC, ABE, LTh, PPT, and SPT) in summer, underline the dynamic nature of plant responses to environmental change. This variability is more pronounced in summer, reflecting broader ecological patterns of increased trait adaptability during warmer seasons when Mediterranean ecosystems experience greater environmental amplitude (Yaoqi, 2020; Weemstra et al., 2022). Such increased trait variability is likely to facilitate morphological adaptations that optimize resource use and resilience under variable environmental stresses.

In both seasons, cuticle thickness on adaxial and abaxial surfaces was highest in the BF population, followed by TMs and BnF, with the lowest thickness observed in MF. This increase in cuticle thickness is likely an adaptive response to drought, aimed at minimizing water loss and increasing drought resistance and protection against damaging radiation (Simões et al., 2023; Yavas et al., 2024). A similar trend is reported by Ait Said et al. (2011) in populations of *Pistacia atlantica* Desf. situated in highly arid regions. During the summer, the *P. lentiscus* population from Tessala Mountains (TMs, AL – 952 m) developed a thicker abaxial epidermis (ABE), which may aid in reducing exposure to intense light and UV-B radiation (Vally et al., 2002; Tiantian et al., 2023).

In both winter and summer, PPT, LTh, and SPT had similar patterns of variation among sites, with higher values recorded in Tessala Mountains (TMs) in the summer and Bouhriz Forest (BF) in the winter. In winter, increased thicknesses of PPT and LTh may support enhanced protection from cold temperatures and increased efficiency in photosynthesis, as leaves have evolved structures to maximize light absorption even under reduced winter light intensity (Niinemets, 2001). Increased LTh may also help reduce winter moisture loss, which is critical for maintaining cell function and frost resistance in cooler conditions (Ait Said, 2011; Sperlich et al., 2015). In summer, the increases in these anatomical traits at Tessala Mountains (TMs) may serve to withstand high irradiance and intense heat. According to Vally et al. (2002) and Tattini (2006), a thicker palisade parenchyma can enhance light capture efficiency, while increased leaf and spongy parenchyma thickness help protect against UV-B radiation and reduce water loss.

Significant seasonal variation was observed in PPT, ADE, LTh, and ABC, suggesting strong physiological responses to environmental factors such as temperature and light. Minimal variation in ADC suggests that it may play a limited role in adaptive responses to seasonal changes, highlighting that the influence of seasons on leaf parameters may vary according to specific habitat conditions (Bacelar et al., 2004; Guerfel et al., 2009). In winter, PPT and LTh were higher than in summer, probably reflecting adaptations for increased protection and photosynthetic efficiency under cooler conditions. Ait Said's (2011) study of the photosynthetic activity in *P. lentiscus* across seasons showed that this species is sensitive to low winter temperatures, highlighting the physiological adjustments necessary for seasonal adaptation. Conversely, the adaxial epidermis was thicker in summer, likely as a response to higher light intensity and UV-B radiation (Vally et al., 2002; Tiantian et al., 2023).

In line with Nevo et al. (2000), Tattini (2006) and Doghbage et al. (2020), our results indicate that *P. lentiscus* exhibits significant anatomical adaptations to environmental variability, particularly in leaf traits, highlighting its high degree of plasticity. Adaptation strategy to spatial variability of *P. lentiscus* was especially reflected in five traits: ADC (adaxial cuticle thickness), ABC (abaxial cuticle thickness), PPT (palisade parenchyma thickness), LTh (leaf thickness), and ABE (abaxial epidermis). This suggests that cuticle thickness and internal leaf structure are key responses for protecting leaves in potentially more arid conditions at specific sites. This aligns with the results of Fletcher et al. (2018), who observed that cuticle and foliar tissue thickness contribute to drought resistance in Mediterranean species. Such spatial plasticity likely allows *P. lentiscus* to maintain physiological function despite variations in water availability and light intensity across different sites (Tattini, 2006).

