

COMPARATIVE ECO-BOTANICAL ANALYSIS OF *PISTACIA LENTISCUS* L. IN ALGERIA THROUGH MORPHOLOGICAL AND ULTRA-STRUCTURAL MARKERS RELATED TO LEAVES AND STOMATA

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Reçu le 07/12/2019, Révisé le 20/05/2020, Accepté le 28/05/2020

Abstract

Description of the subject: In this study, we compared the morphological and ultra-structural characters of *Pistacia lentiscus* L. leaves (epidermis, stomata, trichome and wax) belonging to three populations from Northern Algeria (Djelfa, Médéa and Tizi-Ouzou).

Objectifs: This study aims to establish an analysis of the inter-population variability of *Pistacia lentiscus* in the purpose of identification and knowledge of this species. Despite the ecological and economic features of this species, it remains neglected in the restoration of preforest and forest ecosystems. Besides, it is important to understand the strategies developed by this species according to environmental drivers including altitude and aridity.

Methods: We assessed 14 quantitative and qualitative morphological characters: related to leaf size, number of leaflets, petiole, stomata size, stomata density in abaxial and in adaxial face of the leaf. In addition, the leaf epidermis and stomata were observed under the scanning electron microscope (SEM).

Results: The statistical analysis and scanning electron microscopy observations allowed the separation of the studied populations according to the studied variables and the climatic conditions. The dimensions of the leaf and the terminal leaflet, the number of pairs of leaflets, trichomes occurrence, the dimensions, density, shape, and distribution of the stomata are the most discriminating characteristics.

Conclusion: This approach allowed highlighting new characteristics in species studied such as wax and glandular trichomes occurrence on the leaflets, sunken stomata in the epidermis. These characteristics may afford an initial screening method for classifying species of the genus *Pistacia* in Algeria.

Keywords: *Pistacia lentiscus*; variability; ultrastructure; epidermis; leaf; stomata; adaptation; aridity

ANALYSE ECO-BOTANIQUE COMPARATIVE DE *PISTACIA LENTISCUS* L. EN ALGÉRIE PAR LE BIAIS DE MARQUEURS MORPHOLOGIQUES ET ULTRASTRUCTURELS FOLIAIRES ET STOMATIQUES

Résumé

Description du sujet : Cette étude se propose d'identifier trois populations Algérienne de *Pistacia lentiscus* L. (Djelfa, Médéa et Tizi-Ouzou) dans leur milieu naturel et ceci, en se basant sur des caractères morphologiques et ultra-structurels foliaires (épiderme, stomates, trichomes et cire).

Objectifs : Cette étude vise à établir une analyse de la variabilité inter-population de *Pistacia lentiscus* dans le but d'identifier et de mieux connaître l'espèce qui reste méconnue en Algérie et par conséquent, très peu employée dans la préservation des écosystèmes forestiers et pré-forestiers malgré ses intérêt, aussi bien écologique qu'économique. Elle permet également de comprendre les stratégies développées par cette espèce dans des habitats aux conditions extrêmes, notamment l'altitude et l'aridité.

Méthode : 14 caractères morphologiques quantitatifs et qualitatifs ont été mesurés pour les feuilles et les stomates. En plus de ces caractères, d'autres observations ont été effectuées pour les surfaces foliaires et les stomates sous microscope électronique à balayage (MEB).

Résultats : Les analyses statistiques et les observations au microscope électronique à balayage ont permis de séparer les populations étudiées selon les variables étudiées et les conditions climatiques. Les dimensions des feuilles et des folioles terminales, le nombre de folioles, la présence de trichomes, les dimensions, la densité, la forme ainsi que la répartition des stomates sont les caractéristiques les plus discriminantes.

Conclusions : Cette approche nous a permis de mettre en évidence de nouvelles caractéristiques chez l'espèce étudiée, tel que l'occurrence des cires et des poils glandulaires sur les folioles, de stomates enfoncés dans l'épiderme. Ces critères pourront être ajoutés aux critères morphologiques utilisés dans la classification des espèces du genre *Pistacia* en Algérie qui peuvent donner des résultats satisfaisants dans la lutte contre la désertification.

Mots-clés : *Pistacia lentiscus* ; variabilité ; ultrastructure ; épiderme ; feuille ; stomate ; adaptation ; aridité.

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INTRODUCTION

The environmental conditions and their variations directly affect plants, particularly diversity, distribution and functional traits [1]. In response to these conditions, plants develop adaptation mechanisms reflecting particular morphological characters [2]. In general, water stress is a limiting environmental factor for plant development. This stress worsens in arid semiarid and dry sub-humid areas. Furthermore, individuals, populations do not passively suffer the influence of environmental factors. They present in varying degrees an ecological plasticity allowing them to adapt to the temporal and / or spatial fluctuations of the limiting factors in the environments to which they are attached. Hence, they develop regulatory mechanisms that cause morphological and physiological modifications that allow them to maintain constant and optimal value their internal conditions in the face of the variability of the environment. Several studies have initiated the relationship of environmental conditions with plants using morphological traits. Ait Said *et al.* (2011) showed in their study that the aridity conditions affect the variability of the morphometric traits of *Pistacia atlantica* Desf. Many biometrics works have been based on the study of leaf characteristics.

