



## Comparative Anatomical Sections Of The Atlas Pistachio In Rechaiga, Tiaret (Algeria)

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### Abstract

This work is part of the anatomical study of the leaves of the Atlas pistachio in the Rechaiga region of Algeria and the stomatal density. Transverse sections were made on the central part of the leaflets of male and female individuals. The results obtained showed that the response of the selected individuals to environmental constraints is different and shows a high polymorphism. Thus, they show that this species has a thick cuticle layer and a thick mesophyll to reduce a maximum of water in the presence of drought. They also show that the distribution of stomata varies among the selected trees. *Pistacia atlantica* Desf. is a species of great virtue where it is necessary to appreciate and protect it.

**Keywords:** *Pistacia atlantica* Desf., anatomic section, male and female leaflets, stomata density.

### Introduction

The genus *Pistacia* L. belongs to the family Anacardiaceae. Zohary (1952) recognized 11 species of *Pistacia* based on morphology and divided them into four sections. Some are used as ornamental plants (Chenoune, 2005) and some as fruit trees (Atli et al., 2000).

From an ecological point of view, the Atlas Pistachio or Bétoum (*Pistacia atlantica* L.) is characterised by a high tolerance to climatic variations (Doghbage & Belhadj, 2020), it can grow in relatively low rainfall areas and adapts to all soils (Ghalem, Benhassaini, 2007) due to its strong rooting system (Bouabdelli et al., 2018). Monjauze (1980) and Ozenda (1983) describe the Atlas pistachio as endemic to North Africa. It is found in most semi-arid or steppe regions, alone or in combination with *Ziziphus lotus* or *Pinus halpensis* (Monjauze, 1980). The Atlas pistachio is a common species in Algeria, but it finds its optimum in arid and semi-arid areas, particularly in the high plains where it thrives in the wadi beds and dayas (Kaabeche, 2003, Harfouche et al., 2005).

Indeed, due to its rustic character and its ability to produce abundant humus, the Atlas pistachio could be used as a pioneering reforestation species and for the restoration of highly degraded environments. Moreover, this tree has multiple utility characteristics as fodder for its leaves (Niazi et al., 1999, Boukerker et al., 2020) or as a suitable rootstock for cultivated pistachio (Monastra et al., 1997, Kafkas & Kaska, 1998) and it is cultivated for the production of aromatics (Mosharafa et al., 1999).

The leaves are composed of finely winged rachis and lanceolate leaflets, obuminate at the top (Quezel, Santa, 1963, Yaaqobi et al., 2009) and are tough to the 7-11 leaflets, alternate and are over 12cm in total length (Monjauze, 1980) and rich in essential oils (Sadek Garboui, 2008) and differ qualitatively and quantitatively depending on individuals, male and female. The leaves of female feet are larger than those of male feet. Dimensions are influenced by sex and environment, hence the existence of leaf polymorphism (Mehdeb, 2019). The Atlas pistachio is dioecious. However, a case of monoeciousness has been found in the Rechaiga forest (Mehdeb et al., 2018). The flowers are apetalous (Yaaqobi et al., 2009) which show genetic variation in the reproductive organs within male and female individuals (Mehdeb-Hireche et al., 2021) and reddish in terminal clusters in males and axillary in females (Monjauze, 1980). The fruit yields an excellent edible oil obtained from the seeds, which contain about 55% oil (Daneshard, 1980). It is widely used as an antiseptic, antifungal and for abdominal disorders (Baba Aissa, 2000). It is used for saponification, illumination and in the preparation of cosmetic emollients (Chief, 1982). Its antioxidant oil contains active ingredients that promote wound

healing (Najafias et al., 2023).

The resin oozing from the *Pistacia atlantica* L. tree is widely used in the food industry to prepare chewing gum, in the photographic industry and in dentistry. It is also used as an adhesive. Pistachio resin, called Saqez, is used in various industrial and traditional applications (Zangeneh, 2003, Arefi et al., 2006) and pharmacological proprieties (Ben Ahmed et al., 2021).

The seeds are used as a source of rootstocks and sometimes for consumption of fruits, oil extraction and soap production (Al-Saghir, 2010). *P. atlantica* is also used as a rootstock in Turkey, Syria and Spain (Hadj-Hassan & Kardouch, 1995). It plays an important role in soil conservation (Hosseini et al., 2007).

