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ZIZIPHUS LOTUS (L.) MORPHOLOGICAL DESCRIPTION FROM WILD POPULATIONS IN ALGERIA

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Abstract

Description of the subject: Wild jujube (*Ziziphus lotus* L.) is a xerophytic shrub belonging to the *Rhamnaceae* family widely distributed in arid and semiarid regions in Algeria.
Objective: The present study aimed to analyze the leaves macro and micro morphological variability within and among wild populations sampled from seven different bioclimatic sites and to evaluate the effect of environmental conditions on leaves and stomata morphology.
Methods: 16 quantitative and qualitative morphological and micro morphological characters for leaves and stomata were analysed, the leaf epidermis was observed under the scanning electron microscope (SEM).
Results: The results revealed highly significant differences for all quantitative and qualitative both within and among populations. HAC with Ward's linkage, classified the seven populations in two clusters for leaves traits and three clusters for stomata traits. The environmental conditions did not seem to have effect on leaf traits but stoma characteristics were clearly marked by bioclimatic variation.
Conclusion: This study is the first to investigate morphological characters of leaves and stomata of wild jujube in Algeria. Our results provide new information that can help to better understand the eco-physiological responses of that plant species to different abiotic pressures.
Keywords: *Ziziphus lotus*; leaves; stomata; variability; morphology; populations; Algeria.

DESCRIPTION MORPHOLOGIQUE DE *ZIZIPHUS LOTUS* (L.) PROVENANT DE POPULATIONS NATURELLES EN ALGÉRIE.

Résumé

Description du sujet : Le jujubier sauvage (*Ziziphus lotus* L.) est un arbuste xérophyte appartenant à la famille des Rhamnacées, il se rencontre à l'état sauvage dans les zones arides et semi-arides d'Algérie.
Objectifs : L'objectif est d'analyser la variabilité macro et micro-morphologiques des feuilles inter et intra-populations naturelles provenant de différentes régions bioclimatiques en Algérie et de d'évaluer l'effet des pressions environnementales sur la morphologie des feuilles et des stomates.
Méthodes : 16 caractères qualitatifs et quantitatifs ont été mesurés pour les feuilles et les stomates. Les surfaces épidermiques ont été observées sur Microscope électronique à balayage.
Résultats : Les résultats ont montré une grande variabilité pour tous les caractères quantitatifs et qualitatifs étudiés. ANOVA a montré une grande variation significative entre et à l'intérieur des populations. La CAH (Ward's linkage) a classé les sept populations en deux groups pour les feuilles et trois groupes pour les stomates. Les conditions environnementales n'ont pas eu un grand effet sur la variabilité morphologique des feuilles, par contre, les variables des stomates ont été fortement influencées par la variation bioclimatique.
Conclusion : Cette étude est la première à étudier les caractères morphologiques des feuilles et des stomates du jujubier sauvage en Algérie. Nos résultats offrent de nouvelles informations qui peuvent aider à mieux comprendre les réponses éco-physiologiques de cette espèce aux différentes pressions abiotiques.
Mots clés : *Ziziphus lotus* ; feuille ; stomate ; variabilité, morphologie ; populations.

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INTRODUCTION

Rhamnaceae family counts about 55 genera and 900 species [1].*Rhamnus*, *Ceanothus* and *Ziziphus* are the main genera in the Rhamnaceae. *Zizyphus* genus counts some 40 tree and shrub species. It is represented in Algeria by three main species: *Z. spina-christi* (L.), *Z. mauritiana*(Lam.). and *Z. lotus* (L.) [2]. Wild Jujube (*Ziziphus lotus* (L.)), known as *Sedra*, in local language, is a spiny, deciduous and very thorny shrub, usually under 2.5 m in high [3]. Jujube leaves are dentate, ovate and glabrous, with stipular spines [4]. The fruits, called *Nbeg*, in local language, are brown, globose, sweet and edible [4 and 5]. The flowers are small, yellow and bisexual [6 and 3] with a flowering period expanding from June to August while the fruiting period extends from August to September.

Z. lotus has important medicinal properties; inflammatory, analgesic, antioxidant antispasmodic and antifungal activities have been reported by previous works [7, 8, 9, 10, 11, 3, 12 and 13]. Several scientific reports for health benefit and nutritional potential of bioactive compounds from this plant have been reported [14]. Leaves, fruits and roots are used in traditional medicine as antidiabetic, sedative, bronchitis and antidiarrheal by local populations [15, 16, 17, 3, 18 and 19]. Wild jujube bee honey is a highly appreciated product, for its good quality and medicinal use. Excellent against respiratory infections, cough and sore throats [20, 21 and 22], wild jujube honey is sold out the same season and it is considered as one of the most expensive bee honeys (about, 50 American dollars), in Algeria.

Z. lotus is widely distributed in southern Europe and the semi-desert steppes of northern Mediterranean Africa, northern Sahara, central Sahara and minor Asia [23, 24 and 25]. In Algeria, it is the mostcommon plant in the arid and the semi-arid environments with dry are very long seasons. The shrub provides a specific micro ecosystem and is a home for several flora and fauna species [26]. Besides its use in folk medicine, the plant plays a key role in soil protection against erosion and soil degradation because of its rigorous root system [27]. In addition to that, the spiny branches of the plant provide protection (nurse plant) to the juvenile *Pistacia atlantica*a (Anacardiaceae) seedlings against animal grazing, which occurs frequently in the daya region where *Z. lotus* is common [28,29]. Despite its ecological benefitsand pharmacological value, *Z. lotus* is often neglected in the reforestation programs. Nowadays, this shrub overcomes a severe degradation due mainly to anthropozoogenic factors combined to severe climatic conditions [30 and 26].

The aim of the present study is to investigate the morphological variability within and among seven *Z. lotus* wild populations and to evaluatethe effect of environmental conditions (mainly, soil and bio-climate) on leaves and stomata. Drought is one of the main limiting factors for plants production. Physiological traits such as number and size of stomata are important in selecting for drought resistance in breeding programs [31]. The morphological and Eco-physiological variations recorded in this study may provide a key solution, to the stockholders and local forest administration, in order to promote the reintroduction of this species among other local and resistant species in reforestation programs against the desertification process which undergoes in the steppe and northern Saharan areas in Algeria and North Africa, globally, especially because this plant presents an adaptive capacity to aridity much greater than other tree species from the same ecosystems.

MATÉRIEL ET MÉTHODES

1. Study sites

Z. lotus leaves were collected from seven different populations, widely distributed from northeast to southwest in Algeria (Fig.1): Belkheir (Bl) and Eldir (Ed) located in the North East part of Algeria, south constantinois, while Boughar (Bg), Boussedraia (Bo), Deldoul (Dl) and Hassirmel (Hr) are located in the Center, south algerois. Lahmer (Lh) site is located in western part of Algeria, in the south oranie. These seven locations are situated in different climatic zones (Table 1).