In contrast, adaptation strategy to seasonal variations is primarily expressed through the two traits: PPT (palisade parenchyma thickness) and ADE (adaxial epidermis). The thickness of the palisade parenchyma and epidermis appears to vary with the seasons, which may relate to adjustments to fluctuations in light and temperature between summer and winter. This finding aligns with previous studies, such as those by Sperlich et al. (2015), which showed that seasonal adjustments in leaf structure help optimize photosynthesis in the summer while minimizing water loss during drier months. These results support the idea that *P. lentiscus* adapts its leaf anatomical structure not only to drought conditions but also to the seasonal cycles typical of Mediterranean ecosystems.

The response of *P. lentiscus* to spatial and seasonal variations in this Mediterranean region is expressed through distinct adjustments in certain leaf traits, underscoring an adaptive strategy that enables this species to maintain resilience and survive in heterogeneous environments.

Environmental factors play a crucial role in driving the variation of adaptive anatomical traits in *P. lentiscus*, particularly during the summer. In Mediterranean regions, extreme conditions such as limited water availability (P), high temperatures (TM), and intense solar radiation (AL) impose considerable stress on plants, especially in the hotter months (Giorgi & Lionello, 2008). These climatic pressures prompt adaptations in leaf traits, such as the thickening of the adaxial (ADC) and abaxial (ABC) cuticles, which act as protective barriers to reduce water loss and limit evapotranspiration (Sperlich et al., 2015). This adaptation is particularly notable in Bouhriz Forest, where increased cuticle and leaf thickness suggest a response to higher drought stress, highlighting the plant's resilience under intense seasonal climatic demands.

Seasonal changes in temperature and light also influence traits such as the adaxial epidermis (ADE) and palisade parenchyma, optimizing water retention and photosynthetic efficiency under varying light intensities (Guerfel et al., 2009). These adaptations allow *P. lentiscus* to balance between maximizing photosynthesis in the summer and minimizing water loss during the drier months. Sperlich et al. (2015) demonstrated that seasonal adjustments in leaf structure are essential for optimizing photosynthesis in the summer while minimizing water loss, a pattern observed in the anatomical traits of *P. lentiscus* across different sites.

In addition to climatic and altitudinal influences, edaphic factors, particularly soil properties such as texture, pH, and calcium carbonate content (CaA and CaT), can play a fundamental role in shaping the leaf anatomy of *P. lentiscus*. High levels of CaT are associated with increased adaxial cuticle thickness, enhancing the plant's resilience to arid conditions (Niinemets, 2001; Bacelar et al., 2004). Floret and Pontanier (1982) reported that the silt-limestone combination improves the soil's water-holding capacity, enabling it to retain a reservoir of water during the dry season. At Bouhriz Forest (BF), where $CaCO_3$ and pH levels are higher and the soil texture is silty clay, more pronounced adaptations are observed, such as higher ADC and ABC values. These adaptations suggest that silty clay rich and high-$CaCO_3$ soils enhance the leaf's ability to withstand extreme summer conditions. In contrast, at sites such as MF, where $CaCO_3$ levels and pH are lower and the soil texture is silty clay loam, these resistance traits are less pronounced, potentially limiting the plant's capacity to adapt to seasonal stress.

Moreover, the correlation between soil texture and pH indicates that soil properties directly influence nutrient availability and uptake, which, in turn, affect leaf anatomy in site-specific ways. Alkaline soils rich in $CaCO_3$ support better water retention, enabling the plant to maintain crucial traits such as palisade thickness (PPT) and leaf thickness (LTh), which optimize photosynthesis and improve drought resilience even in water-limited conditions.

Conclusion

The results of this study highlight the significant influence of environmental factors, both climatic and edaphic, on the anatomical variations of *P. lentiscus* leaves in western Algeria. Adaptation to spatial variability is primarily reflected in modifications of cuticle and palisade parenchyma thickness, while responses to seasonal variations are mainly driven by adjustments in the epidermis and leaf parenchyma. These adaptations enable *P. lentiscus* to survive and maintain its physiological functions in the heterogeneous conditions characteristic of Mediterranean ecosystems in this region.