Indeed, many authors explain the variation of certain biometric characters by the influence of the stationary conditions. We cite those of *Cedrus atlantica* and *Pistacia atlantica* [3, 4]. Plants promote different functional traits in order to minimize the impact of drought [5, 6]. In arid environments, the leaves of xeromorphic plants are often smaller and covered with trichomes and wax.

The density of waxes and trichomes is also a characteristic influenced by ecological conditions. Altitude and positive values of minimum temperatures can play an important role in their distribution and density on the leaves [4]. On the other hand, little work has been done on the leaf characteristics of *Pistacia lentiscus*. Although, contributions have been made to the description of the morphology of the leaf epidermis, leaves.

Only a few species of the genus *Pistacia* have been examined with the light microscope and very little with the scanning electron microscope (SEM).

The genus *Pistacia* remains poorly defined systematically and ecologically [7].

Moreover, very little information is available on the leaf morphological characters of *P. lentiscus* occurring in different climatic regions in North Africa.

In this context, it is important to use the functional approach including foliar and stomatal micro and macromorphological to characterize *Pistacia lentiscus*. *Pistacia lentiscus*. (lentisque pistachio) is a typical Mediterranean thermophilic species; it is a dioecious shrub with persistent paripinnate leaves. This species is distinguished from the two other spontaneous species of Algeria of the same genus; *P. atlantica* Desf and *P. terebinthus* L [7, 8, 9]. It can reach 3 m in height, belonging to the family of Anacardiaceae which consists of more than eleven species [7]. The pollination is anemophilous, the fruit is a drupe [8, 10]. *P. lentiscus* known in the Mediterranean countries by its resin (putty) used since antiquity for the manufacture of certain products and the treatment of gastric diseases, ulcers, bacterial infections and canker sores [11]. The aqueous leaf extract of *P. lentiscus* is widely used as a beverage in North African countries and increasingly in the world for its special health benefits [12]. In addition, *Pistacia* oil is widely recognized in the Algerian traditional medicine [13]. These species are adapted to climatic hazards and can be used in the reforestation of eroded soils. *Pistacia lentiscus* is one of those species with great potential. Unfortunately, its use is often hampered by insufficient or fragmentary knowledge of its diversity, its ecology and the techniques of its multiplication. *P. lentiscus* has a wide geographical and bioclimatic distribution, ranging from wetlands to arid zones [14]. This elasticity is due to its capacity to develop morphological and physiological adaptation strategies with respect to different degrees of water stress (reference). Particularly, these adaptation strategies are related to mechanisms based on stomatal control and reduction of leaf area and size [15]. *P. lentiscus* occurs in isolated stands or in association with other species such as the Aleppo pine, the Prickly Juniper, the carob tree, the olive tree, the terebinth, the holm oak and the cork oak [16]. This species grows on different soil types in the sub-humid and semi-arid climates [17].

Our study is based on the morphological and ultrastructural features of the leaf, which is the most exposed organ of the plant to environmental conditions; this study aims to determine how the three populations of *P. lentiscus* respond to climatic variations by their micro and macromorphological traits. Especially, the survey aims to elucidate the effects of climatic variables on the morphological and ultra-structural characteristics of the leaves (epidermis, stomata, trichome and wax) and the observation of the leaf epidermis and stomata under the (SEM) across a climatic gradient that includes semi-arid climate, semi-humid climate and humid climate.

MATERIAL AND METHODS

1. Sampling sites

The sampling sites of *Pistacia lentiscus* leaves are situated according to a climatic gradient south–north of Algeria (Fig. 1). We selected three sites to assess the differences in morphological and ultra-structural traits, Taksebt (Tizi-Ouzou province), Cherrata (Medea province) and Sidi Bayazid (Djelfa province). We used the De Martonne aridity climate index as an aridity gradient for the choice of the sampling sites from semi-arid climate to humid climate. - The site of Taksebt (36.665° N, 4.116° E, altitude is 200 m a.s.l.). The climate data were obtained from the nearest meteorological station of Tizi-Ouzou for the period (1999- 2012), these data were provided by the National Meteorological Office. The climate of this site is humid with a temperate winter. The average temperature is 18.30°C with the highest value of 28.03°C recorded in August and a minimum value of 10.11°C for January. The rainy month was December with 127.55 mm, and the average rainfall was 780.13 mm. The climate is characterized by two periods, a dry period between June and September, and a humid period from October to May.

- The site of Cherrata (36.177° N, 2.89° E, altitude is 1040 m a.s.l.). The climate data used belongs to the nearest meteorological station of Ben-Chicao. These data were issued from the climate software New_Loc Clim: Local Climate Estimator version 1.10 (http://www.fao.org/nr/climpag/pub/en3_051002_en.asp). The program includes the FAOCLIM database of 28100 stations worldwide. The climate of this site is humid with a hot winter. The average rainfall was 615 mm, mostly occurring in November, December and January. The average temperature is 13.75°C.