The diversity of the species' habitat makes it a candidate for colonisation of areas prone to vegetation degradation and difficult climatic conditions.

To enable this species to fully play these multiple roles, ecological, social and economic, it is necessary to rehabilitate it and protect its settlements where the importance of exploration.

The aim of this work is to assess the species variability by studying and identifying the anatomical parameters of the leaves to better exploit this species, the maintenance of genetic diversity, conservation and prospects for its development.

### Material and methods

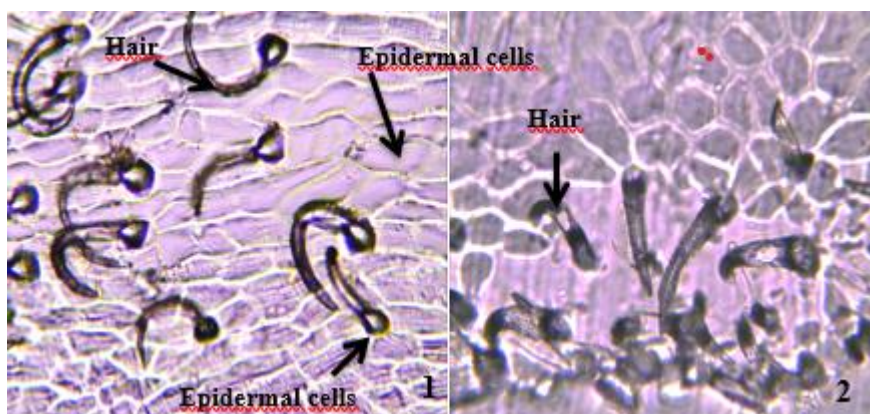
The Atlas pistachio leaves were collected in Rechaiga, Tiaret (west of Algeria), located in the southern Algerian steppe. It extends over an area bounded to the north by the Tellian chains, to the south by the vast Sahara, to the west by the plains of South Oran (chott chergui) and to the east by the Hodna basin. The studied population is located 6 km from the capital of the Rechaiga commune with the following coordinates Latitude 35° 18' 09" N and 35° 22' 50" N and longitude : 02° 06' 37" E and 02° 08' 45 " E.

The selection of the samples was random. In order to count the stomata distributed on both sides of the leaflet, we used the method of DOHMAN et al. (1991), applied by DENDEN and LEMEURE (1999 & 2000). It consists of taking leaf fingerprints by applying a layer of clear nail polish on the two epidermis of the Atlas pistachio leaflet. After drying, we also applied a transparent adhesive tape to remove the leaf prints, which we placed on a ZEISS microscope slide.

The anatomical study was carried out on histological sections of transverse leaflets from twenty-five males and females. The central part of the lamina was removed and immediately fixed in a mixture of ethanol and acetic acid (17, 3) for 24 hours. The samples were then rinsed in running water for a time equal to the fixation time. The samples were then dehydrated by passage through three increasing concentrations of ethanol (70%, 100% and 100%) for 15 minutes at each concentration. The samples were impregnated in a bath of toluene paraffin (50%, 50%) for 4 hours and another of pure paraffin for 12 hours at the melting temperature of paraffin (60°C). Finally, the samples were embedded in paraffin blocks and sectioned to a thickness of 7µm using a LEICA microtome.

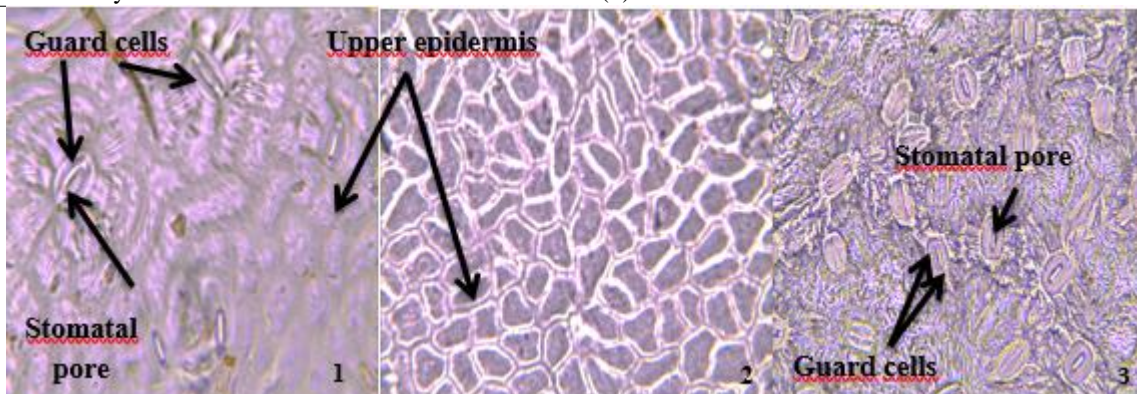
The measurements focused on different structural parameters of the leaflet, which are as follows: the thickness of the mesophyll, the thickness and number of cell files of the palisade parenchyma, the number of cribro-vascular rays at the central rib, the length of the epidermal cells, the thickness of the outer cell wall coating and, finally, the importance of the sclerenchyma on both sides of the lamina.

