Biodiv. Res. Conserv. 58: 1-12, 2020

BRC
www.brc.amu.edu.pl

DOI 10.2478/biorc-2020-0005

Submitted 05.06.2020, Accepted 30.06.2020

Effect of environmental conditions on morphological variability of leaves and fruits of five populations of *Pistacia atlantica* Desf. in North Algeria

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Abstract. *Pistacia atlantica* has a lot of medical, pharmaceutical and economic benefits, and its variability shows its evolutionary potential. The objective of this study was to investigate morphological and micro-morphological variability of these trees within different ecological regions. This study offers a general description of sites, an analysis of morphological variability of twenty quantitative and qualitative parameters based on the impact of natural and artificial conditions, and leaf anatomical analysis. The results showed that the population exhibited heterogeneity in all parameters of the leaf related to changes in soil, density, climate and slope. Variability in nut size was also demonstrated which was due to the effects of climate, type of soil and topographic factors. The results of electron microscope scanning of leaf anatomy showed the existence of large micro-morphological variability between study sites.

Key words: morphology, micro-morphology, environmental impact, biodiversity, Anova, ecological zone

1. Introduction

Geographical situation and multitude of climates in Algeria created perceptible biodiversity of flora and fauna. *Pistacia atlantica* constitutes a kind of notable morphological biodiversity, as it shows rare plasticity attracting attention of specialists to its current knowledge and its interaction with different environments. This tree is characterized by ecological amplitude since it occurs from inside the Sahara to margins of humid bioclimate (Quézel & Médail 2003). It adapts to low rainfalls of about 150 mm and sometimes less (Benhssaini & Belkhodja 2004). This species does not require much rainfall, it is satisfied with very low rainfalls ranging from 200 to 400 mm isohyet (Khaldi & Khoudja, 1996), the Atlas pistachio is a heliophilous species present where average temperature is between 7 and 7.5 °C (Larouci 1987), it is indifferent to soil type (Zohary 1996), thus it grows well in clay or silty soil (Khaldi & Khoudja 1996). *Pistacia atlantica* occupies a large area and can be found in the Canary Islands, in Libya (Cyrenaica) in Cyprus and the Middle East (Quézel & Médail 2003). However, Baba Aissa (2000) adds that this tree is an endemic North African species (Morocco, Algeria, Tunisia).

Pistacia atlantica tree is called "Betoum" in Algéria, "Botma" in North Africa and "Boutmela" in the Middle East and it is also known as wild pistachio or false pistachio (Monjauze 1980). It is described as a strong tree which can reach 20 m of height (Benhssaini & Belkhodja 2004) having an impressive aspect in adult age with a large and rounded crown of tight foliage. Leaves are deciduous, composed, alternate and pennate made up of 7 to 11 leaflets per leaf (Larouci 1987) or 3 to 15 leaflets per leaf (Belhadj 1999), more or less leathery. They are from 2.5 to 6 cm in length, rarely – 12 cm, and 0.5 to 1.5 cm in width. It is a monoecious tree in which male flowers are gathered in terminal clusters and female flowers in axillary clusters of greenish-yellow colour.

Flowering occurs between February and March, the appearance of fruits between March and May depending on space conditions. Fruits are reddish at ripeness and become dark green, black or brownish by the end of August, September and beginning of October. However, economic, ecological and even