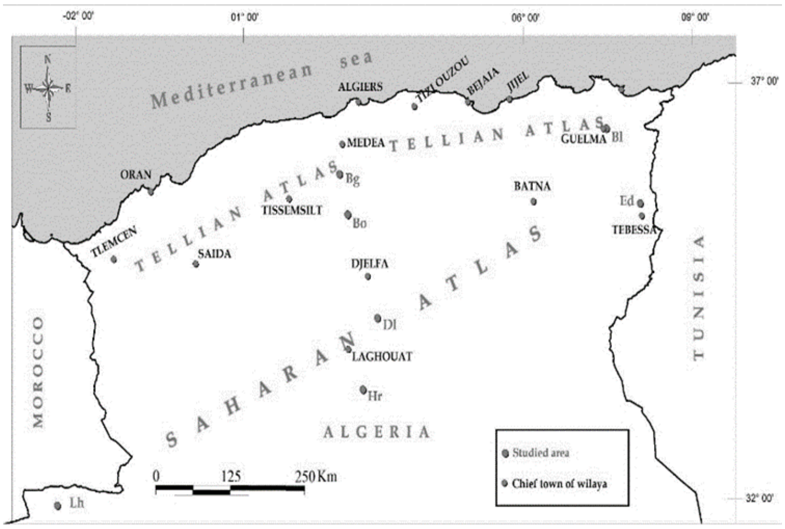


Figure 1: Localization of study sites (Bl: Belkheir; Ed: Eddir; Bg: Boughar; Bo: Boussedraia; Dl: Deldoul; Hr: Hassirmel; Lh: Lahmer).

Table 1: Location and climatic zone of the study sites.

Study sites	Latitude	Longitude	Altitude (m)	Rainfall (mm)	M (˚C)	m (˚C)	Q_3	Emberger climate zone[1]	Seasonal Musset type[2]	Aridseason length (months)
Belkhir (Bl)	36˚27′N	07˚29′E	210	605.9	34.9	3.3	65.9	Sub-humidtemperate	WFSpSu	4
Boughar (Bg)	35˚54′N	5˚54′E	860	438	35.4	3.7	52.3	Semi-aridtemperate	FSuWSp	6
El dir (Ed)	35˚33′N	0˚11′E	818	302.4	35.4	2.7	31.2	Semi-aridfresh	WSpFSu	4.5
Boussedraia (Bo)	35˚25′N	2˚51′E	737	276	35.8	0.5	18.9	Aridfresh	WSpFSu	6
Deldoul (Dl)	34˚10′N	3˚37′E	810	172	36.3	1.3	17.5	Aridfresh	WFSpSu	10
HassiRmel (Hr)	33˚19′N	03˚08′E	808	110	40.1	2.3	10.1	Saharianfresh	FSpWSu	12
Lahmer (Lh)	31˚55′N	-2˚13′E	882	75,6	41	2.5	6.9	Saharianfresh	FSuWSp	12

1. Rainfall. average annual precipitation. M. mean maximum temperature of the warmest month; m. mean minimum temperature of the coldest month. Q_3: pluviothermicquotient.Source of climatic data: National Office of Meteorology -Algeria, 2.Each season is defined according to the first letter: (F ; Fall. W ; Winter. Sp ; Spring. Su ; Summer).

2. Bioclimaticstudy

For the bioclimatic study two meteorological elements, were taken in accounts (temperature and precipitation) for laps of time of 30 years (according to the rules of the World Meteorological Organization). The total precipitation average, the seasonal rainfall regime, the average annual temperature, the average monthly temperature, the dry season, De Martonne aridity index [32], the Continentality Index of Gorbunov [33], the summer drought index, and the Emberger pluviothermic quotient Q_3 [34] were calculated (Tables 1 and 2).

Table 2: Climate types, aridity index of De Martonne (AI) and continentality index of Gorbunov (CI) of the studied sites.

Sites	Belkheir (Bl)	Eddir (Ed)	Boughar (Bg)	Boussedraia (Bo)	Deldoul (Dl)	Hassir'mel (Hr)	Lahmer (Lh)
De Martonne AI	40.4	21.7	20.9	19	11.9	10.8	10.5
	Humid	Subhumid		Semi-arid			
Gorbunov CI	31.5	33.2	31.7	32.4	33.6	37.4	37.3
	Semi-continental					Continental	

3. Soilsampling

Soil samples were collected beneath the shrubs in two replicates for each site, using an auger at depths between 0 and 20 cm. In the laboratory, samples were air-dried, pulverized and sifted through a 2mm sieve. Soil pH, electrical conductivity (EC) [35], total lime content [36], active lime using Drouineau method [37], humidity, organic matter using Walkey & Black method [38] and soil texture were studied.

4. Morphologicalvariables

Mature leaves were sampled between August and October, a random sampling of 25 shrubs per site was selected, distance between samples did not exceed 20 m. Thirty (30) leaves per plant were randomly collected and conserved in paper bags until use, (the sampling totalized 175 shrubs and 5250 leaves). In the laboratory, leaves were dried, and then measured. The characterization of the leaves is performed using the *Pistacia* descriptor of the International Plant Genetic Resources Institute [39] as well as previous studies conducted on morphological variation of *Pistacia atlantica* [40 and, 29]. The descriptor for Carob tree [41] was useful as well.

Twelve quantitative and qualitative variables were measured. After that, stomatawere characterized in a random sample of dehydrated leaves (5 shrubs per site and 10 leaves per shrub) on clear nail polish imprints [42, 40 and 29] taken from abaxial and adaxial leaf surfaces. Stomatal size and density were then calculated on images taken with a digital camera connected to an optical microscope using Scion Image program for 10 field view per polish imprints of the leaf surface. Eight (8) quantitative variables were described (Table 3). For the epidermal micromorphology study, leaf samples were prepared with standard methodology for scanning electronic microscope (FEI/Philips XL-30 Field Emission ESEM). Three leaf samples from each population were placed in ethanol (90°) for 5h, then allowed to dry under natural conditions. Three more samples per population were used without pre-treatment. The samples were covered with a thin layer of gold and placed on stubs for their study under SEM. The adaxial and abaxial leaf surface were observed under different magnifications.

Table 3: Morphological variables measured for *Z. lotus* leaves and stoma.

Qualitative variables	Leaf shape (L): 1- Broad lanceolate; 2- Ovate ; 3- Elliptic ; 4- Round ovate; 5- Roundish.
	Petiole shape (P) : 1- Rounded ; 2- Flattened ; 3- Rounded straight adaxially.
	Leaf base (B): 1- Attenuate ; 2- Obtuse ; 3- Truncate ; 4-Oblique.
	Leaf apex (A): 1- Acuminate ; 2- Mucronate; 3- Obtuse ; 4- Mucronulate; 5- Retuse.
	Leafmargen (M): 1-Entire ; 2-Serrulate ; 3-Dentate.
	leafepicuticular wax (W): 0-Absence ; 1- unusual ; 2-remarkable. ; 3- Abundant: on the abaxial surface.
	Leaf trichomes (T) : 0-Absence ; 1-unusual; 2-remarkable ; 3- numerous: on the abaxial surface.
Quantitative variables	Leaf lenght (LL) in cm.
	Leaf width (LW) in cm.
	Leaf Lenght/width ratio(R1).
	Petiole lenght (PL) in cm.
	Stoma lenght on the adaxial surface (DSL) in µm.
	Stoma width on the adaxial surface (DSW) in µm.
	Lenght/width ratio (R2) on the adaxial surface.
	Stomatal density (DSD) (stomata/mm²) on the adaxial surface.
	Stoma lenght on the abaxial surface (BSL) in µm.
	Stoma width on the abaxial surface (BSW) in µm.
	Stoma Lenght/width ratio (R3) on the abaxial surface.
	Stomatal density (BSD) stomata/mm² on the abaxial surface.

5. Statistical analysis

The data obtained were subjected to statistical analysis using Statistica 10 software. Normality and equality of variances were checked out through the Kolmogorov–Smirnov test. Morphometric data were analysed using Tukey's HSD test. Nested analysis of variance was usedto determine the variance level within and among populations (and within each individual). Correlation analysis was performedto reveal relationship between morphological traits and environmental variables. A discriminant analysis (DA) and anascendant hierarchical classification (AHC) using Ward linkage with square Euclidean distance measure were usedto evaluate the variability among populations.