In this study, we found that the hairiness of the leaves is concentrated at the level of the main vein on the ventral surface of all the leaves. Thus, we noticed a large amount of hairiness on the margins of the leaves on the dorsal surface and (1 and 2 in Fig. n°01) and (2 in Fig. n°5).



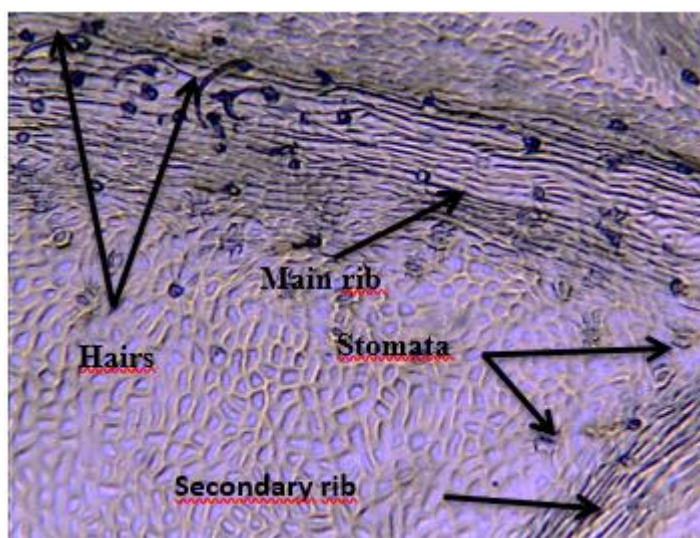
**Fig. n ° 01:** Hairiness of the Atlas pistachio leaflets  
(At the level of the main rib 1 and on the margins of the leaflets 2) (Grx40)

By studying the stomatal density on both sides of the leaf, we observed that the number of stomata on the dorsal surface is greater than that on the ventral surface. In fact, this number is balanced between two minimum and maximum values of 15 to 46 stomata per unit area.



**Fig. n ° 02:** Presence (1) and absence (2) of stomata on the ventral surface, dorsal aspect (3) of the Atlas pistachio leaflets (Gr.x40)

Thus, they have an average density of 4.81 on the ventral surface. Thus, the number of stomata varies from 1 to 11 and are mainly located on the periphery of the central rib and the secondary ribs, as shown in Fig.03.



**Fig. n ° 03:** Concentration of the stomata in the ventral aspect in the Atlas pistachio leaflets

## Results and Discussion

In the genus *Pistacia*, the epidermal cells on the leaf have trichomes (Belhadj et al., 2008) and are covered with a thick layer of wax (Al-Saghir, 2006). This is an adaptation to dry conditions.

A good characteristic that distinguishes the betum from other species of *Pistacia* is the presence of a line of almost microscopic hairs, curved towards the apex and parallel to each other, in the plane of the leaf and on the edges of each leaflet. However, there are Algerian Atlas pistachio without these hairs, in particular Desfontaine's specimen. In Palestine, *P. atlantica* and *P. mutica* do not show it (Monjauze, 1980). The results obtained by (Alvarez et al., 2008) showed the existence of two types of trichomes, unicellular and glandular, in *P. terebinthus*. (Lin et al., 1984) studies, showed that pistachio of Atlas leaflets are hairless, but (Komarov, 1949) indicated that leaflets main vein is covered by hairs while the margins are rarely ciliated. The hairs are concentrated along the midrib on the ventral side of the leaflets and on the margins on the dorsal surface.