Two periods characterize this site, a humid period of 8 months from October to May, and a dry period from June to September.

- The site of Sidi Bayazid (34.933° N, 3.383° E, altitude is 1080 m a.s.l.). The climate information was obtained from the nearest meteorological station of Djelfa for the period (1984- 2013). The climate of this site is humid with a temperate winter. The average rainfall was 311.41 mm, with a maximum of 34.93 mm recorded in May and a minimum of 9.59 mm recorded in July. The average temperature is 14.73°C. The dry period extends from June to October, and the humid period extends from November to May. **2. Plant Materials**

At each sampling site, ten trees were randomly selected for each population. For each tree, 30 leaves were harvested; in total 300 leaves in each sampling site and 900 leaves for the whole study area. Sampling was performed between June and September 2012.

The leaves were dried in the open air and kept in paper bags in the laboratory for trait measurements and analysis.

3. Traits measurement

We followed the method described in the I.P.G.R.I [18], reference for biometric and macromorphological measurements of the leaves. Hence, seven quantitative morphological characters were measured: (i) leaf length (cm), (ii) leaf width (cm), (iii) leaflet length (cm), (iv) leaflet width (cm), (v) petiole length (cm), (vi) number of pairs of leaflets, and (vii) ratio of the length to the width of the terminal leaflet. We used the epidermal printing method for biometric stomatal measurements. This method involves printing or molding the surface of the leaf epidermis on a colorless adhesive tape. This adhesive tape is glued on a microslide to count the number of stomata under a light microscope (magnification × 40). Stomatal size measurements (length and width) were performed on the abaxial face of *P. lentiscus* leaves. On the other hand, the stomatal densities were evaluated on both sides. These measurements were made using an optical microscope equipped with a digital camera connected to the computer, as well as the software Motic Image Plus "version 2.0. For the micromorphological study of leaf surfaces (shape, distribution and position of stomata on the epidermis, trichomes, waxes), we used a scanning electron microscope (SEM) (PHILIPS XL 30 ESEM) with a secondary electron detector to characterize the microstructure of our samples.

Sample preparation for SEM observations was carried out at the Mediterranean Institute of Biodiversity and Marine and Continental Ecology at Aix Marseille University.

4. Statistical analysis

Data were assessed with one-way ANOVA in order to compare the differences between macro and micromorphological traits in the three populations. Tukey's multiple comparisons followed the analysis to separate means. We retained statistical significant levels at $p < 0.05$. In addition, principal component analysis (PCA) was performed to assess the relationships between trait variables of different populations. Statistical test and models were performed using the SPSS software.

RESULTS

1. Macromorphological variability of the leaves

1.1. The leaves

The dimensions of the leaves vary between 5 to 14.50 cm concerning the length (an average of 9.71 cm), and 2.2 to 12 cm for the width (an average of 5.24 cm). The leaves are long (10.46 cm) and wide (5.33 cm) at Cherrata (C), shorter (8.77 cm) and narrower (5.11 cm) at Taksept (T). The leaves have between 4 and 16 leaflets for all provenances, with an average of 9.92 leaflets (Table 1). ANOVA indicated that there are significant differences between the three populations of *P. lentiscus* in leaf length ($F_{(2, 897)}=163.9$, $p < 0.001$), and leaf width ($F_{(2, 897)}=4.52$, $p < 0.05$). The analysis of variance showed that the number of leaflets was significantly different for the three populations ($F_{(2, 897)}=11.81$, $p < 0.001$). A Tukey post hoc test revealed a statistically significant difference between the three groups of sites concerning leaf length. For leaf width and number of leaflets, Tukey's test revealed two homogeneous groups (Table 1).

1.2. The terminal leaflet

32.77% of the leaves had the terminal leaflet for the three populations studied, 20% in (T), 38.66% in (S) and 39% in (C). Significant differences were recorded ($F_{(2, 897)}=6.41$, $p < 0.01$) for the terminal leaflet length between the three population of *P. lentiscus*. The same observation was indicated by the ANOVA for the terminal leaflet width ($F_{(2, 897)}=4.23$, $p < 0.05$), and for the length/width ratio ($F_{(2, 897)}=13.65$, $p < 0.001$). Tukey's post hoc test indicated two homogeneous groups for three mentioned traits (Table 1).

1.3. The petiole

The petiole length varied significantly ($F_{(2, 897)}=34.7$, $p < 0.001$) between the three stations. The mean value of petiole length in (S) was 1.61 cm, whereas in (C) it was 1.65 cm, and 1.36 cm recorded in (T). The comparison of the means with the Tukey test reveals the presence of two distinct groups (Table 1).