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References

Ait Said, S. (2011). Stratégies adaptatives de deux especes du genre *Pistacia* (*P. lentiscus* L. et *P. atlantica* Desf.) aux conditions d'altitude, de Salinité et d'aridité: Approche morpho-anatomique, phytochimiques et ecophysiologiques [Adaptive strategies of two species of the genus *Pistacia* (*P. lentiscus* L. and *P. atlantica* Desf.) to conditions of altitude, salinity and aridity: Morpho-anatomical, phytochemical and ecophysiological approach] University Mouloud Mammeri, Tizi-Ouzou (in French).

Ait Said, S., Fernandez, C., Greff, S., Derridj, A., Gauquelin, T., & Mevy, J. P. (2011). Inter population variability of leaf morpho-anatomical and terpenoid patterns of *Pistacia atlantica* Desf, ssp. *atlantica* growing along an aridity gradient in Algeria. Flora. Morphology. Distribution. Functional Ecology of Plants, 206, 397–405.

Alyafi, J., (1979). Approche systématique et écologie du genre *Pistacia* L. dans la région Méditerranéenne [Systematic approach and ecology of the genus *Pistacia* L. in the Mediterranean region]. Université d'Aix, Marseille (in French).

Bacelar, E. A., Correia, C. M., Moutinho-Pereira, J. M., Gonçalves, B. C., Lopes, J. I., & Torres-Pereira, J. M. (2004). Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiology, 24(2), 233–239.

Boudy, P. (1950). Economie forestier Nord-Africaine: Monographie et traitement des essences forestier [North African forest economy: Monograph and treatment of forest species] (in French).

Correia, O., & Diaz Barradas, M. C. (1998). Photosynthetic characteristics of leaves of a dioecious species (*Pistacia lentiscus* L.) under summer stress conditions. In: Garab, G. (Ed.). Photosynthesis: Mechanisms and Effects. Springer, Dordrecht. Pp. 4039–4042.

De Martonne (1926). Une Nouvelle fonction climatologie: L'indice d'aridité [A new climatology function: The aridity index]. La météo. Pp. 449–459 (in French).

De Mico, V., & Aronne, G. (2012). Anatomy and lignin characterization of twigs in the chaparral shrub *Rhamnus californica*. IAWA Journal, 33(2), 151–162.

Doghbage, A., Belhadj, S., Belhouadjeb, F.A., Boukerker, H., Mevy, J.P., Gauquelin, T., Tonetto, A., Merdas, S., Touati, B., & Saimi, F. (2023). Leaf morphological and epidermal traits variability along environmental gradients in ten natural populations of *Pistacia lentiscus*. Life, 13, 1617.

Doghbage, A., Belhadj, S., Derridj, A., Mevy, J. P., Gauquelin, T., Merdas, S., Tonetto, A., & Foulla Hassen, F. (2020). Comparative eco-botanical analysis of *Pistacia lentiscus* L. in Algeria through morphological and ultrastructural markers related to leaves and stomata. Revue Agrobiologia, 10(1), 1826–1836.

Drouineau, G. (1942). Dosage rapide du calcaire actif du sol: Nouvelles données sur la séparation et la nature des fractions calcaires [Rapid determination of active limestone in soil: New data on the separation and nature of limestone fractions]. Annales Agronomiques, 12, 441–450 (in French).

Emberger, L. (1952). Sur le Quotient pluviothermique [On the rainfall quotient]. C.R. Académie Science: Paris, 234, 2508–2510 (in French).

Filella, I., & Peñuelas, J. (1999). Altitudinal differences in UV absorbance, UV reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. Plant Ecology, 145, 157–162.