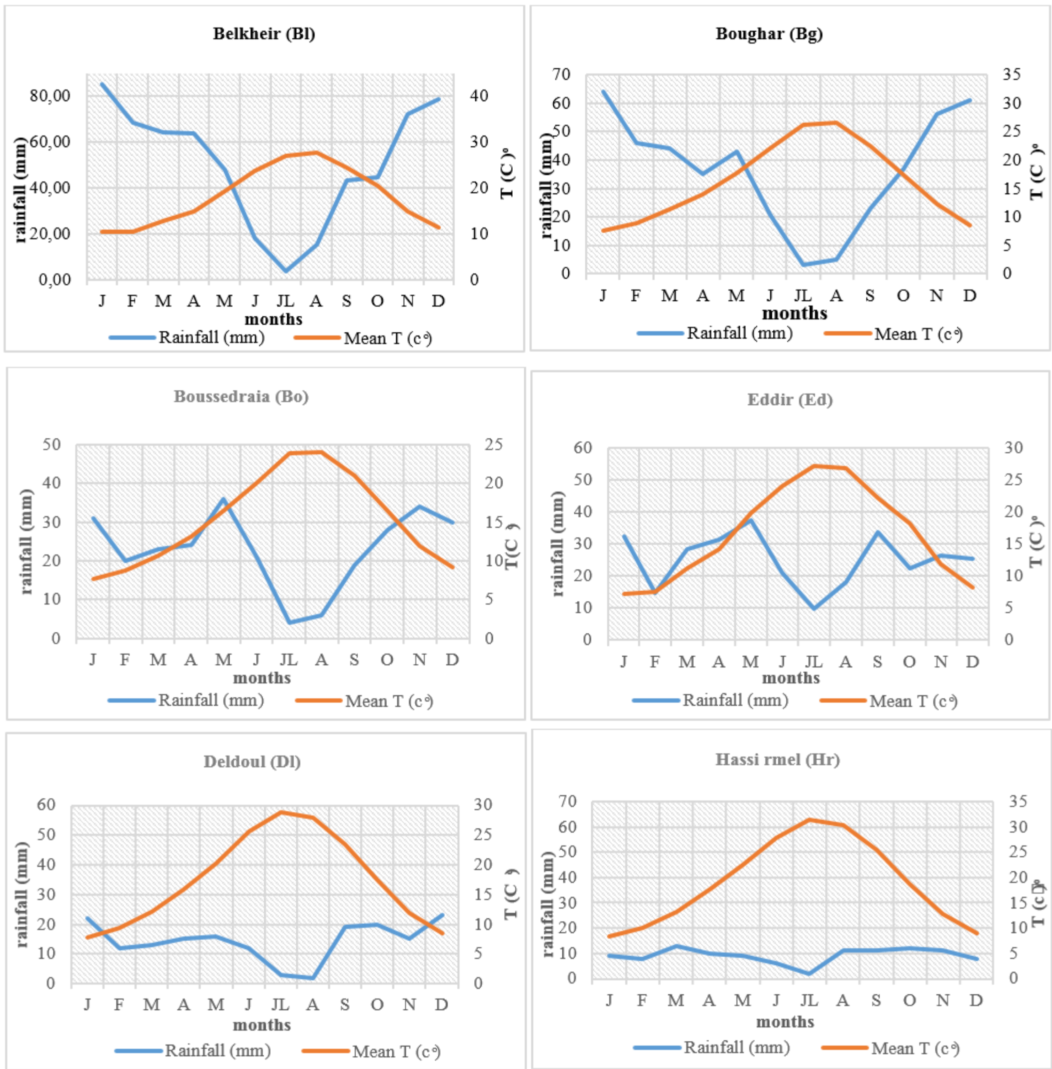
RÉSULTATS

1. Bioclimatic data

The bioclimatic study showed that the rainiest site is Belkheir with 605.9 mm/year of the total average rainfall. The rainiest month is January at Belkheir and Boughar, December at Deldoul, November at Eddir and Boughar and finally March at Hassirmel and Lahmer. At Belkheir, Boussedraia, Boughar and Deldoul the maximum of cumulative rainfall occurs during the winter season. On the other hand, at Lahmer, Eddir and Hassirmel fall and summer are the rainiest seasons.

The maxima values for temperature were recorded between July and August and the minima values, between December and January, for all the sites.

Based on Emberger'spluviothermal index Q_3, Belkheirwas classified under sub-humid and temperate climate, moreover, Boughar and Eddir are, both, under semi-arid bio-climate with temperate and fresh winter, respectively. Djelfa sites, Boussedraia and Deldoul, are under arid fresh bioclimate. Finally, Hassirmel and Lahmer sites are under a Saharianbioclimate with fresh winters (Table 1). The ombrothermic diagram showed, for Boussedraia and Boughar sites, a long dry season lasting for six months, while Eddir and Deldoul sites presented even longer dry season (ten months). Hassirmel and Lahmerwere characterized by the longest dry season (12 months). The remaining sites showed a dry season less than six months (Fig. 2 and Table 1).



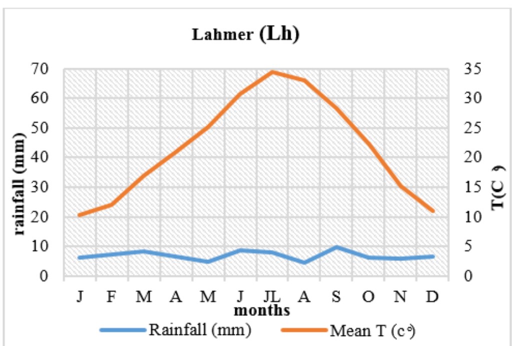


Figure 2: Ombrothermic diagrams of studied sites (Bl: Belkheir; Ed: Eddir; Bg: Boughar; Bo: Boussedraia; Dl: Deldoul; Hr: Hassirmel; Lh :Lahmer) and their arid season.

Environment conditions varied greatly among sites, The De martonne aridity index, allowed the identification of a humid climate for Belkheir site, a sub humid climate for Eddir and Boughar. The remaining sites, Boussedraia, Deldoul, Hassirmel and Lahmerwere classified under a semi-arid climate. The continentality index of Gorbunov showed that five sites are under semi-continental climate (Belkheir, Eddir, Boughar, Boussedraia and Deldoul) while the two remaining sites (Hassirmel and Lahmer) are under continental climate (Table 2). Concerning the seasonal type, winter is the rainiest season for BI, Ed, Bo and DI sites, while Fall was the rainiest for Bg, Hr and Lh sites (Table 1).

2. Soil analysis

For the soil samples description, Belkheir site is characterized by a clay loamy soil, particularly rich in organic matter with a low electrolyte and limestone fraction, while Boussedraia, Boughar and Eddir soils were composed mostly of sand. However, Deldoul, Hassirmel and Lahmer soils contained more sand than other components, poor in organic matter with higher limestone amounts. Soils were slightly to moderately alkaline, the maximal pH value was recorded at Deldoul site (Table 4).

Table 4: Soil properties of the studied sites (Values are means ± standard deviation).

