Foliar microphytodermal characterization of *Pistacia lentiscus* under different bioclimates from Northwest Algeria

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Received: September 09th, 2016; Accepted: February 15th, 2017

Abstract: Very abundant in North Africa, *Pistacia lentiscus* extends from Morocco and Spain to Minor Asia and Palestine. It reappears as a distinct breed, *emarginata* variety Ang. in Eastern Africa. *P. lentiscus L.*: the mastic tree of *Pistacia* genus (Anacardiaceae family) is a medicinal taxon. This study aims to reveal the microphytodermal structure of *P. lentiscus* leaflets from three sites (Maghnia, Beni-Saf and Tessala) under different environmental conditions (Arid semi-continental and semiarid littoral and continental) from Northwest Algeria. Samples were treated and observed under light and scanning electron microscopy (SEM). The results revealed a highly diversified structure for epidermal hypoamphystomatic leaflets. The stomata are in pericytic, actinocytic, paracytic and desmocytic types, never mentioned as stomatal complex of mastic tree before . Capitate and pelted glandular trichomes were observed. The climate impact is reflected differently by rugueues to smooth stomatal surfaces, strongly to slightly covered by wax particles. Continentality of the climate and high altitudes (989 m) are expressed by low stomatal density (272,50 st /mm^2).

Keywords : Continentality, hypoamphystomatic, microphytodermal structure, *Pistacia lentiscus*, stomatal density, trichomes, wax.

1. Introduction

Mastic: *Pistacia lentiscus* is a species of *Pistacia* genus (Anacardiaceae family). It is an evergreen shrub. The tough leaves with visibly winged rachis wear 2-3 pairs of ovate to elliptic or oblong leaflets (Zohary 1996). Flowering of male and female plants overlap between March and May. Male flowers are grouped in inflorescences of 8 to 10 flowers, and female flowers are combined into inflorescences of 4 to 21 flowers (Jordono 1988). The fruit is about 3-4 mm in diameter, red at first, then turns black at maturity. Although it is easily distinguished by its strong smell of resin, mastic is a main essence of the Mediterranean

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maquis thermophylle at low altitude (0-500m) (Zohary 1952). Its general distribution encompasses all Mediterranean and Macaronesian regions (Mazola et al. 1996).

Abundant in North Africa, it extends from Morocco and Spain to Minor Asia and Palestine. He reappears as a distinct race: *emarginata* Ang. variety in Eastern Africa (Zohary 1952). Four taxa of variety: *P. lentiscus* var. *mastic*, var. *Latifolia* Coss. And var. *Massiliensis* Mill (Mazola et al. 1996) and *P. lentiscus* var. *Mastichophora* (Rouskas 1996) were cited.Taxonomic interspecific interaction is indicated by a cross between the mastic tree and the terebinth named *P.* x *saportae* Burnat. *P. lentiscus* has been the subject of several studies including the treatment of its genetic and phenotypic variability (Zografou et al. 2010), the study of its phytochemical and pharmacological properties (Tassou and Nychas 1995, Benhammou et al. 2008, Amri et al. 2012, Nahida et al. 2012) and the effect of ozone on the histology of the leaves (Armiñana et al. 2004). El-Oqlah (1996) carried out morphological, anatomical and palynological studies in species of the genus Pistacia. Few works that treated its microphytodermal variability with the exeption of those of Smail-Saadoun (2005) and Al-Saghir and Porter (2005). This study aims to identify the microphytodermal structure of leaflet's of *Pistacia lentiscus* and a comparison of 03 sampling sites in different bio-climate from Northwest Algeria.

2. Materials and methods

2.1. Plant Material

Three (03) mastic stations have been prospected. Leaf samples were collected the period 2013- 2014 from Beni-Saf (BS) and Tessala (Te) stations in semi-arid bioclimatic , and Maghnia (Mg) station in arid bioclimatic (Figure 1). According to the index of continentality (Debrach 1953) established on thermal differences (M-m), Beni-Saf station is influenced by a coastal climate, that of Maghnia is marked by a semi-continental climate. Tessala station has a continental climate (Figure1). For the three prospected sites, climatic data are represented by Table 1. For each study site, a sample of 10 healthy and mature leaflets was done. Fives (05) repetitions for each site was performed.