Fletcher, L. R., Cui, H., Callahan, H., Scoffoni, C., John, G. P., Bartlett, M. K., Burge, D. O., & Sack, L. (2018). Evolution of leaf structure and drought tolerance in species of Californian Ceanothus. American Journal of Botany, 105, 1672–1687.

Fletcher, L. R., Cui, H., Callahan, H., Scoffoni, C., John, G. P., Bartlett, M. K., Burge, D. O., & Sack, L. (2018). Evolution of leaf structure and drought tolerance in species of Californian ceanothus. American Journal of Botany, 105, 1672–1687.

Floret, C., & Pontanier, R. (1982). L'aridité en Tunisie présaharienne: Climat, sol, végétation et aménagement [Aridity in pre-Saharan Tunisia: Climate, soil, vegetation and management]. Travaux et documents de L'Orstom, 150 (in French).

Fritz, C. O., Morris, P. E. & Richler, J. J. (2012). Effect size estimates: Current use, calculations, and interpretation. Journal of Experimental Psychology: General, 141(1), 2–18.

Galmés, J., Flexas, J., Savé, R., & Medrano, H. (2007). Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: Responses to water stress and recovery. Plant and Soil, 290, 139–155.

Gaouar, A. (1980). Hypothèses et réflexions sur la dégradation des écosystèmes forestiers dans la région de Tlemcen (Algérie) [Hypotheses and reflections on the degradation of forest ecosystems in the Tlemcen region (Algeria)]. Forêt Méditerranéenne, 2(2), 131–146 (in French).

Gee, G., & Or, D. (2002). Particle size analysis. In: Dane, J. H., & Topp, G. C. (Eds.). Methods of soil analysis. Part 4. Physical methods. SSSA Book Series Madison, Wisconsin. Pp. 255–293.

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. Global and Planetary Change, 63, 90–104.

Guerfel, M., Baccouri, O., Boujnah, D., Chaibi, W., & Zarrouk, M. (2009). Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in two main Tunisian olive (*Olea europaea* L.) cultivars. Scientia Horticulturae, 119, 257–263.

Haffani, S., Mezni, M., Nasri, M. B., & Chaibi, W. (2017). Comparative leaf water relations and anatomical responses of three vetch species (*Vicia narbonensis* L., *V. sativa* L., and *V. villosa* Roth.) to cope with water stress. Crop and Pasture Science, 68, 691–702.

Iozia, L. M., Crisafulli, V., & Varone, L. (2023). Climatic variations along an aridity gradient drive significant trait intraspecific variability in Mediterranean plant species. Journal of Arid Environments, 217, 105042.

Lambers, H., Chapin, F. S. III, & Pons, T. L. (2008). Plant physiological ecology. 2nd ed. Springer.

Limousin, J. M., Misson, L., Lavoir, L. M., Martin, N. K., & Rambal, S. (2010). Do photosynthetic limitations of evergreen *Quercus ilex* leaves change with long-term increased drought severity? Plant, Cell and Environment, 33, 863–875.

Liu, S., & Zheng, J. (2024). Adaptive strategies based on shrub leaf stem anatomy and their environmental interpretations in the Eastern Qaidam Basin. BMC Plant Biology, 24, 323.

Mahroug, S., Courdavault, V., Thiersault, M., St-Pierre, B., & Burlat, V. (2006). Epidermis is a pivotal site of at least four secondary metabolic pathways in *Catharanthus roseus* aerial organs. Planta, 223, 1191–1200.

Miranda, J. D., Armas, C., Padilla, F. M., & Pugnaire, F. I. (2011). Climatic change and rainfall patterns: Effects on semi-arid plant communities of the Iberian Southeast. Journal of Arid Environments, 75(12), 1302–1309.

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In: Sparks, D. L. (Eds.). Methods of soil analysis. Part 3. Chemical Methods. SSSA Book Series Madison, Wisconsin. Pp. 961–1010.