Study sites	Belkhir (Bl)	Boughar (Bg)	Edir (Ed)	Boussedraia (Bo)	Deldoul (Dl)	HassiRmel (Hr)	Lahmer (Lh)
Soil type	Clay loam	Clay loam	Loamysand	Loamysand	Sandy loam	Sandy loam	Sandy loam
Soil pH/H_2O	7.8 ± 0.02	7.9 ± 0.12	8.1 ± 0.12	8.1 ± 0.1	8.2 ± 0.15	8.1 ± 0.08	7.85± 0.18
Soil EC (mS/cm)	0.8 ± 0.19	0.8 ± 0.15	0.6 ± 0.15	1.2 ± 0.16	1.3 ± 0.19	1.5 ± 0.15	1.2 ± 0.12
Humidity (%)	1.8 ± 0.12	1.3 ± 0.14	0.6 ± 0.12	0.4 ± 0.12	0.4 ± 0.12	0.6 ± 0.19	0.3 ± 0.15
Total limestone (%)	7.4 ± 0.1	13.0 ± 0.19	15.8 ± 0.15	20.7 ± 0.19	20.1 ± 0.15	17.5 ± 0.15	7.8 ± 0.12
Active limestone (%)	0.1 ± 0.12	3.8 ± 0.1	4.0 ± 0.16	4.3 ± 0.15	3.8 ± 0.19	3.1 ± 0.12	1.4 ± 0.15
Organicmatter (%)	2.6 ± 0.19	1.0 ± 0.12	1.1 ± 0.12	1.7 ± 0.19	0.6 ± 0.16	0.2 ± 0.12	0.4± 0.19

3. Morphological analysis

Concerning the descriptive analysis of the morphological study, significant differences occurred almost in all the studied leaf and stoma variables ($p<0.01$). Boughar site had the longest leaves with a mean value of 2.14cm. While, the largest leaf size was observed at Belkheir site with an average of 1.24cm. The lowest variation was recorded in petiole length (Table 5).

Z. lotus Leaves are amphistomatic, the stomata are present, both, on the adaxial and abaxial leaf surface. Stomatal size and density showed differences between adaxial and abaxial leaf surfaces. Stomatal lenght and width on adaxial surfaces were greater than those of the abaxial surfaces, inversely, the stomatal density was higher than to the adaxial face. Belkheir presented higher stomatal density values for both leaf surfaces than the other sites. While the highest adaxial stoma size was observed at Boughar (Table 4).

Coefficients of variation (Cv), ranging from 5.82% to 52.77%, were higher in Lahmer population for most leaf and stoma measurements. ANOVA analysis showed highly significant variation ($p<0.01$) both within and among populations (Table 6), although, no significant variation was observed within leaves of the same shrub for all the measured variables (variance within individuals).

The correlation matrix (Table 7) indicated strong significant correlation between most of the leaves and stomata variables. Significant correlations were recorded between stoma widths on both leaf surfaces (r=0.94; $p<0.001$) and between stoma length and stoma width on the adaxial surface (r=0.93; $p<0.001$). Significant and positive correlations were recorded between leaf length and leaf width (r=0.83; $p<0.01$), between stomatal densities on the abaxial and adaxial surfaces (r=0.80; $p<0.01$) and between stomatal size and stomatal density on both surfaces. However negative correlation between petiole length and the remaining leaf and stoma characters, were recorded.

The first DA of individuals based on leaves characters (Fig. 3a) showed that individuals of Boussedraia, Boughar, Deldoul, Eddir and most individuals of Belkheir were more similar to each other and formed one cloud. Thus, the DA allowed us to distinguish Hassi rmel population and most individuals of Lahmer from the other populations. Populations were differentiated by leaf length (LL) and petiole length (PL) with partial Wilks'-λ of 0.5998 and 0.5758, respectively. The first HCA dendrogram based on leaves morphological similarity (Fig. 3b) revealed two different clusters. The first group divided onto two subgroups: the first one composed of Boussedraia population characterized by the highest value of the leaf length and the second subgroup consisted of Boughar, Deldoul, Eddir, Lahmer and Belkheir sites characterized by a medium leaf size. The second group consisted of Hassi rmel populations which had the smallest leaf size.

Table 5: Quantitative characterization of *Ziziphus lotus*leaves and stomata in the studied sites.

Variables	Belkhir (Bl)	Boughar (Bg)	El dir (Ed)	Deldoul (Dl)	Bousedraia (Bo)	Hassirmel (Hr)	Lahmer (Lh)	Mean Values
Leaflength (LL) (cm)	2 e ± 0.3 0.8 - 3.2 (16.9)	1.92 b.c ±0.3 0.9-3.1 (17.11)	1.9a.b ± 0.3 0.6-3 (18.72)	1.9 c ± 0.2 1.3 - 3.1 (15)	2.1 f ± 0.3 1.1 - 3.3 (19.2)	1.5 d ± 0.3 1 - 2.7 (16.8)	1.8 a ± 0.3 0.6 - 3.1 (18.8)	1.9 ± 0.3 1.1-3.3 (15.9)
Leaf width (LW) (cm)	1.2 c ± 0.3 0.7 - 2.2 (23.6)	1.1 a ± 0.2 0.6 - 2.2 (19.3)	1.1 c± 0.2 0.5 - 2.6 (20.1)	1.1 a.b± 0.2 0.5 - 2.2 (20.3)	1.1 a.b ± 0.2 0.5 - 2.2 (22.4)	0.8 d ± 0.1 0.5 - 1.9 (21.7)	1.1 b ± 0.2 0.4 - 2.6 (24.1)	1.1± 0.2 0.5 -2.6 (22.1)
Leaf L/W ratio (R1)	1.6 a± 0.3 0.4 - 2.5 (22.3)	1.77 a ± 0.3 0.7 - 2 (17.1)	1.6 c ± 0.3 0.5-2.6 (19.5)	1.7 a ± 0.2 1- 2.6 (14.3)	1.9 b ± 0.3 0.9 - 2 (16.8)	1.9 b ± 0.3 0.1 - 2.2 (20.2)	1.8 d ± 0.3 1.1 - 2 (18.9)	1.7 ± 0.1 0.1 -2.6 (19.4)
Petiole length (PL) (cm)	0.2 a.b± 0.08 0.1 - 0.5 (40.9)	0.17 a ±0.05 0.08 - 0.4 (33.1)	0.2 a.b± 0.1 0.5 - 1 (47.2)	0.1 a ± 0.06 0.1- 1.4 (37.8)	0.2 b ± 0.08 0.7 - 1.5 (31.5)	0.2 a.b ± 0.07 0.08 - 0.9 (36.9)	0.2 a.b ± 0.06 0.08 - 1 (29.9)	0.2± 0.1 0.1 - 2.5 (48.7)
Adaxial stoma lenght (DSL)(µm)	28.6 d ± 5 15.3 - 44.5 (17.7)	34.9 f ± 9.4 18.2 - 37.9 (26.9)	31.2 e ± 3.2 20.8 - 38.8 (10.4)	25.6 a ± 3.7 19 - 31.6 (14.5)	21.6 b ± 2.9 13.5 - 29 (13.6)	25.1 c ± 2.3 15.5 - 28.7 (9.2)	27.6 a ± 6.2 12.9 - 44.5(22.6)	26.2 ± 2.1 12.9-31.6 (8.3)
Adaxial Stoma width(DSW)(µm)	18.9 f ± 2.7 10.6 - 27.3 (14.3)	23.7 h ± 5.2 16.8 - 46.1 (22)	21.7 g ± 2.1 17 - 32.6 (9.7)	18.2 e ± 1.9 14.1 - 25 (10.8)	11.6 d ± 1.8 7 - 19.5 (15.6)	12.4 a ± 2 9.1 - 23 (16.1)	17.4 b ± 5 7 - 46.1 (28.8)	15.4 ± 1.9 11.5 - 23.1 (12.7)
Adaxial Stoma L/W ratio (R2)	1.5 c ± 0.2 0.9 - 2.4 (16.8)	1.4 b ±0.2 0.9 - 2.2 (17)	1.1 a.b ± 0.09 0.8 - 1.6 (8.1)	1.4 a ± 0.2 0.9 - 1.9 (15.6)	1.8 d ± 0.2 1 - 2.6 (13.6)	2 e ± 0.3 1 - 2.9 (17.5)	1.6 f ± 0.3 0.8 - 2.9 (20.7)	1.7 ± 0.2 0.8 - 2.2 (12.5)
Adaxial stomatal density(DSD) (stomata/µm²)	120.3 c ±18.3 73.6 - 152.6 (29.2)	117.4 c ± 23.4 63.1 -173.6 (19.9)	104.2 a ± 11.5 68.4 - 173.6 (11)	108.1 b ± 10.8 68.4 - 142.1 (10)	110.3 a ± 13 68.4 -142 (11.8)	83.8 b ± 10.4 57.8 -100 (12.4)	106.8 d ± 21.3 42.1- 194.7(19.9)	103.1 ± 30.1 42.1 - 194.7 (29.2)
Abaxial stoma lenght (BSL)(µm)	26 b ± 4.1 18.1 - 39.4 (15.7)	25.4 b ± 4.4 17.8 - 44.9 (17.6)	22.6 a ± 2.1 18.9 -28.8 (9.1)	24 e ± 3.2 18.1 -31.5 (13.4)	21.2 a ± 2.2 14.8 -28 (10.7)	23.3 c ± 2.9 15.1 -29.8 (12.4)	23.5 d ± 3.5 14.8- 44.9 (15.1)	22.3 ± 2.6 16.5 - 29.2 (11.8)
Abaxial Stoma width (BSW)(µm)	19.6 a ±3 13 - 29.5 (15.2)	19.4 a ±2.9 14.3 - 33.2 (15)	19.6 a ± 0.1 13.9 - 22.8 (5.8)	18 e ± 2.1 13.1 - 24.3 (11.9)	13.8 d ± 1.5 9.3 - 19.1 (11.4)	15.2 b ± 1.9 9.6 - 24.7 (12.7)	17.4 c ± 3.1 9.3 -33.2 (17.8)	16.2 ± 2 11.9 - 23 (12.7)
Abaxial Stoma L/W ratio (R3)	1.3 a ± 0.1 0.9 - 1.7 (12.3)	1.3 a ± 0.1 0.8 - 1.7 (12)	1.4 c ± 0.1 1 - 1.8 (11.1)	1.3 a ± 0.1 1 - 1.8 (12.4)	1.5 d ± 0.1 0.9 -2 (12.3)	1.5 b ± 0.2 0.9 - 2.2 (15.2)	1.3 b ± 0.2 0.8 - 2.2 (15.8)	1.3 ± 0.1 1 - 2 (12.6)
Abaxial stomatal density(BSD) (stomata/µm²)	488.1 e ±28.8 405.2 - 584.2 (14)	413.8 d ± 36 342.1 - 536.8 (8.7)	423.9 b ± 38.1 352.6 -521 (8.99)	354.8 a ± 27.1 278.9 - 415.7 (7.6)	357.8 b ± 22.9 278.9 - 415.7 (5.9)	318.9 a ±35 268.4 - 415.7 (6.4)	396 c ± 6.7 268.4-584.2(11)	396.9 ± 59.3 257.8 -573.6 (16.1)