2. Comparative analysis of leaf epidermis and stomatal characters

2.1. Density and size of stomata in the epidermis

The mean of stomatal length recorded in (C) is 25.93 μm , and 29.46 μm in (S), and 26.14 μm in (T). ANOVA indicated significant differences for this parameter ($F_{(2, 746)}=87.87$, $p < 0.001$), and Tukey's post hoc test showed two groups. Whereas the mean value of stomatal width revealed significant differences between the three stations ($F_{(2, 746)}=114.29$, $p < 0.001$), the stomatal width was 19.71 μm in (T) and 23.27 μm in (S) and 20.80 μm in (C) (Table 2). For the density, the values vary between 105.09 st/mm^2 in (T) and 197.83 st/mm^2 in (C), on the abaxial face, and 0.33 st/mm^2 in (S) and 3.97 st/mm^2 (T) on the adaxial face (Table 2). ANOVA analysis indicated significant differences ($P < 0.001$) between the different populations of *Pistacia lentiscus* concerning these variables (Table 2).

2.2. Distribution, position, form of stomata

The leaves are amphistomatic for the three studied populations (the stomata are present on the abaxial and the adaxial face of the leaf). The number of stomata on the abaxial face is higher than to the adaxial face. Our results show that the stomata are distributed over all abaxial leaf surfaces for the three populations, while the rare stomata of the adaxial face are distributed near the main and secondary veins (Fig. 2). In this study, the stomata are embedded in the epidermis (S) while they are at the same level to slightly embedded in the epidermis for the other populations (C), (T) (Fig. 3). The stomata have different shapes; they are elliptical in (S) and (T) and sub-rounded in (C) (Fig. 3).

Principal component analysis (PCA) (Fig. 4A) separated the populations into three distinct groups based on stomatal sizes and densities. Axis 1 is determined by the size of the stomata and explains 44.68 % of the total variance, and axis 2 represents the stomatal density and explains 29.38% of the total variance (74.06%).

The figure revealed a negative correlation between leaf stomata dimensions, while the number of stomata on the abaxial face is positively correlated.

The projection of the variables related to stomata traits (Fig. 4B) reveals an association between traits of the same population characterized by the environmental conditions. The stomata traits of Cherrata (C) are correlated positively to the first axis. However, those of Taksebt (T) are negatively correlated to the same axis. The stomata traits of the population sampled in Sidi Bayazid (S) are correlated negatively to the second axis.

2.3. Trichomes and waxes

Trichomes were observed in *P. lentiscus* with low intensity, especially for the population (T), SEM observations show the presence of two types of trichomes on the abaxial and the adaxial face of the leaves for all populations. Glandular hairs scattered throughout the leaf surface (abaxial and adaxial) and rarely at the level of the midrib. On the other hand, the non-glandular hairs are elongated and distributed along the main vein (nerve hair), they are absent on the leaf margin (Fig. 5). SEM observation also allowed us to notice wax deposits on all samples (Fig. 5). This observation was not possible under the binocular microscope or optical microscope.