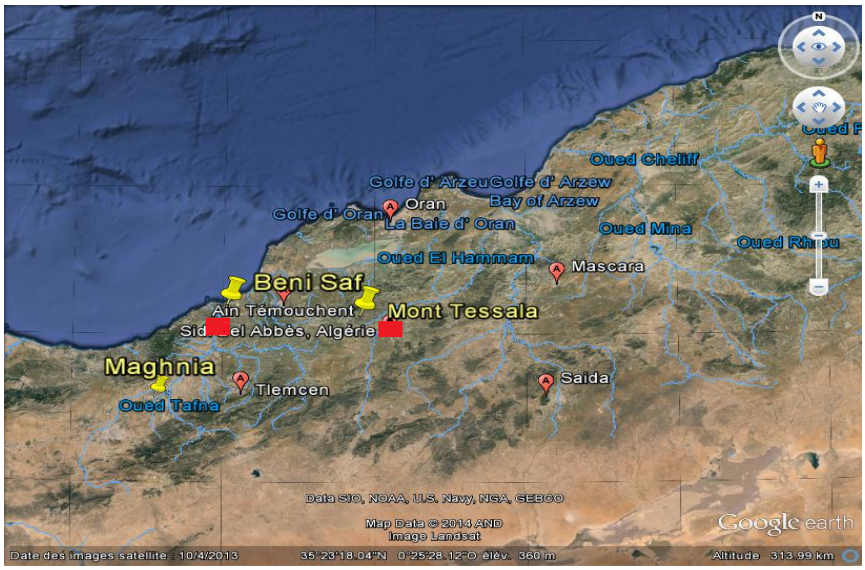

Figure 1: Location of sampling sites of *Pistacia lentiscus* (Google Earth, 2014)

Table 1: climatic and geographical coordinates of stations study

Station	Latitude	Longitude	Altitude (m)	M (C°)	m (C°)	Ann. prec. (mm)	Q_3	Climate type
Beni-Saf (Bs)	35° 18`N	1° 21`W	68 m	30,58	10.09	362,68	60,71	Semi-arid « littoral »
Maghnia (Mg)	34° 81`N	1° 78`W	428 m	35.01	3.21	249.93	26,95	Arid « Semi-continental »
Tessala (Te)	35°16'21`N	0°47'13"W	989	25,01	8,91	231,96	49,43	Semi-arid « continental »

M: average of maximum temperatures of the warmest month, **m**: average of minimum temperature of the coldest month, **Ann**. **prec**: annual precipitation, Q_3: pluviothermic quotient

2.2. Light and scanning electron microscopy

For light microscopy, a thick layer of clear nail varnish is spread on both sides of a fresh leaflet. Once the varnish layer is dried in ambient air, using a pincer, we remove the peel of the varnish which copies the topography of the leaflet and it is spread out on a wetted slide of a drop of water beforehand. The observations were made with (x10) and (x40) magnifications. When the scanning electron microscope (SEM), the samples are previously cleansed and dried. Foliar surfaces of 1 mm2 were cut and glued on the pads with the carbon double-sided tape, and the edges have been brought into contact with the Ag lacquer. Afterwards, a golden metallization (25 um) by sputtering of the samples was made. The microphotographs were

performed using SEM, type *Ieol 6320 F* (*FEGSEM*) in Marseille Interdisciplinary Nanoscience Centre, CINaM, France.

2.3. Statistical treatment

The results were statistically treated by principal component analysis (PCA) to the "Staistica 6" and (ANOVA 1) in EXCEL.

3. Results and discussion

3.1. Results

The microphytodermal characterization of leaf samples of *P. lentiscus* of 03 sites examined is represented by the table (2)

Table 2: Microphytodermal characterization of 03 study sites

Parameters	Sites	*P. lentiscus* (Bs)	*P. lentiscus* (Mg)	*P. lentiscus* (Te)
Length (L)/ width (l) of the stomata	Ad	nm	nm	nm
	Ab	16,67ǂ 2,22 / 15,78ǂ 2,27 P***	16,44ǂ 2,87 / 12,00ǂ 1,99 P***	16,89ǂ 1, 64 / 12,00ǂ 1,45 P***
Stomatal density (Ds) (Nb/mm²)	Ad	nm	nm	nm
	Ab	485± 10,00 P***	404,9± 12,85 P***	272,50± 33 ,63 P***
Stomatal size (Ss) (L x l) (µm2)	Ad	--	--	--
	Ab	263,82± 26,18 P***	195±17,09 P***	203,35± 16, 12 P***
Stomatal shape (SHs) (L/ l)	Ad	--	--	--
	Ab	1,07± 0,10 P***	1,41±0,17 P***	1,42± 0, 09 P***
Epidermal cells		Polygonales		
Stomatal complex		Peri., Actin., Para.., Desmo.		
Trichomes		T. G. pelted , T. G. capitate		

nm : Unmeasurable, **Ad** : adaxial, **Ab** : abaxial, **L**: length, **l**: width, **nb**: number, **Peri**. : pericetic, **Actin**. : actinocytic, **Para**. : paracytic, **Desmo**. : desmocytic **P***: very highly significant at 5%, (P<0,01)