Mean±Standard Deviation; The extreme values (the lowest and the highest value), C.V.: coefficient of variation (%), Fisher test : a.b.c.d.e.f.g.

Table 6: ANOVA test for the leaf and stoma morphological variables within and among *Ziziphus lotus*populations.

		SS	d.f	MS	F	*p*	Significance
Leaflenght (LL)(cm)	Populations	128.38	6	25.68	238.2	0.000000	***
	Individuals	40.20	24	1.68	34.2	0.000000	***
	Leaves	8.10	29	0.28	2.1	0.0004	***
Leafwidth (LW)(cm)	Populations	67.47	6	13.49	63.88	0.000000	***
	Individuals	351.34	149	2.49	32.63	0.000000	***
	Leaves	16.93	29	0.58	2.63	0.000004	***
Petiolelenght (PL)(cm)	Populations	16.71	6	3.34	1.69	0.13	NS
	Individuals	349.29	149	2.34	1.19	0.05	NS
	Leaves	58.6	29	2.02	1.02	0.42	NS
Adaxial stoma lenght (DSL)(µm)	Populations	29131	6	4855	214.81	0.000000	***
	Individuals	31933	29	1101	51.76	0.000000	***
	Leaves	0.448	4	112	2.87	0.021	*
Adaxial stoma width (DSW)(µm)	Populations	30898.8	6	5149.8	659.55	0.000000	***
	Individuals	30875.4	29	1064.7	134.32	0.000000	***
	Leaves	150.8	4	37.7	1.48	0.20	NS
Adaxial stomatal density (DSD)(stomata/µm²)	Populations	21481.9	6	3580.3	107.73	0.000000	***
	Individuals	28704.7	29	9898	33.58	0.000000	***
	Leaves	335.5	4	83.9	1.85	0.11	NS
Abaxial stoma lenght (BSL)(µm)	Populations	4410.4	6	735.1	70.92	0.000000	***
	Individuals	7728.4	29	266.5	31.1	0.000000	***
	Leaves	280.7	4	70.2	5.52	0.0002	***
Abaxial stoma width (BSW)(µm)	Populations	8439.2	6	1406.5	289.0	0.000000	***
	Individuals	9176.6	29	316.4	70.3	0.000000	***
	Leaves	74.8	4	18.7	1.94	0.12	NS
Abaxial stomatal density (BSD)(stomata/µm²)	Populations	4867.2	6	8112.14	5720	0.000000	***
	Individuals	4837.6	29	4837.62	114.7	0.000000	***
	Leaves	1717.7	4	1717.7	1.02	0.39	NS

Table 7: Matrix of correlation between leaf and stoma morphological variables.

	LL	LW	PL	DSL	DSW	DSD	BSL	BSW	BSD	Alt	P	M	m	pH	EC	Hum	Total lime	Active lime	OM
LL	-	0.83*	-0.09	0.39	0.37	0.00	0.45	0.49	0.40	-0.32	0.46	-0.55	-0.31	-0.18	-0.81*	0.22	-0.25	0.32	0.39
LW		-	-0.07	0.60	0.63	0.14	0.63	0.81*	0.54	-0.48	0.65	-0.59	0.09	-0.36	-0.61	0.37	-0.22	0.38	0.53
PL			-	-0.43	-0.59	-0.48	-0.44	-0.43	-0.09	-0.23	-0.16	0.68	-0.09	-0.04	0.1	-0.18	-0.18	-0.4	-0.03
DSL				-	0.93**	0.39	0.62	0.85*	0.50	-0.05	0.67	-0.78*	0.57	-0.45	-0.54	0.5	-0.28	0.44	0.44
DSW					-	0.55	0.62	0.94**	0.60	-0.12	0.68	-0.88*	0.46	-0.34	-0.42	0.47	-0.04	0.65	0.43
DSD						-	0.47	0.52	0.80*	-0.54	0.74	-0.72	0.34	-0.53	-0.31	0.75	-0.17	0.71	0.69
BSL							-	0.72	0.42	-0.54	0.76*	-0.67	0.56	-0.76*	-0.52	0.79*	-0.62	0.11	0.77*
BSW								-	0.67	-0.37	0.77*	-0.82*	0.43	-0.43	-0.43	0.54	-0.1	0.61	0.57
BSD									-	-0.76*	0.93*	-0.68	0.29	-0.67	-0.66	0.76*	-0.38	0.67	0.85*
Alt										-	-0.75	0.28	-0.23	0.74	0.39	-0.76*	0.52	-0.23	-0.88*
P											-	-0.77*	0.5	-0.83*	-0.69	0.91*	-0.56	0.53	0.94*
M												-	-0.21	0.41	0.67	-0.61	0.17	-0.74	-0.59
m													-	-0.71	0	0.62	-0.51	-0.12	0.48
pH														-	0.53	-0.95**	0.87*	-0.01	-0.93*
EC															-	-0.57	0.6	-0.34	-0.64
Hum																-	-0.74	0.26	0.96**
Total lime																	-	0.34	-0.72
Active lime																		-	0.3
OM																			-