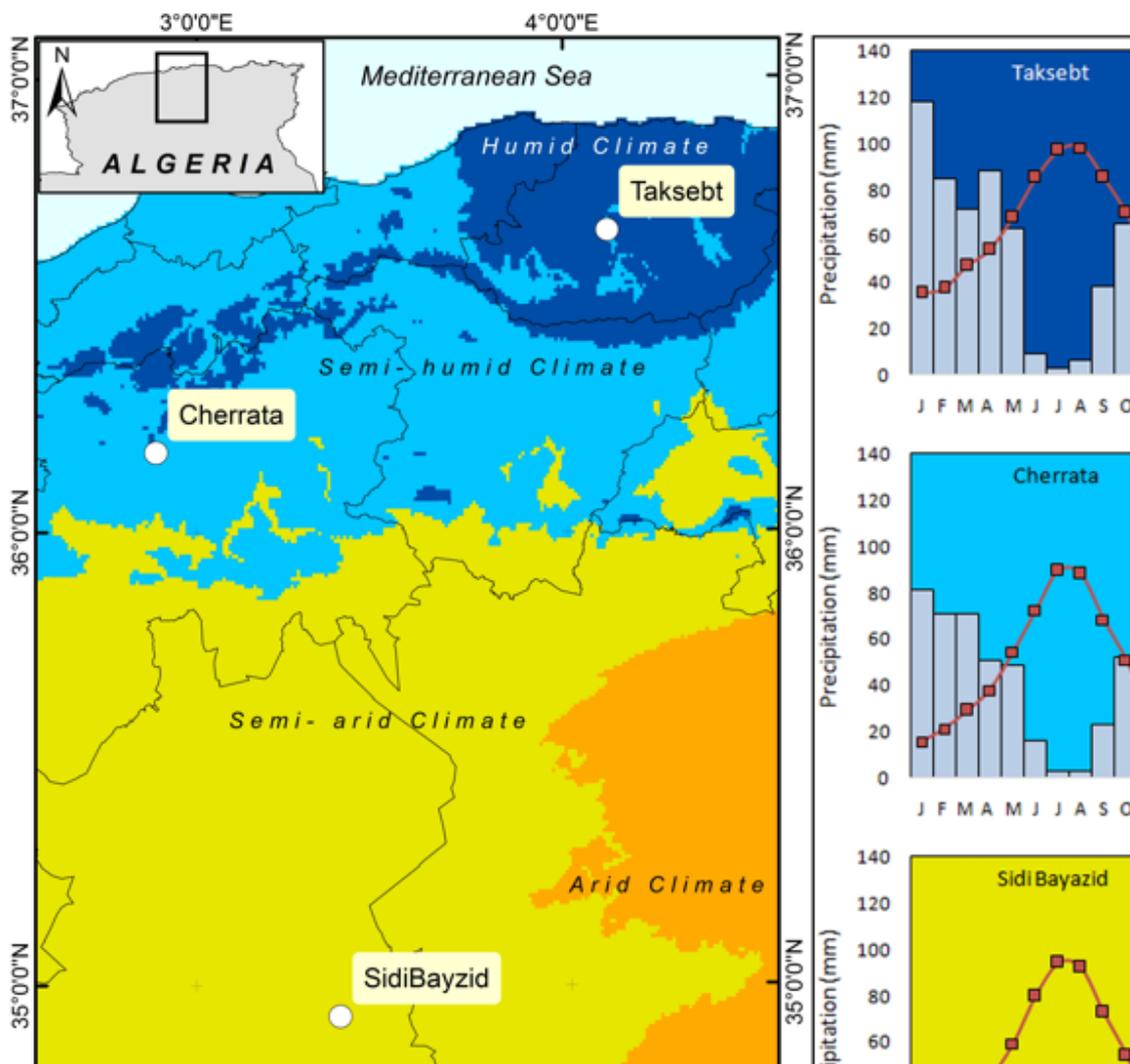


Figure 1: The location of sampling sites (○) according to a climatic gradient, Taksebt (Tizi-Ouzou province) humid climate, Cherrata (Medea province) semi-humid climate and Sidi Bayazid (Djelfa province) semi-arid climate. The diagrams represent climate conditions for each sampling site (precipitation in a blue bar chart and mean temperatures in orange squares).

Table 1: Morphological characteristics of *Pistacia lentiscus* leaves from different sampling sites (M ± SD,R,CV)

Characters	Sidi bayzid (S)	Cherrata (C)	Taksept (T)
Leaf length (cm)	9.9±0.85 a ^{***} 7.8–12.10 (8.61)	10.46±0.76 b ^{***} 8.7–12.6 (7.27)	8.77±1.66 c ^{***} 5–14.5 (18.99)
Leaf width(cm)	5.27±0.82 ab [*] 3.5–10.1 (15.56)	5.33±0.89 a [*] 3.3–12 (16.75)	5.11±1.06 b [*] 2.20–8.6 (20.77)
Number of leaflets	10.32±2.03 a ^{***} 4–16 (19.7)	9.70±1.54 b ^{***} 5–14 (15.94)	9.75±1.61 b ^{***} 5–15 (16.56)
Terminal leaflet length (cm)	2.31±0.66 a [*] 0.7–4.4 (28.82)	2.37±0.58 a [*] 1,1–4,6 (24,56)	2.66±0.63 b [*] 1.1–4 (23.91)
Terminal leaflet width (cm)	1.02±0.44 a [*] 0.2–2,10 (43.38)	0.98±0.39 a [*] 0.5–1.9 (39.9)	0.84±0.28 b [*] 0.2–1.6 (33.89)
length / width ration of terminal leaflet	2.58±1.04 a ^{**} 1.16–8.5 (40.49)	2.66±0.85 a ^{**} 0.88–5.20 (31.95)	3.32±0.89 b ^{**} 1.5–6 (27.01)
Petiole length (cm)	1.61±0.44 a ^{***} 0.7–3.3 (27.30)	1.56±0.37 a ^{***} 0.8–2.7 (23.91)	1.36±0.37 b ^{***} 0.5–2.8 (27.49)

^{a,b,c} Separation of groups of populations by the Tukey test ($p < 0.05$). Values marked with the same letter are not significantly different. Mean: M; Standard deviation: SD.; Range: Min-Max; Coefficient of variation (%) CV.

Table 2: Stomata characteristics of *Pistacia lentiscus* leaves from different sampling sites (M ± SD, R, CV)

Characters	Sidi bayzid (S)	Cherrata (C)	Taksept (T)
Length of stomata on the abaxial face (µm)	29.46±4.15 a ^{***} 12.71–42.67	25.93±3.17 b ^{***} 15.49–39.44	26.14±2.47 b ^{***} 17.49–31.21
Width of stomata on the abaxial face (µm)	23.27±2.76 a ^{***} 14.98–31.09	20.80±3.05 b ^{***} 14.40–37.74	19.71±2.23 c ^{***} 13.22–29.15
Number of stomata on the abaxial face (st/mm ²)	194.86±48.62 a ^{***} 78.95–321.05	197.83±50.67 a ^{***} 78.95–352.63	105.09±35.09 b ^{***} 47.36–236.84
Number of stomata on the adaxial face (st/mm ²)	0.33±1.53 a ^{***} 0–10.53	0.48±1.73 a ^{***} 0–10.53	3.97±4.67 b ^{***} 0–15.78

^{a,b,c} Separation of groups of populations by the Tukey test ($p < 0.05$). Values marked with the same letter are not significantly different. Mean: M; Standard deviation: SD.; Range: Min-Max; Coefficient of variation (%) CV.