In the micromorphological study of the leaves of *Pistacia atlantica*. We observed the presence of a pubescence at the level of the two leaf faces, but with a different distribution. Our results confirm those of (Amara et al., 2016), but with a slightly different distribution, but in agreement with the work of Tires et al. (2014). The hairs are concentrated along the main vein on the ventral side of the leaflets and on the margins on the dorsal surface.

Stomata play an important role in regulating the water balance of plants. Reducing water loss through stomatal closure is a means of adapting plants to drought (Slama et al., 2005). The density of stomata is usually greatest in the epidermis below the underside of leaves (Roland et al., 2008).

Our results show that the stomatal density is higher on the dorsal surface than on the ventral surface, which responds to different climatic changes, which agrees with the work of (Al-Saghir, 2005) and recalls the distribution of stomata in dicots. Their distribution is concentrated in the periphery of the main vein, which is consistent with the work of (Tires et al., 2014). This is a form of xericity of the plant. This shows that the leaves of the Atlas pistachio present a great variability micromorphological, which has been confirmed by (Taib & Sitayeb, 2020).

The study of the histological sections made on the leaves of male and female plants revealed peculiarities. The structure of

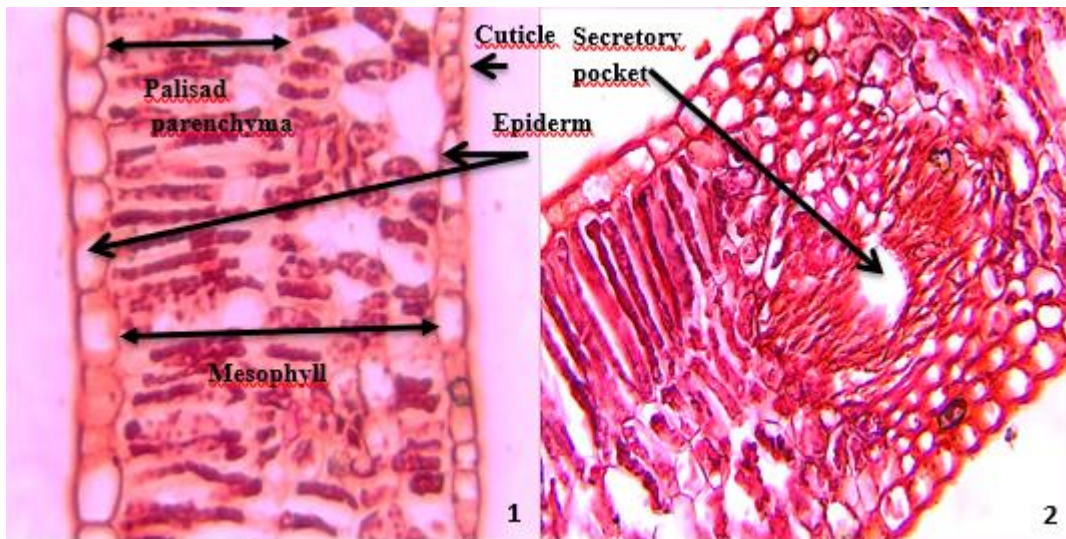
Comparative Anatomical Sections Sections Of The Atlas Pistachio In Rechaiga, Tiaret (Algeria)

the leaves was studied in terms of the following parameters:

The leaf is covered by upper and lower epidermis. The surfaces of the epidermal cells facing outwards are covered with a cuticle. The photosynthetic tissue is located between the two epidermal layers. The uppermost tissue, the palisade, is made up of three layers of elongated cylindrical cells. Below this is the spongy parenchyma, which has irregular cells and intercellular air spaces (Hopkins, 2003, Judd et al., 2002). The Atlas Pistachio belongs to the Anacardiaceae or Terebinthaceae family, where the existence of pockets and secretion channels at the level of the leaflets, and we can smell this on touching the leaflets (fig. n°04). In the adaxial epidermis, the cells have an irregular shape. Measurements of the thickness of the outer wall of the epidermal cells show that the average is 3.80  $\mu\text{m}$  and varies between 1.19 and 6.6  $\mu\text{m}$  in selected male plants, while in female plants the average is 3.45 and varies between 2.34 and 6.17  $\mu\text{m}$ .