Level of significance : *. $p< 0.05$; **. $p< 0.01$; ***. $p< 0.001$; NS : Not significant

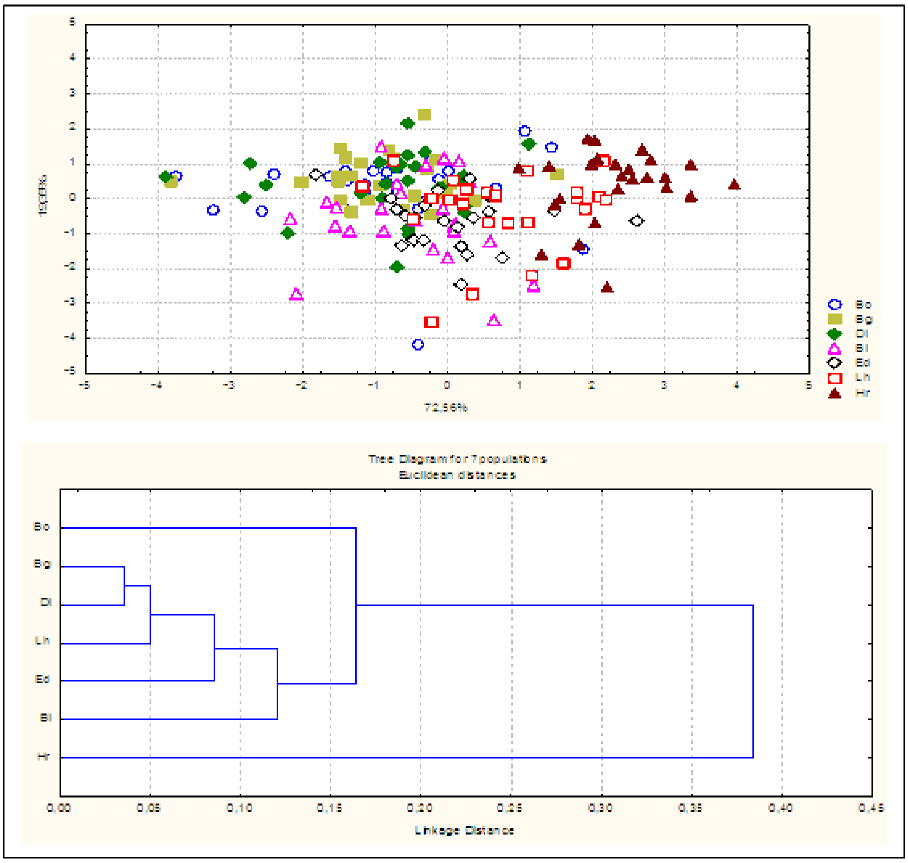


Figure 3: Results of descrimination analysis for *Z. lotus* leaves caracters (a) ; Classification of study sites based on leaves morphological data (b) (Bl: Belkheir; Ed: Eddir; Bg: Boughar; Bo: Boussedraia; Dl: Deldoul; Hr: Hassirmel; Lh : Lahmer).

The second DA of individuals based on stomata characters (Fig. 4a) showed that individuals of Hassi rmel population were closer to those of Lahmer and Deldoul populations. While, the majority of Boussedraia individuals were closer to those of Belkheir, Eddir and Boughar populations. Groups of populations were differentiatied mostly by adaxial stoma length, adaxial stoma width, adaxial stomatal density and abaxial stomatal density (DSL, DSW, DSD and DSB) with partial Wilks' - λ of 0.027, 0.040, 0.030 and 0.063, respectively. The second HCA dendrogram based on the stomatal character's similarity (Fig. 4b) allowed the subdivision of the populations onto three different groups: Boussedaia site formed the first group, the second group divided onto two subgroups (the first one consisted of Boughar population which had the highest stomatal size and the second one consisted of Belkheir and Eddir populations). The third group, which had the lowest values of stomatal density, is separated onto two subgroups (the first one consisted of Deldoul and Hassirmel populations and the second one of Lahmer population). The common characteristic between these populations is that they are situated in arid environment with the longest dry season.

For the qualitative leaf characteristics, the results showed differences within and among the different sites. The ovate to round-ovate leaf shape were the most common in Belkheir site, while broad lanceolate to elliptic leaves were recorded in Boughar, Eddir and Boussedraia sites. Ovate leaves are common in Deldoul and Hassi rmel sites while more broad lanceolate to ovate leaves are recorded in Lahmer site. For the petiole, rounded straight adaxially to rounded shapes were recorded in all the sites. However, the most common shapes for the leaf apex recorded in Belkheir, Deldoul, Hassirmel, Eddir and Lahmer sites were acuminate to mucronate, while the mucronulate shape was the most important in Boussedraia site. The leaf base was obtuse in five sites (Belkheir, Boughar, Boussedraia, Deldoul and Hassi rmel), and the attenuate shape was recorded in Eddir and Lahmer sites. Moreover, the leaf margins were serrulate to dentate in Eddir and Boussedraia sites and sinuate to entire in the remaining five sites (Table 8. Fig. 5).

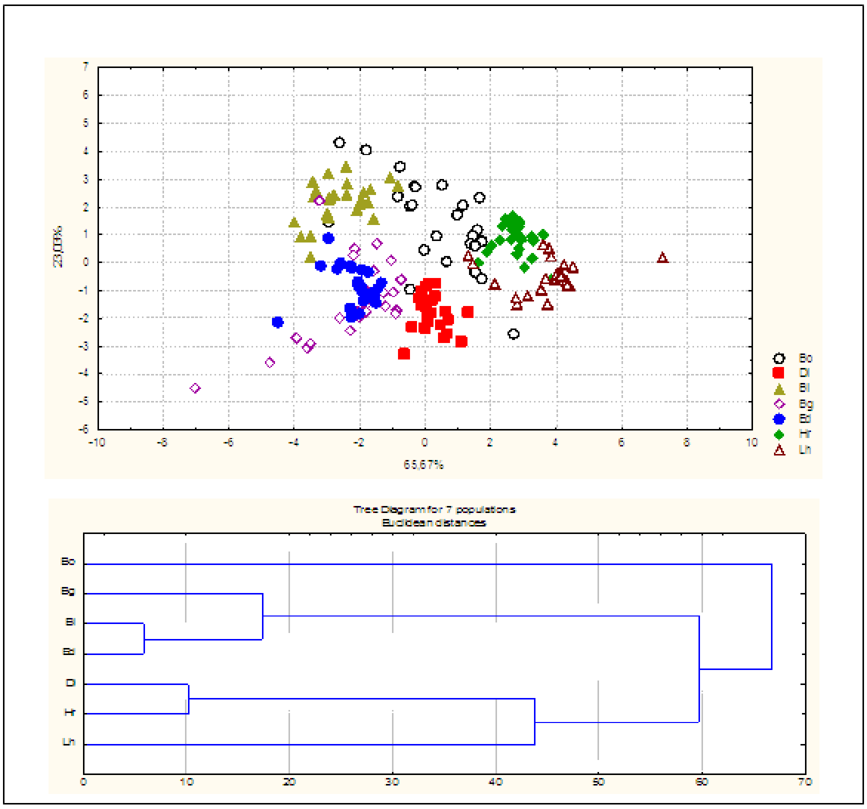


Figure 4: Results of descrimination analysis for Z. lotus stomata caracters (a); Classification of study sites based on stomata morphological data (b) (Bl: Belkheir; Ed: Eddir; Bg: Boughar; Bo: Boussedraia; Dl: Deldoul; Hr: Hassi rmel; Lh: Lahmer).