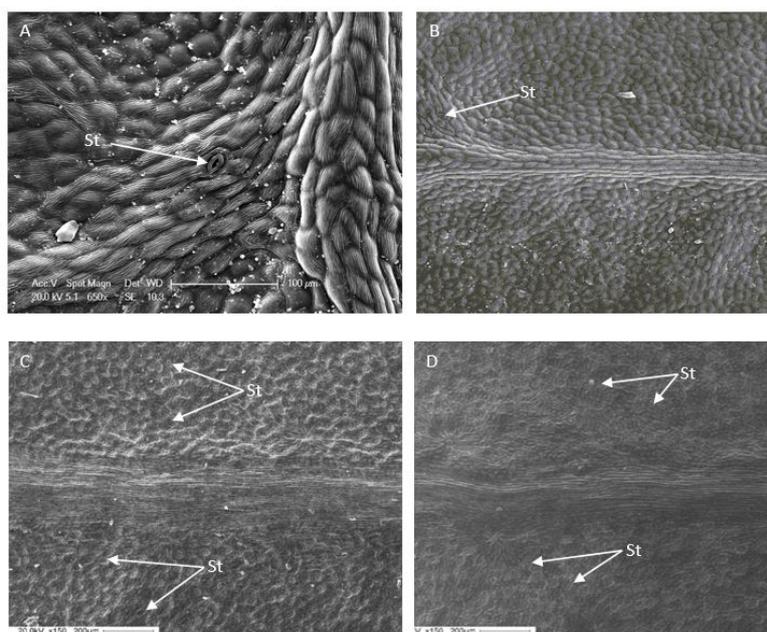


Figure. 2: Scanning electronmicrographs of abaxial and adaxial leaf surfaces showing stomata distribution: (A) and (B) midrib on the adaxial surface, (C) et (D) midrib on the abaxial surface.

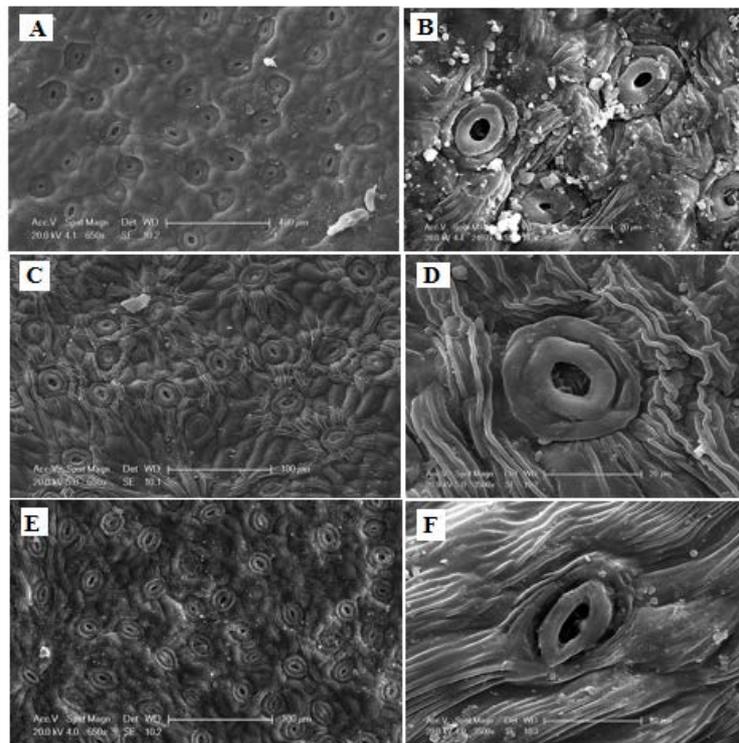


Figure. 3: Scanning electronmicrographs of adaxial leaf surfaces showing stomata position and shape (A) (B) Sidi bayzid, (C) (D) Cherrata and (E) (F) Taksept.