The microphytographies samples of *P. lentiscus* of the 03 sites examined, observed under scanning electron microscope (SEM) revealed a rough and rigid structure on the adaxial side (Figure 2. A, C, E). At (6000) magnification, this roughness is accentuated by the abundance of epicuticular waxes in the form of flakes on samples of Beni Saf (Bs), Tessala (Te) and in the form of a mass in samples Maghnia (Mg), with the absence of stomata on the observed surfaces 03 (Figure 2, B, D, F). On the abaxial surface, the median nervue is projecting from leaf samples of 03 sites, less reduced width on samples (Mg) (Figure 3. A, C, E). Stomata are

sunk into the epidermal surface samples (Bs) (Figure 3. B), slightly submerged in the sample (Mg) and (Te) (Figure 4. D, F). The surface of the stomata is less smooth on samples (Bs), smooth over those of (Mg), steep and covered with epicuticular wax flakes on samples (Te) (Figure 3 A, C, E). Stomata samples of the (Bs) reveal external stomatal slightly raised ridges, thick lined with little resin particles, smooth in (Mg) samples. Those samples (Te) are thick and slightly crenellated (Figure 4. A, C, E). External crests of the stomatal ostiole are slightly and irregularly pleated on the stomata (Bs), highly pleated longitudinally on the stomata of (Mg) and (Tc) (Figure 4 B, D, F). Outer stomatal ledges of ostiole are slightly and irregularly pleated on the stomata (Bs), highly pleated longitudinally on the stomata of (Mg) and (Te) (Figure 4 B, D, F).