Table 8: Qualitative variables measured for *Ziziphus lotus* leaves for the studied sites.

Main variables	Sub-variables	Mean for the species (%)
Leaf shape (L)	Broad lanceolate (1)	31.9
	Ovate (2)	39.4
	Elliptic (3)	15.3
	Round ovate (4)	11.4
	Roundish (5)	2.0
Petiole shape (P)	Rounded (1)	42.1
	Flattened (2)	13.4
	Rounded straight adaxially (3)	44.5
Leaf baseshape (B)	Attenuate(1)	29.0
	Obtuse (2)	54.6
	Truncate(3)	7.1
	Oblique (4)	9.3
Leaf apex shape (A)	Acuminate(1)	52.1
	Mucronate (2)	22.1
	Obtuse (3)	12.7
	Mucronulate (4)	11.5
	Retuse (5)	10.6
Leaf margin (M)	Entire (1)	28.5
	Serrulate (2)	52.5
	Dentate (3)	19.0
Leaf epicuticular wax (W)	Absent (0)	0.03
	Rare (1)	0.00
	Remarkable (2)	35.4
	Abundant on the abaxial surface (3)	64.5
Leaf trichomes (T)	Absence (0)	63.5
	Rare (1)	29.7
	Remarkable (2)	4.9
	Numerous on the abaxial surface (3)	1.8

Figure 5: Comparison among provenances of *Z. lotus* leaves in terms of: (A) Leaf shapes, (B) Apex shapes, (C) Base shapes, (D) Margin shapes and petiole shapes (E).

Scanning electron microscope images showed that *Z. lotus* leaf surfaces (abaxial and adaxial) were covered with epicuticular waxes on all samples, moreover, waxes density was higher in Lahmer, Boughar and Eddir sites, with a snowy white appearance covering the epidermis cells and the stomata (Figs 6G and 6H). On the other hand, the epidermal surfaces in Belkheir site looked glossy (Figs 5E and 5F). Stomata varied in shape, size and distribution between the abaxial and adaxial leaf surfaces. On the adaxial surface, very few stomata were irregularly arranged on both sides of the main vein (Figs 6A and 6B). While, On the abaxial surface, the stomata were oval shaped and typically larger, distributed over all the abaxial leaf surface (Figs 6C and 6D).SEM observations allowed the identification of two types of trichomes, however, trichomes were not found on the adaxial surface, they were present on the abaxial surfaces, non-glandular trichomes were observed with low density especially around the veins (Figs 7E and 7F), their density was higher in Boussedraia (Figs 7A and 7B). In contrast, glandular trichomes were rarely observed on the abaxial surface (Figs 7C and 7D).

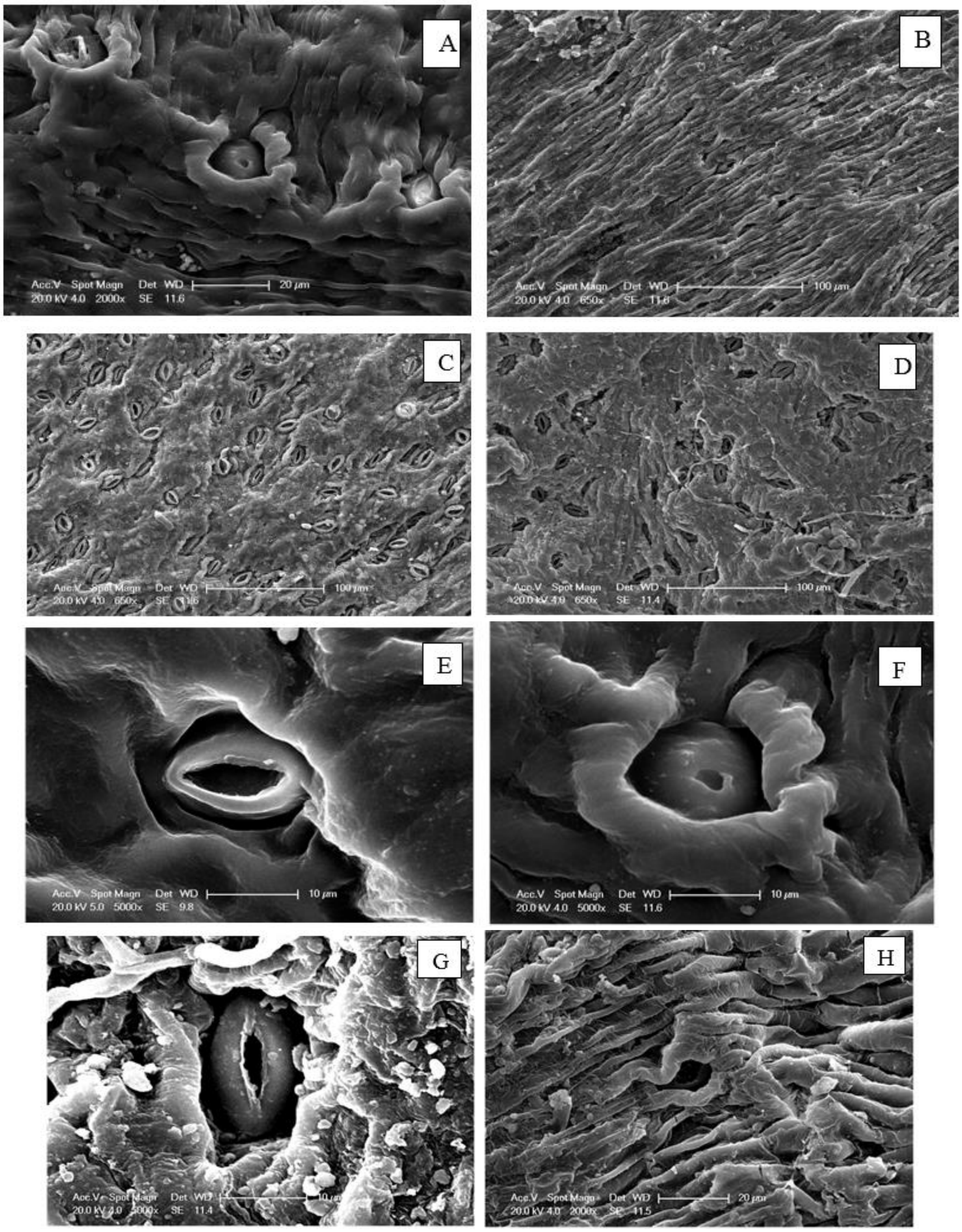


Figure 6: SEM micrographs showing: stomata low density and their distribution on the adaxial surface in (A) Bl and (B) Bo, stomata high density and their distribution on the abaxial surface in (C) Bg and (D) Hr, stomata in Bl (E) on the adaxial surface (F) on the abaxial surface, (G) and (H)waxes