Regarding the thickness of the mesophyll, the results show that the average thickness of the male leaf mesophyll varies between 143.91 and 233.53  $\mu\text{m}$  with a mean value of 179.57  $\mu\text{m}$ . However, the same observation recorded for female individuals showed that the thickness of the mesophyll varies between individuals and has a mean value of 173.92  $\mu\text{m}$  that varies between 129.54 and 224.99  $\mu\text{m}$  (Fig. 04).

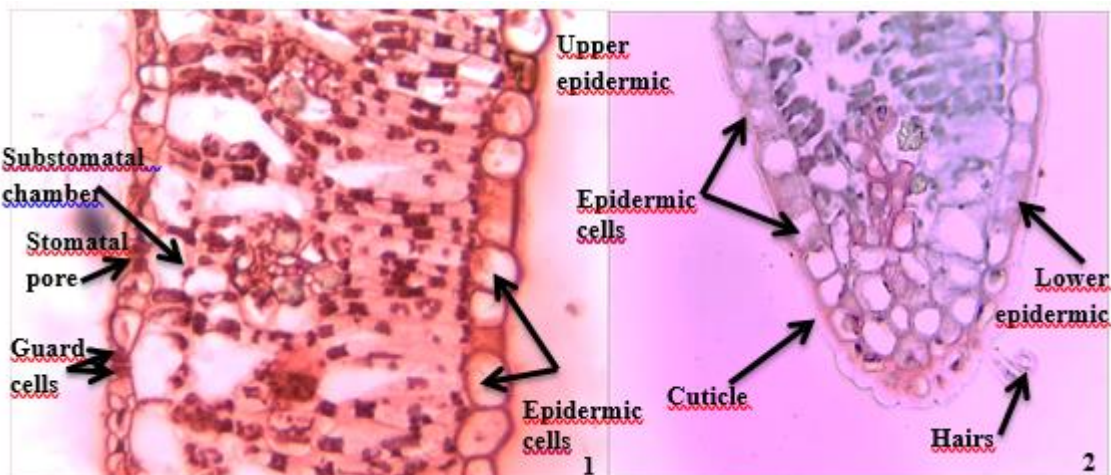
Following the analyses carried out on the thickness of the palisade parenchyma, we recorded a mean value of 80.12  $\mu\text{m}$  and measurements ranging between 58.80 and 144.35  $\mu\text{m}$  for the leaflets of male plants. For female individuals, the values ranged from 55.10 to 106.16  $\mu\text{m}$  with a mean of 73.81  $\mu\text{m}$  (Fig. 04).



**Fig. n°04:** Thickness of mesophyll and palisade tissue (1) and secretory pocket (2) of section the Atlas pistachio leaflets (x10)

Pistacia forms a type in which xeric species have advanced development of palisade parenchyma. The genus Pistacia, known as a xerophyte, has an advanced development of palisade parenchyma (Belhadj et al., 2007). Research on leaf anatomy has shown that Pistacia vera is covered by a single cell line that forms the epidermis and the palisade tissue consists of two layers, each containing many cells (Al-Saghir et al., 2005).

The measurements of the thickness of the palisade parenchyma cells show a difference between the males and females of this population. Thus, we find that these measurements vary between 9.30 and 20.84  $\mu\text{m}$ , with an average value of 14.36  $\mu\text{m}$  for male trees, and between 8.43 and 21.45  $\mu\text{m}$  for the leaves of female trees, with an average value of 13.89  $\mu\text{m}$ , which was confirmed by the work of (Mokhfi et al. (2017).