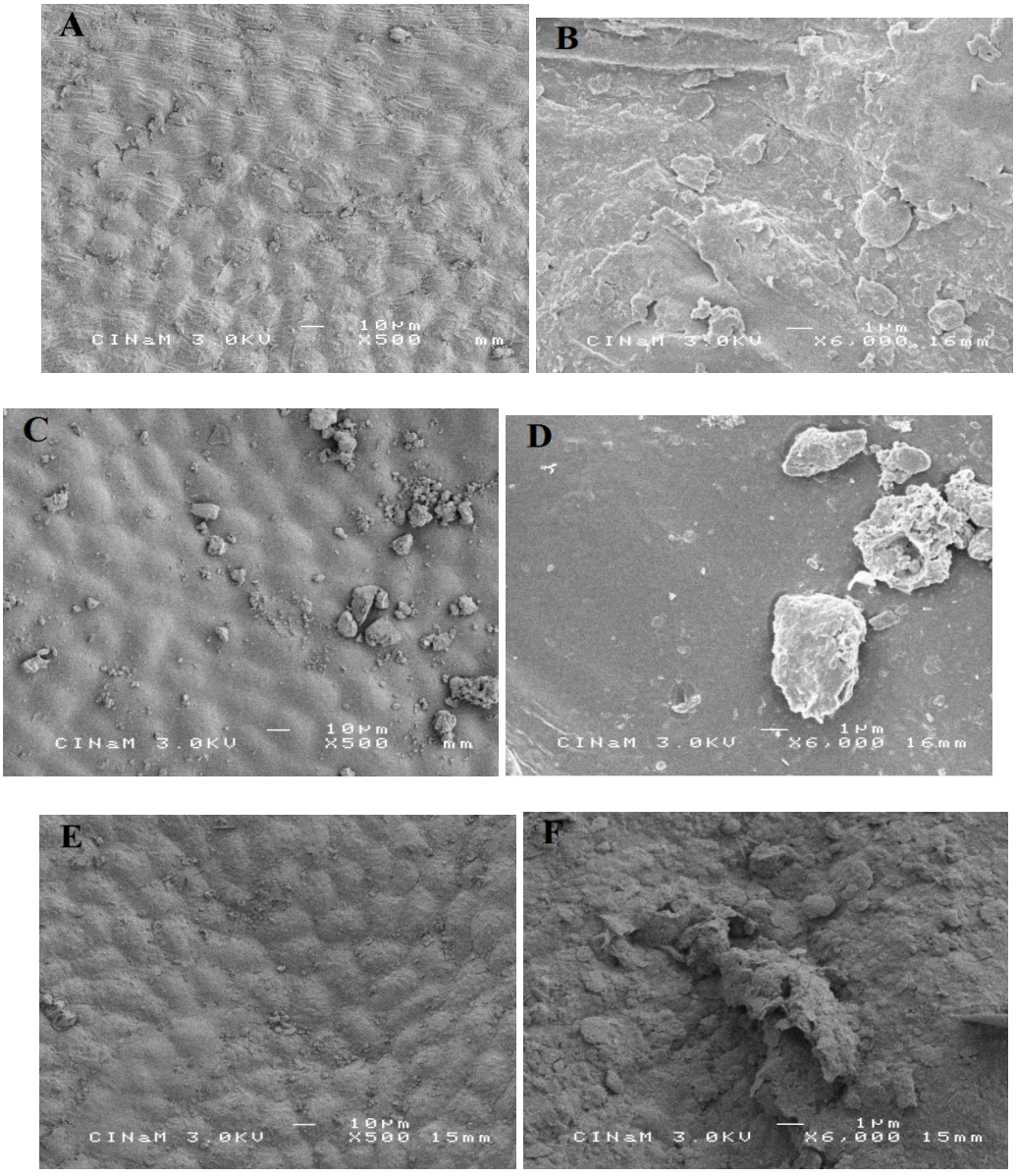

Figure 2: SEM photographs of the adaxial side of the *P. lentiscus* leaflets's (**A**, **B**): Beni Saf, (**C**, **D**): Maghnia, (**E**, **F**): Tessala