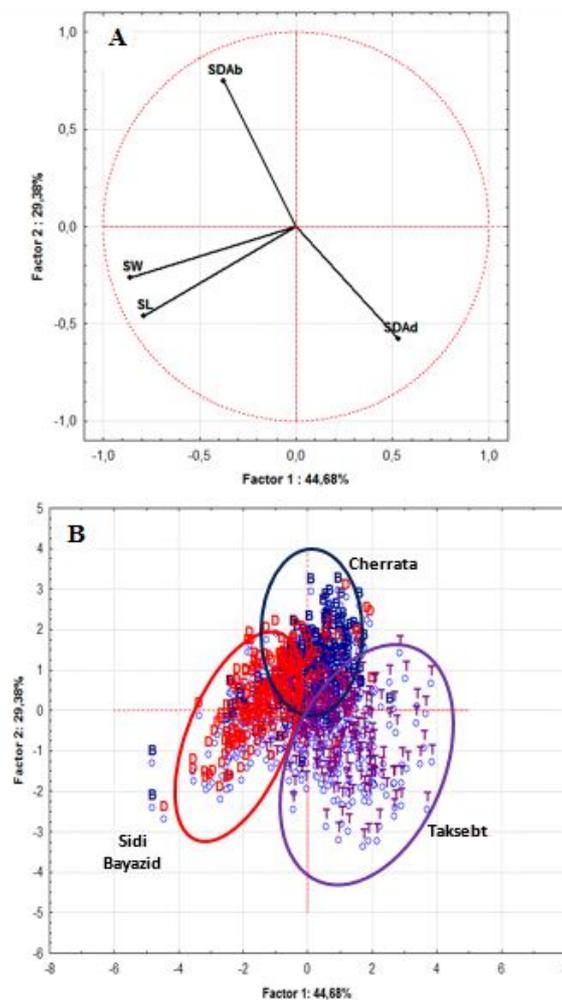


Figure 4: Principal Components Analysis (PCA) of the investigated *P. lentiscus* populations and their stomatal characteristics (SDAb: stomatal density in the abaxial face, SDAd: stomatal density on the adaxial face, SW: stomata width, SL: stomata length).

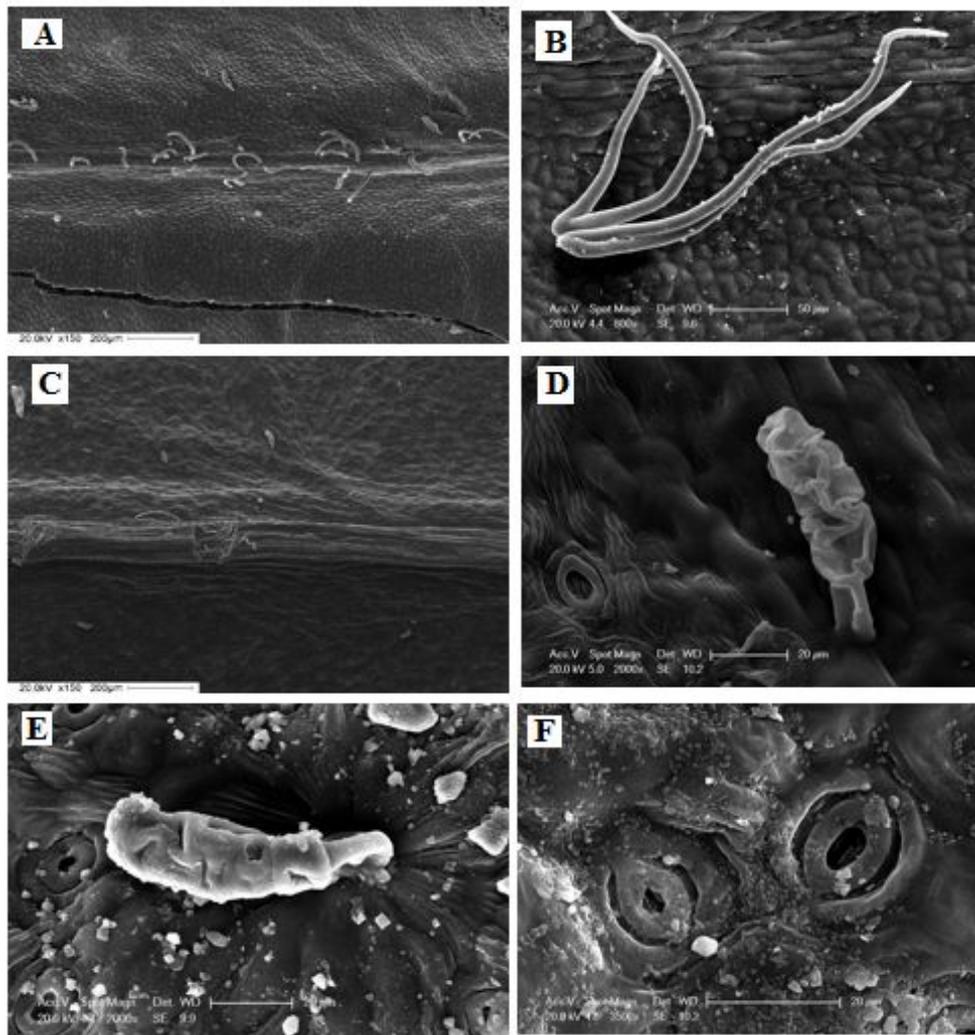


Figure 5: Scanning electronmicrographs of wax and different types of trichomes on leaf surface of *P. lentiscus* (A) (B) ciliated hair, (C) (D) glandular hair and (E) (F) wax.