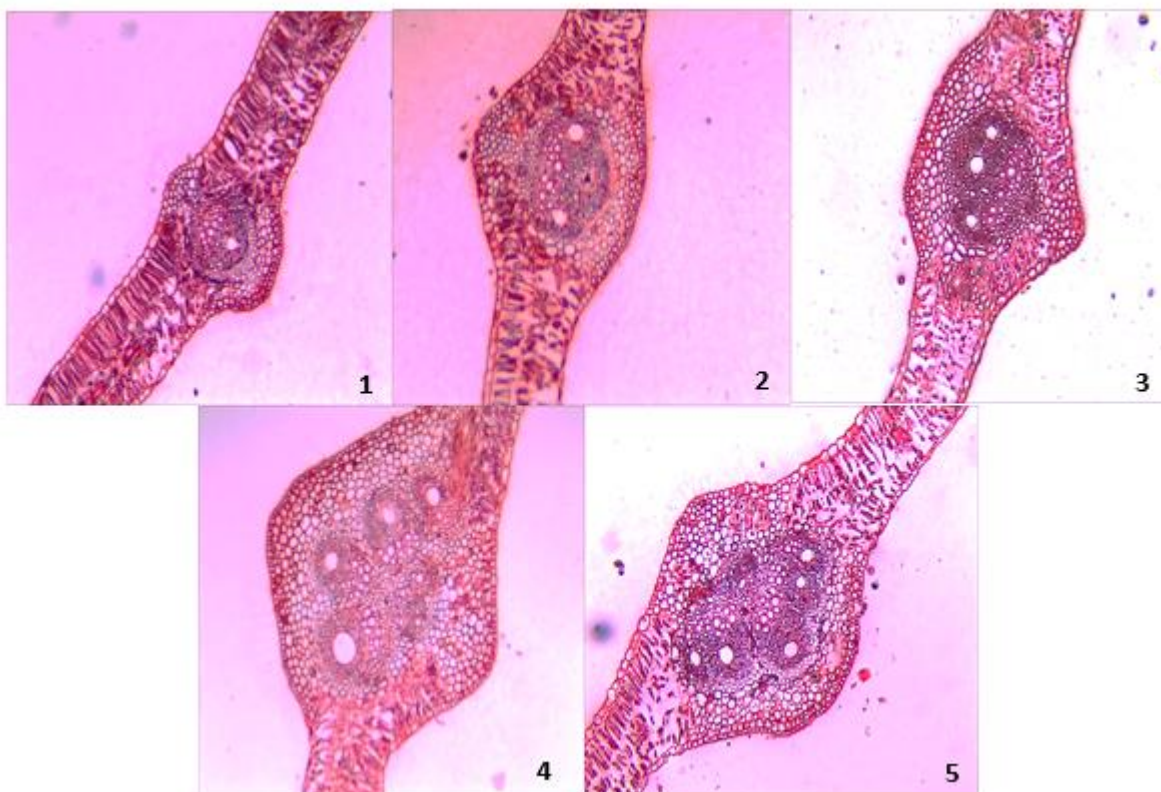
**Fig. n°05:** Anatomical section of the Atlas pistachio leaflets (palisade cells) (x40)

The genus *Pistacia* is xerophytic and characterized by the presence of different types of adaptation to drought, as the great development of the palisadic parenchyma and the extensive root. Moreover, the formation of the characteristic stratified palisadic tissue remains among the changes that accompany the dryness of the air and/or the increasing lack of water (Al Saghir et al., 2006). The palisade tissue that grows in a dry environment is composed of narrow and elongated cells, probably due to adaptation to light intensity rather than to drought (Oppenheimer, 1961) (Fig. n°05).

We note that the number of palisade parenchyma cells files varies between 3 and 6, and 46.67% of individuals have five cells, 33.33% have six cells, 13.33% have four cells and finally 6.67% have three cells; for male trees. The most frequent number is six cells in female individuals with 44%, then 5 cells and finally 16% of the population have four cells. These cells are narrow and elongated according to their number; when the number is low, their size increases, which can be explained by the plant's response to water deficit and high exposure to light intensity. The increase in the length of the cells therefore affects their number.

While the vascular bundles show a number that ranges from 2 to 5 vascular bundle drivers for all males. It turns out that in two individuals; one contains two and the other five. Those with three rays represent the majority with 72.41% and finally 20.69% for those with four vascular bundles.

In female individuals, the number varies between 1 and 5; the number 3 is the most frequent with 44%, the second most frequent with 24% are individuals with two vascular bundles, others with 4 with a rate of 20%, a 2-beam with 8% and the last one with a single beam (Fig. 06).



**Fig. n°06:** Number of vascular bundles in the Atlas pistachio leaflets (x40)

The dimensions of the palisade cells vary between 6.69 and 33.46  $\mu\text{m}$  in males, with an average of 22.22  $\mu\text{m}$ , while in females the average is 21.42  $\mu\text{m}$ , with a minimum of 10  $\mu\text{m}$  and a maximum of 33.62  $\mu\text{m}$ .