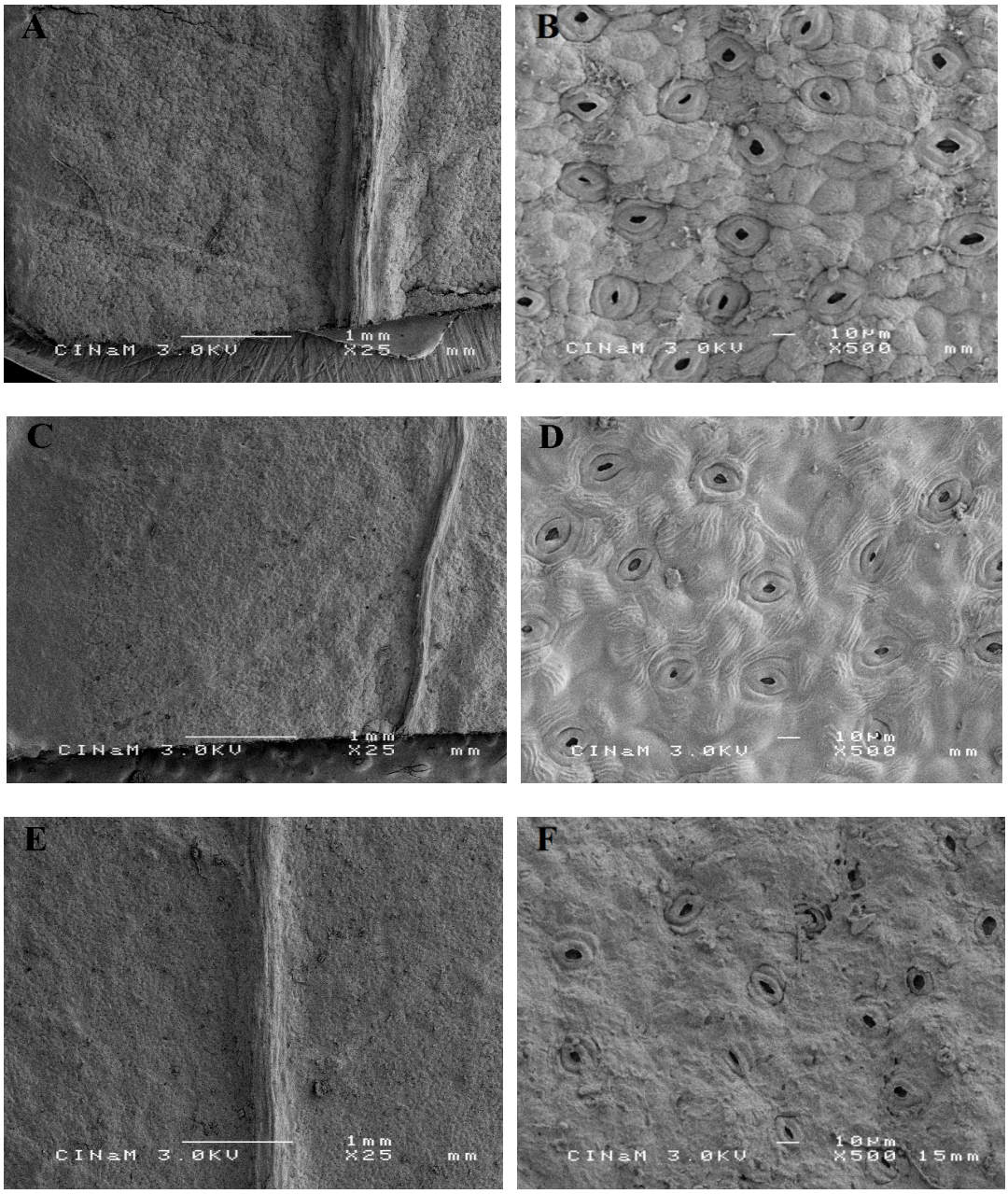

Figure 3: SEM photographs of the abaxial side of the *P. lentiscus* leaflets's (**A**, **B**): Beni Saf, (**C**, **D**): Maghnia, (**E**, **F**): Tessala