DISCUSSION

For the Macromorphological variability of the leaves, we recorded a large interpopulation variability between (T) on the one hand and (S), (C) on the other hand for the majority of quantitative variables measured. Barazini *et al.* [19], in their study of three populations of *Pistacia lentiscus* in Cyprus, Tunisia, and Spain, reported a smaller leaf length (3-7.8 cm) compared to our results (5- 14.5 cm). However, the work of Al-Saghir [10] reported very similar values compared to our results concerning the dimensions of the leaf and terminal leaflet as well as the number of leaflets. This heterogeneity can be justified by the different environmental drivers especially climatic conditions of the sampling sites. Reduced leaf size is correlated with reduced transpiration [20], as aridity increases and leaf size decreases.

This finding is contradicted by our results where we recorded higher values in semi-arid and semi-humid stations (S, 311.41 mm) (C, 615 mm) and lower values in (T, 780.13 mm) on the dimensional plan. We find that climate is a determining factor since the large values recorded for the size of the leaves, number of leaflets as well as for the length of the petiole. Other factors, such as edaphic conditions, can decisively influence leaf morphology. The presence or absence of the terminal leaflet is important in distinguishing species of the genus *Pistacia*. According to our study, the presence of the terminal leaflet was recorded for all the studied populations. Kafkas *et al.* [21], reported the presence of a mixture of paripinnate and imparipinnate leaves on the same tree.

In addition, it is also possible to add the difference of sexes between subjects (trees). Most authors state that the length of the leaves and leaflets of female subjects is slightly higher than that of males, this is consistent and demonstrated for *P. vera* [22], *P. terebinthus* [21] and *P. lentiscus* [19, 23]. Nevertheless, the influence of the genetic factor is not to be omitted [24]. For the leaf epidermis and stomatal characters, Generally, little work has been done on the comparative study of species of the genus *Pistacia* micromorphologically. Nevertheless, we can mention the comparative study of the micromorphology of the leaf epidermis of eight populations of *Pistacia atlantica* from Algeria [4, 25, 26]. The study of the variability of leaf characteristics and stomata of four species of the genus *Pistacia* in Algeria [23], and the study conducted by Ait Said *et al.* [27] on the morpho-anatomical variability and the terpene composition contained in the leaves of *Pistacia atlantica*. The last study demonstrated that the studied populations exhibited very great macro and micromorphological morphological variability in the response of the aridity gradient, both in the leaves and in the stomata. Al-Saghir and Porter [28] suggested that the distribution of stomata could be related to the ecological plasticity of *Pistacia* species on a wide range of environmental conditions. In fact, our samples were taken from different bioclimatic stages, from semi-arid (S) to humid (T), with annual rainfall ranging from 311.41 mm (S) to 780.13 mm (T). In addition, the altitude varies from 1211 m (S) to 182 m (C). It should be noted that Calamassi [29], indicated that forest species of different origins are characterized by high stomatal density and have a better ability to withstand drought. Indeed, Sculler [30], reported that plant species from dry habitats generally had higher stomatal densities than species from humid habitats.

Our study confirms this hypothesis, where the two stations (S) and (C) with an average rainfall of between 311.41 mm/ yr and 615 mm / yr, have a high density of 194.86 st / mm² and 197.83 st / mm². On the other hand, the station (T) which is characterized by average precipitation exceeding 780.13 mm/ year had the lowest density of 105.09 st / mm². This characteristic seems to be one of the features of *Pistacia lentiscus* under the conditions of increasing aridity. Few data are available regarding the position of stomata in the epidermis, and our results are the among the first data reporting this feature for the studied species. Other criteria, such as pubescence and the presence of wax are commonly used in the classification in the genus *Pistacia* are also unstable [25], some families can be easily identified by the presence of a particular type of hairs. In other cases, the hairs are important in the classification of genera and species [31, 20]. In this study, the leaves of *P. lentiscus* are pubescent at the level of the main vein, the abaxial and adaxial leaf surface. However, their margins are not ciliate, which is consistent with the results of Doghbage [23], where the results showed the presence of trichomes in *P. lentiscus* with a low intensity unlike the leaves of other species of the genus *Pistacia*.

CONCLUSION

The functional approach is suitable in providing solutions on the distribution of organisms along environmental gradients. This implies many aspects as; the identification of the patterning drivers of the assembly of communities, the understanding of how those functioning are reflected in ecosystems and how controls some of the goods and services they provide to human societies. This approach allowed us to highlight new characteristics of the species studied, such as the occurrence of waxes and glandular hairs of the leaflets, stomata sunk into the epidermis. These criteria may be added to the morphological criteria used in the classification of species of the genus *Pistacia* in Algeria.

Acknowledgments

Our thanks go to the anonymous reviewers for their corrections, and relevant comments.

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