The results of the thickness of the ventral sclerenchyma show that there is significant variation between individuals. The values vary between 23.76 and 42.36  $\mu\text{m}$  with an average of 33.26  $\mu\text{m}$  in males. In females, the values vary between 20.07 and 42.45  $\mu\text{m}$  with an average of 32.30  $\mu\text{m}$ .

In contrast, measurements of the thickness of the dorsal sclerenchyma on the leaves of male trees show that the thickness varies between 12.67 and 30.16  $\mu\text{m}$  with an average of 22.18  $\mu\text{m}$ . In female trees, the average thickness is 24.51  $\mu\text{m}$  and varies between 18.75 and 32.53  $\mu\text{m}$ .

In females, according to Table n° 01, there is a positive correlation between the thickness of the mesophyll and the palisade tissue of a leaflet. There is also a positive correlation between the length of the epidermal cells and the thickness of the outer wall, between the length of the epidermal cells and the thickness of the dorsal sclerenchyma, and between the thickness of the ventral and dorsal sclerenchyma. However, there is a negative correlation between the number of cribrovascular bundles and the thickness of the palisade cells, i.e. the number of cribrovascular bundles increases as the thickness of the palisade cells decreases, which may be xeric in character. Table n°01 also shows that the thickness of the ventral sclerenchyma is negatively correlated with the number of cribrovascular bundles.

Comparative Anatomical Sections Sections Of The Atlas Pistachio In Rechaiga, Tiaret (Algeria)

**Table n ° 01:** Correlation of the anatomical parameters (females) marked correlations are significant at  $p < .05000$

	<b>THIK-MESO</b>	<b>THIK – PALISS – T</b>	<b>PALISS – CEL – THIK</b>	<b>NUM-CV-BEAMS</b>	<b>NUM-PALISS – CEL</b>	<b>LENG-EPID – CEL</b>	<b>OUTER – WALL – THIK</b>	<b>THIK-VENT-SCLER</b>	<b>THIK-DORS – SCLER</b>
<b>THIK-MESO</b>	1,0000	,7704*	,3819	,0144	-,1023	,2724	,3530	,3030	,1548
<b>THIK – PALISS-T</b>	p= ---	p=,000	p=,060	p=,946	p=,626	p=,188	p=,083	p=,141	p=,460
<b>PALISS-CEL-THIK</b>		1,0000	,4652*	,0401	-,0875	,2632	,3296	,2597	,1963
<b>NUM-CV-BEAMS</b>		p= ---	p=,019	p=,849	p=,677	p=,204	p=,108	p=,210	p=,347
<b>NUM-PALISS – CEL</b>			1,0000	-,5006*	,0241	,0960	-,1036	,1752	,0269
<b>LENG-EPID – CEL</b>			p= ---	p=,011	p=,909	p=,648	p=,622	p=,402	p=,899
<b>OUTER-WALL-THIK</b>				1,0000	-,0705	-,3480	-,1008	-,4087*	-,2157
<b>THIK-VENT-SCLER</b>				p= ---	p=,738	p=,088	p=,632	p=,042	p=,300
<b>THIK-DORS – SCLER</b>					1,0000	,1680	-,0255	,3052	,1512
					p= ---	p=,422	p=,904	p=,138	p=,471
						1,0000	,5509*	,3126	,3977*
						p= ---	p=,004	p=,128	p=,049
							1,0000	,2770	,2001
							p= ---	p=,180	p=,338
								1,0000	,5837*
								p= ---	p=,002
									1,0000
									p= ---

Parameters studied are: Thickness of mesophyl (THIK\_MSEO), Thickness of palisade tissue (THIK \_ PALIS\_ T), Palisade cellular Thickness of palisade cells (PALIS\_ CEL \_THIK), Number of cribrovascular beams (NUM\_ C V\_ BEAMS), Number of palisade cells (NUM-PALISS –CEL), Length of epidermal cells (LENG-EPID –CEL), Thickness of the outer cell wall (Thik-OUTER-WALL), Thickness of ventral sclerenchyma (THIK-VENT-SCLER) and thikness of dorsal sclerenchyma (THIK-DORS –SCLER)

Table n°02 between shows that there are positive correlations between the parameters studied. These correlations show that the species studied has xerophytic characteristics.