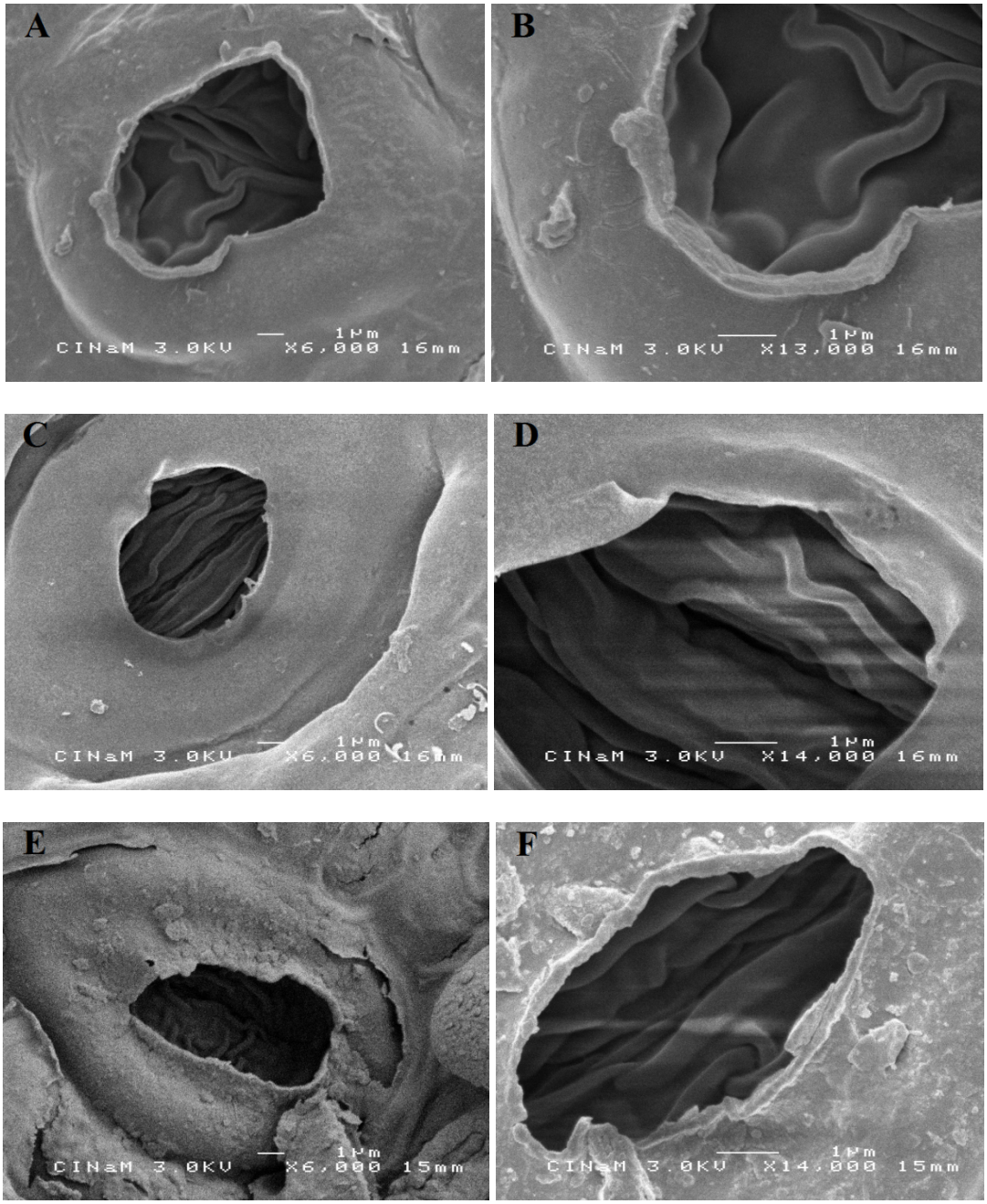

Figure 4: SEM photographs showing the structure of stomata on the *P. lentiscus* leaflets's (**A**, **B**): Béni-Saf, (**C**, **D**): Maghnia, (**E**, **F**): Tessala

The abundance of stomata on the abaxial surface of leaf samples of *P. lentiscus* from 03 sites examined under SEM gives it the hypoamphystomatic character, the samples seen under light microscope occasionally reveal stomata (unmeasurable) meadow midrib on the adaxial side (Figure 5, F). The epidermal cells are of a polygonal geometric shape and of unequal size

(Figure 5, E). The stomata have a rounded schape and the poles where the guard cells are meeting have circular shape .

Other forms can substitute as the obtuse shape, rarely elliptical shape. A variety of stomatal complexes was recorded in 03 sampling sites of *P. lentiscus* namely pericytic, actinocytic, paracytic and desmocytic types (Figure 5, A, B, C, D). SEM photographs revealed the presence of the peltate and capitate glandular trichomes on the lower surface of samples (Te) and (Mg) respectively (Figure 6, A, B).

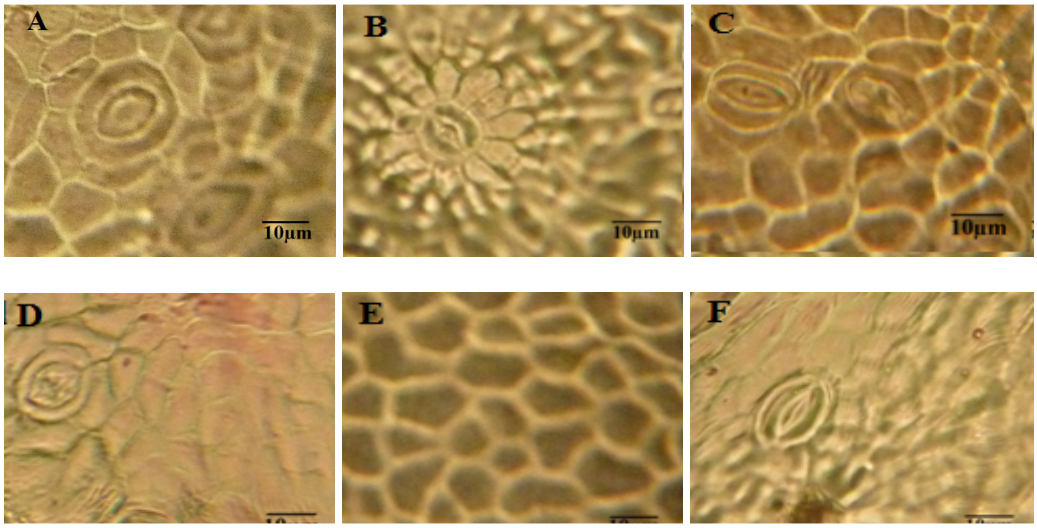

Figure 5: Stomatal types of *Pistacia lentiscus* (Light microscopy), **A** : pericytic stomata, **B** : actinocytic, **C** : paracytic, **D** : desmocytic, **E** : polygonal shape of epidermal cells (adaxial side), **F** : rare stomata near the midrib (adaxial side)