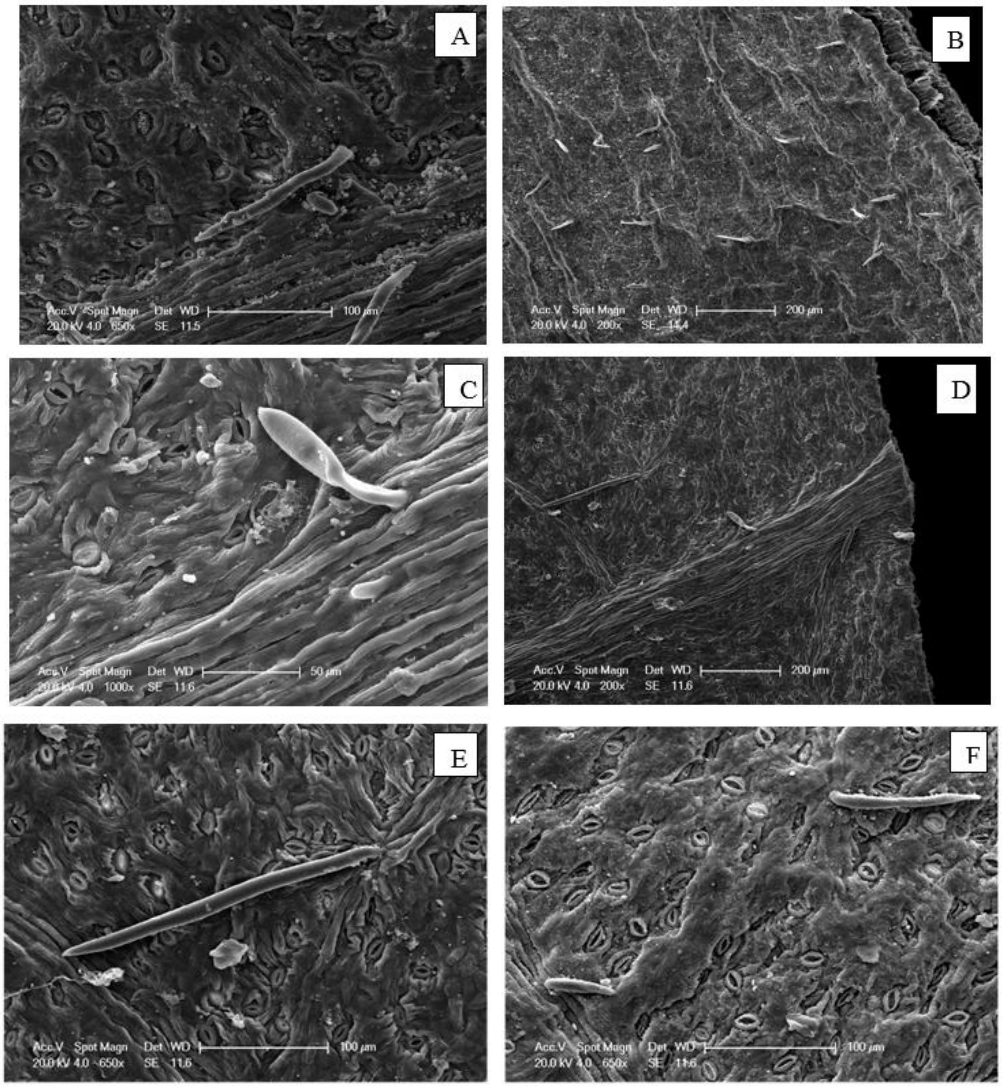


Figure 7: SEM micrographs showingtrichomes on leaf surface of *Z.lotus* : (A) and (B) Non-glandular trichomes on the abaxial surface and the adaxial surface in Bo (C) and (D)) glandular trichome on the abaxial surface in Bl, (E) and (F) abaxial leaf surface showed hight stomatal density and trichomes in Bl and Bg.

4. Morphological and environmental conditions correlations

Significant correlations between stoma traits and environmental variables were recorded (Table7). Positive correlation was observed between abaxial stomatal density and average annual precipitation (r=0.93, p<0.01). Abaxial stoma length and width were negatively correlated with mean maximum temperature (r=0.88, p<0.01; r=0.82, p<0.01), respectively. While, all variables were negatively correlated with soil pH, soil EC and soil total limestone amounts; also, abaxial stomatal density and abaxial stoma length were positively correlated with organic matter (r=0.85, p<0.01; r=0.77, p<0.01), respectively.

DISCUSSION

The wild jujube occupies a wide geographical distribution which demonstrate its great ability of adaptation to different climates and environmental conditions [43], it's a good exemple of plasticity to adapting in arid regions, especially at leaves level [44]. In this study *Z. lotus* leaves micro and macro morphological variability were evaluated in seven wild populations in Algeria, based on quantitative and qualitative leaf and stoma traits. On the other hand, we investigated the effect of ecological factors on leaf morphology variation (soil and bioclimate conditions). The results revealed a high variability among and within populations.

The quantitative traits were significantly correlated with the variation of ecological conditions, the longest leaves were located in boussedraia (arid bioclimate) characterized by lower precipitation and a long dry season, while, the largest leaves were located in Belkheir (subhumid bioclimate) characterized by higher precipitation. However, the smallest leaves were located in hassi rmel (Saharan bioclimate) characterized by the longest dry season. Our results are similar to those recorded by Rais et *al.* [26], in their study conducted on three *Z. lotus* populations from different bioclimatic zones in marroco, when, the smallest leaves camed from the arid region, and the longest ones from the semi-arid region.

Our results reported that the abaxial stomata presented significant differences in size and density in the different populations, this variation induced by environmental conditions, such as precipitation, temperature and soil composition, the longest leaves sampled from Boussedraia site had the smallest stomata, however the leaves of Hassirmel had the lowest stomata densities, Belkheir had the biggest stomata and the highst stomatal densities in both leaf surfaces. Our results are similar to those recorded by Kang et *al.* [45], with the decrease of water content in the habitats, *Ziziphus jujube* var. *spinose* stomata became smaller gradually, and their density increase gradually. This hypothesis is supported by other studies conducted, in Algeria, on *Z.lotus* fruits diversity and the influence of the environmental conditions in Algeria [43 and 46], which revealed that the environment has a considerable effect on the morphological variability among the populations for the majority of the studied traits. Tree elasticity is due to itscapacity to develop morphological and physiological adaptation strategies to water stress, especially, these adaptation strategies are based on stomatal control and reduction of leaf size [47].

On the other hand, qualitative traits were not clearly affected by the environmental conditions; hight variation in leaf shape were observed even in each specimen in the same population, Tatari et *al.* [48], confirms that climatic conditions have an important impact on the morphological traits of *Z. jujuba* ecotypes.

Azam-Ali et *al.* [49], reported that the genus *Ziziphus*, presents a hight phynotypic and genotypic diversity within the same population. The differences within the same population might reflect the genotypic effects [50]. The observation of *Z.lotus* in different phenological states showed a high variation among and within sites. Variations among sites could be explained by the influence of temperature, soil moisture, photoperiod, exposure, soil texture and drought [51].

No information is available in the literature concerning *Z. lotus* stoma and epedermis morphology, Scanning electron microscope images provided supplementary information about the leaf micromorphology. Bothadaxial and abaxial leaf surfaces were covered by waxes. Stomatal densities were higher in the abaxial surface, non-glandular trichomes were observed on the abaxial surface, especially arround the veins, but, they were absent on the margins of leaves. In arid environments, xenomorphic plants reduce the leaves size and cover them with trichomes and waxes to reduce loss of water by transpiration [52]. Similar results were recorded for plants from the genus *Pistacia.* For instance, *P. atlantica* which occurs in similar environmental conditions as *Z .lotus*, in arid and semi-arid lands [40].

CONCLUSION

The current study allows the identification of more morphological characteristics of wild jujube in Algeria (leaf size, petiole, leaf shapes, stoma size, stomata density and leaf epidermis surface). Our results showed a high morphological variability within and among pouplations for the majority of leaf and stoma traits. The variation was clearly marked by the environmental conditions. The present study may be useful to provide a data base for morphological description of *Ziziphus lotus* L. and help to understand the different adaptation mechanisms and strategies of the plant in the different habitats.

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