**Table n ° 02:** Correlation of the anatomical parameters (males) marked correlations are  $p < .05000$

	<b>THIK-MESO</b>	<b>THIK – PALISS – T</b>	<b>PALISS – CEL – THIK</b>	<b>NUM-CV-BEAMS</b>	<b>NUM-PALISS – CEL</b>	<b>LENG-EPID – CEL</b>	<b>OUTER – WALL – THIK</b>	<b>THIK-VENT-SCLER</b>	<b>THIK-DORS – SCLER</b>
<b>THIK-MESO</b>	1,0000	0,7481*	0,1770	0,2240	0,4523*	0,1298	0,1942	0,3071	0,3544
<b>THIK – PALISS-T</b>	p= ---	p=,000	p=,358	p=,243	p=,014	p=,502	p=,313	p=,105	p=,059
<b>PALISS-CEL-THIK</b>		1,0000	0,2673	0,3921*	0,4291*	-0,0321	0,1408	0,1601	0,2488
<b>NUM-CV-BEAMS</b>		p= ---	p=,161	p=,035	p=,020	p=,869	p=,466	p=,407	p=,193
<b>NUM-PALISS – CEL</b>			1,0000	0,2149	-0,2436	-0,0826	0,1821	-0,0596	0,0568
<b>LENG-EPID – CEL</b>			p= ---	p=,263	p=,203	p=,670	p=,344	p=,759	p=,770
<b>OUTER-WALL-THIK</b>				1,0000	0,1369	-0,0014	-0,0988	0,3131	0,1550
<b>THIK-VENT-SCLER</b>				p= ---	p=,479	p=,994	p=,610	p=,098	p=,422
<b>THIK-DORS – SCLER</b>					1,0000	0,2747	0,0217	0,0246	0,2530
					p= ---	p=,149	p=,911	p=,899	p=,185
						1,0000	-0,0501	-0,0653	0,1048
						p= ---	p=,796	p=,736	p=,588
							1,0000	0,4637*	0,4658*
							p= ---	p=,011	p=,011
								1,0000	0,5043*
								p= ---	p=,005
									1,0000
									p= ---

Parameters studied are: Thickness of mesophyl (THIK\_MSEO), Thickness of palisade tissue (THIK \_ PALIS\_ T),

*Palisade cellular Thickness of palisade cells (PALIS\_ CEL \_THIK), Number of cribrovascular beams (NUM\_ C V\_ BEAMS), Number of palisade cells (NUM-PALISS –CEL), Length of epidermal cells (LENG-EPID –CEL), Thickness of the outer cell wall (Thik-OUTER-WALL), Thickness of ventral sclerenchyma (THIK-VENT-SCLER) and thikness of dorsal sclerenchyma (THIK-DORS –SCLER)*

This shows that *Pistacia atlantica* Desf. has xerophytic characteristics, which always contribute to the protection of the plant with respect to the arid climate where it is located, it is an adaptation to drought to conserve water inside the plant or to minimise the loss of the plant to water.

### Conclusion

The Atlas pistachio, with its nutritional, medicinal and ecological properties, represents an important genetic and forestry heritage in the North African region. Its peculiarity lies in its plasticity, which enables it to survive in the harshest conditions. This species is currently threatened by several factors that contribute to its degradation.

Our results show that the leaf of the Atlas pistachio has a hairiness on the main vein on the ventral surface and on the edges of the dorsal surface, and the stomatal density shows an interspecific variation. The adaptation to the climate shows a reduction of stomata and a thick layer of cuticle to limit transpiration. The climate requires this species to adapt to water stress and high light intensity to have xeric characteristics in order to reduce the phenomenon of transpiration.

Our anatomical study, carried out on the leaves of male and female Rechaiga individuals, also shows that the discrepancy showing a high polymorphism and the existence of a compact mesophyll is a response to the surrounding conditions. The leaves developed a rather large palisade parenchyma, the number of its cells reached six and they developed a rather pronounced ventral and dorsal sclerenchyma. This study showed that the species is very sensitive to drought.

Moreover, the results obtained will undoubtedly contribute to the valorization of the species in question. Indeed, the development of the Atlas pistachio will make it possible to protect barren land, stabilize soils and increase resources in semi-arid areas.

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