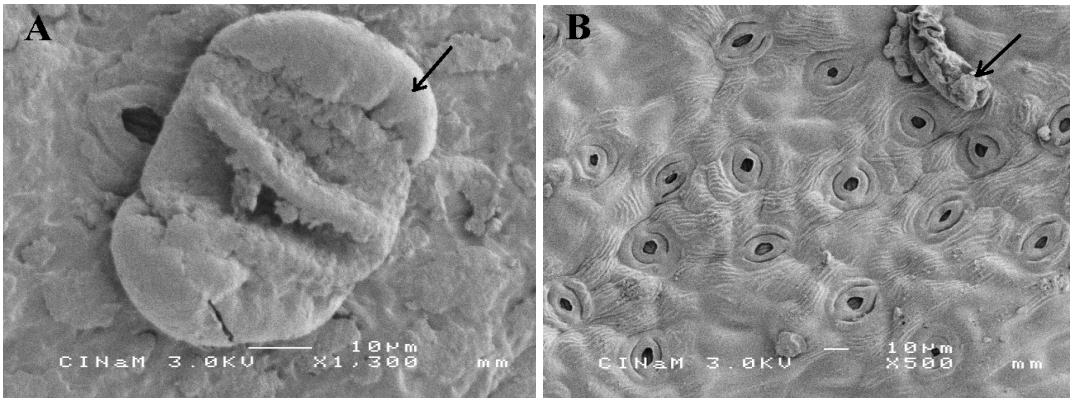

Figure 6: Types of trichomes : **A** : Pelted glandular trichome (Te), **B** : Capitate glandular trichome (Mg)

Stomatal density (Ds) given by the number of stomata on 1mm^2 of the leaf area is between 272,50 st / mm^2 and 485 st / mm^2 at both sites (Te) and (Bs) respectively. It is of the order of 404.9 st / mm^2 in the samples (Mg). Stomatal size (Ts) defined by the product of the length

and width of the stomata is estimated at 203.35 um^2 and 263.82 um^2 in samples (Te) and (Bs) respectively. The smaller stomatal is granted to samples (Mg) estimated of 195 um2. The ratio of length to width of the stoma defines stomatal shape (Fs). It is of the order of 1.41 and 1.42 in the sample (Mg) and (Te) respectively. Stomata samples (Bs) has a shape estimated of 1.07 (Table 2).

These results were statistically treated by (ANOVA1) in EXCEL and revealed a highly significant difference P*** (F cal 38,134 ≥ Fth 8,892) between the studied parameters and stations. Thus, the projection of variables on both axes F1 (77.39%) and F2 (22.61%) by a principal component analysis (PCA) revealed two groups (Figure 7). The G1 group reveals a positive correlation between samples (Mg), (Te) and two parameters namely altitude and abaxial stomatal shape. The G2 group revealed a negative correlation between samples of Bs, the Q_3, stomatal density and stomatal size on the abaxial side.

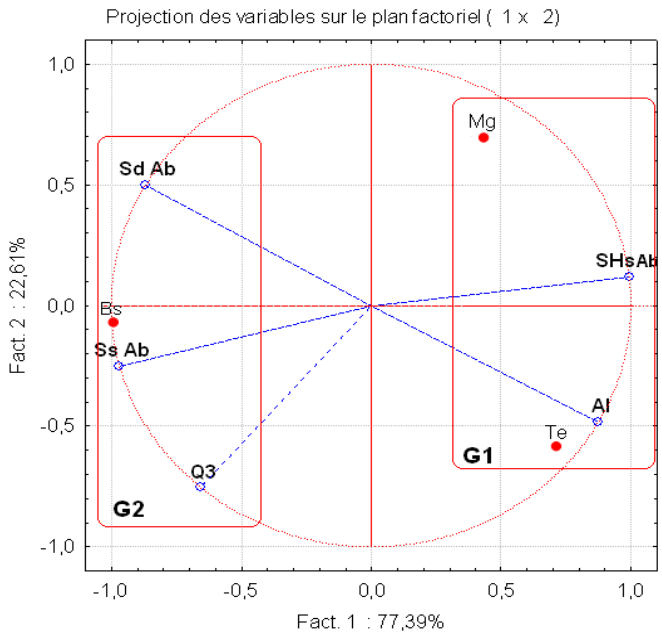

Figure 8: Circle of correlation between microphytodermal variables studied for three sites of *P. lentiscus*

3.2. Discussion

Mediterranean ecosystems are particularly fragile and characterized by two periods of stress during the year, namely the drought of summer and the cold of winter. The lack of water is the main limiting factor in these ecosystems (De Micco and Aronne 2012). In response to the diversity of climatic conditions, the mediterranean vegetation has developed a range of adaptations to water stress, resulting in a great diversity of life forms and growth. These commonly called xeric plants have common features that include: a small ratio of leaf