Nevo, E., Bolshakova, M. A., Martyn, G. I., Musatenko, L. I., Sytnik, K., Pavlíèek, T., & Beharav, A. (2000). Drought and light anatomical adaptative leaf strategies in three woody species caused by microclimatic selection at "Evolution Canyon", Israel. Israel Journal of Plant Sciences, 48(1), 33–46.

Niinemets, U. (2001). Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology, 82(2), 453–469.

Sardans, J., & Peñuelas, J. (2013). Plant-soil interactions in Mediterranean forest and shrublands: Impacts of climatic change. Plant and Soil, 365, 1–33.

Sardans, J., Peñuelas, J., & Rodà, F. (2006). Plasticity of leaf morphological traits, leaf nutrient content, and water capture in the Mediterranean evergreen oak *Quercus ilex* subsp. *ballota* in response to fertilization and changes in competitive conditions. Écoscience, 13(2), 258–270.

Simões, R., Miranda, I., & Pereira, H. (2023). Variation in leaf cutin content and chemical composition along one annual cycle in the Mediterranean cork oak (*Quercus suber* L.). Forests, 14(2), 334.

Sperlich, D., Chang, C. T., Peñuelas, J., Gracia, C., & Sabaté, S. (2015). Seasonal variability of foliar photosynthetic and morphological traits and drought impacts in a Mediterranean mixed forest. Tree Physiology, 35(5), 501–520.

Tattini, M., Remorini, D., Pinelli, P., Agati, G., Saracini, E., Traversi, M. L., & Massai, R. (2006). Morpho-anatomical, physiological and biochemical adjustments in response to root zone salinity stress and high solar radiation in two Mediterranean evergreen shrubs, *Myrtus communis*, and *Pistacia lentiscus*. New Phytologist, 170(4), 779–794.

Tian, M., Yu, G., He, N., & Hou, J. (2016). Leaf morphological and anatomical traits from tropical to temperate coniferous forests: Mechanisms and influencing factors. Scientific Reports, 6(1), 19703.

Tiantian, C., Junjie, P., Minjie, Q., Xian, S., Jingjia, D., Feng, L., & Kaibing, Z. (2023). The effects of enhanced Ultraviolet-B radiation on leaf photosynthesis and submicroscopic structures in *Mangifera indica* L. cv. 'Tainong N°1. Horticulturae, 9(1), 83.

Vally, L., Janet, F. B., & George, K. (2002). The ability of abaxial and adaxial epidermis of sun and shade leaves to attenuate UV-B radiation in relation to UV absorbing capacity of the whole leaf methanolic extracts. Physiologia Plantarum, 117(1), 33–43.

Weemstra, M., Roumet, C., Cruz-Maldonado, N., Anthelme, F., Stokes, A., & Freschet, G. T. (2022). Environmental variation drives the decoupling of leaf and root traits within species along an elevation gradient. Annals of Botany, 130(3), 419–430.

Wei, T., & Simko, V. (2024). R package 'corrplot': Visualization of a Correlation Matrix (Version 0.95).

West, A. G., Dawson, T. E., February, E. C., Midgley, G. F., Bond, W. J., & Aston, T. L. (2012). Diverse functional responses to drought in a Mediterranean-type shrublands in South Africa. New Phytologist, 195, 396–407.

Yaoqi, L., Dongting, Z., Nawal S., Xiaoting, X., Qinggang, W., Wen, J., & Zhiheng, W. (2020). Spatiotemporal variation in leaf size and shape in response to climate. Journal of Plant Ecology, 13(1), 87–96.

Yavas, I., Jamal, M. A., Ul Din, K., Ali, S., Hussain, S., & Farooq, M. (2024). Drought-induced changes in leaf morphology and anatomy: Overview, implications and perspectives. Polish Journal of Environmental Studies, 33(2), 1517–1530.

Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., & Gómez-Cadenas, A. (2018). Plant adaptations to the combination of drought and high temperatures. Physiol Plantarum, 162(1), 2–12.