area by reducing sweating, tissue mechanically reinforced to prevent wilting (De Micco and Aronne 2012), and high resistance to cavitation of the xylem (Vilagrosa et al. 2003). However, the xeromorphisme is not limited to the xerophytes and not all xerophytes develop xeromorphic features (Rheda et al. 2009). From this study, the leaflets of *P. lentiscus* have manifested differently surfaces covered with epicuticular wax on the adaxial face. On the other side, the stomata are slightly sunken on the leaf epidermis. epicuticular wax particles are dispersed more or less on the stomata samples of (Mg) and (Bs). According Saleha Tahir and Rajput (2009), the elevation and sinking of stomata on the leaf epidermis, presence or absence of wax on the guard cells and subsidiary cells provide a taxonomic tool genetic. Samples (Te) have imprints continentality of its environment through stomata strongly covered with epicuticular wax particles. According Rudall (2007), the wax particles are variously oriented and sometimes occurring in characteristic patterns. Gostin (2009) believes that the quality and quantity of the wax are a response of the species to its environment. It thus grants thickening of adaxial cuticle to species evergreen. epidermal cells have manifested a polygonal shape. Tripati and Mondal (2012) reported that the shape of epidermal cells varies slightly with the growth of the port. The shrub species have epidermal cells polygonal to pentagonal shaped. The form of the epidermal cells of shrub species have differently shaped or undulating irregularly. The 03 *P. lentiscus* sites are hypoamphystomatic. The stomata are found in large quantities on the abaxial sides. The adaxial sides reveal little stomata near the midrib. This is in contradiction with the results of Al-Saghir and Porter (2006) and Smail-Saadoun (2005) where they noted a total absence of stomata on the upper side. The results of Rotondi et al. 2003 about the study of leaf xeromorphic adaptaions in some species of the mediterranean ecosystem including mastic tree have granted an absence of stomata on the adaxial face. Similarly, El-Oqlah (1996) noted the presence of stomata only on the underside of leaves in *Pistacia* species in Jordan. According to Smail-Saadoun (2005), this lack of stomata on the upper side is an adaptation of mastic tree in relation to the environment water stress. In the *Pistacia* genus, the density of stomata per square millimeter varies according to species (Fahn, 1974).The lower stomatal density (272, 5 st / mm^2) given by samples Tessala is probabment granted to the conditions of continental climate and altitude. Rotondi et al. (2003) estimated an abaxial stomatal density on the order of 254 st / mm^2 for mastic tree with a total absence of stomata on the adaxial face. According to Al-Saghir and Porter 2006, the density can reach 312,5 st / mm^2 or even up to 420 st / mm^2 (Galmes et al. 2007). However, samples (Bs) in semi-arid coastal bioclimatic show the highest density of stomata on the order of 485 st /

mm2. In this context, and according to the biosystematic study established by El-Oqlah (1996) on species of the genus *Pistacia* in Jordan, stomatal density can vary from 70 to 240 st / mm2 in the mastic tree. According Vaten and Bergman (2012), the frequency and positioning of stomata are specific characters to organs and species, but are also affected by environmental factors. Pericytic stomata are frequently observed, completely surrounded by a single subsidiary cell (Prabhakar 2004, Ibrahim and Ayodel 2013), also called stoma floating (synonym: adetostome) according Guyot (1985) or amphipericytic stoma (synonym: copericytic) according Prabhakar (2004). Actinocytic stomata is typical in its form as defined by Metcalf and Chalk (1950). The guard cells are surrounded by 11 subsidiary cells in the form of a rosette. According Armiñana et al. (2004), the typical actinocytic stoma for mastic tree is the result of the effect of ozone that affects his foliar anatomy. On the basis of the comparison of modern species, it appears that the original stoma is paracytique type of dicots, and other are derived (Chen et al. 2008). The presence of different types of stomata among species have taxonomic interest (Essiette et al. 2010) and its characteristics such as type, number, size and frequency are of diagnostic value (Kameshwari Shiva 2011). In comparison the great diversity and abbondance of trichomes in the Atlas pistachio collected from different eco-geographical areas (Ait-Said et al. 2011, Tirse et al. 2014), mastic tree showed capitate and / or peltate glandular trichomes and is devoid of aglandulaires trichomes. Metcalfe and Chalk (1950), attributed the power of exudation's oleoresin and considerable quantities of essential oils in *Anacadiaceae* and precisely *Pistacia* species to glandular trichomes.

4. Conclusions

Microphytodermals traits characterizing the leaf samples of 03 sites of *Pistacia lentiscus* show the same hypoamphystomatic distribution mode on leaf surfaces. The absence of stomata on the upper side is an adaptation character of mastic tree to the xericity conditions of its environment including lack of water. The pronounced diversity of stomata complexes can be used as a classification tool to solve in overlapping taxonomic entities and / or to study the adaptive strategies of species with respect to the xericity of their environments.

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Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.org ISSN 2458-5920