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medicinal values of *Pistacia atlantica* tree remain less known, although it is a very useful and productive tree at the same time (Monjauze 1967) and has many medicinal, pharmaceutical, and economic benefits and even plays an important role in landscape conservation. In the oriental part of the Mediterranean zone, it has been already used in production of resin – mastic for medical purposes and as a rootstock in the cultivation of pistachio vera, for preservation purposes of arid and semi-arid regions. Its wood is used in production of soap (Benabid 1986). In Algeria, this interest grows with its exploitation by steppe populations in particular, who use it for themselves and their livestock. Leaves and bark are used in decoration, as medicine for stomachaches and gastric pains via inhalation (Baba Aissa, 2000); leaves are used as a febrifuge, galls are used as powder, alone or with round nutmeg as anti-diarrhea and stomachic powder. The lipid extracted from pistacia grains is rich in triglycerides, unsaturated fatty acids and phytosterols which are responsible for hypocholesterolemic effects (Mahmoud *et al.* 2015). Drupes play an important role in prevention of some metabolic problems, cardiovascular diseases and cancer (Rezaei *et al.* 2012). It was confirmed that essential oil and resin show antibacterial activities and phenolic and lipid extracts were discovered to show antileishmanial properties (Taran *et al.* 2010).

This abundant species continues to regress year after year due to anthropogenic factors as well as problems with natural regeneration (Ait Said 2011). Other factors such as devastation, diseases and drought contributed to its degradation (Benhssaini & Belkhodja 2004).

The objective of the study was to investigate morphological variability and characterise *Pistacia atlantica* in Algeria and to show the impact of environmental conditions on this variability. Our study was based on leaf morphological and micromorphological variability of the best populations in Algeria in different ecological regions (Batna, Tlemcen, Saida, Tissemssilt, Laghouat) focusing on environmental factors that contribute to this variability. In addition, variability at different levels was measured: interpopulation – to recognise variability that exists between several regions found in different environmental conditions and intrapopulation – to measure variability inside each population.

2. Materials and methods

2.1. Study area

The study area covered a large geographic area of the best population of this tree in different ecological zones (from North to South and from West to East of the Atlas Tellian Mountains) (Fig. 1). After targeting the best populations of *Pistacia atlantica* in the five sites, it was decided to establish circular plots of 25 m radius in each site.

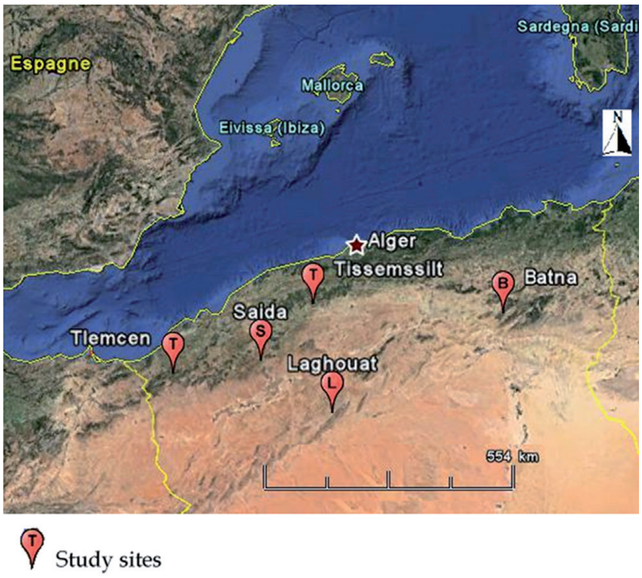

Fig. 1. Location of the study area (base map: Google Earth)

In the west site, Tlemcen and Saida are located in Tellian Atlas. The site of Saida is represented by the Dhaya Mountains and the northern limit of the high plain in semi-arid climate. They include soils of the region classified into three groups: limestone soils, red soil and the litho soils (S.A.T.E.C. 1976; B.N.D.E.R. 2008; Pietracarprina 1988; Halitim 1988). The site of Tlemcen is situated in the Northwest of Algeria. It belongs to a semi-arid Mediterranean climate characterized by two contrasting seasons, hot and dry summer and cold winter with abundant rainfall depending on altitude.

In the East, the site of Batna is located between the Tellian Atlas and the Saharan Atlas, in arid climate and on poorly evolved soils; they are characterized by shallow depth and are often rich in limestone. Slopes are often steep, up to about 18%. This site experiences abundant grazing and moderate competition.

Tissemssilt site is located in the centre of the country, in the national park of Theniet El Had, in subhumid climate and cold winters. The soil here is thin, less profound (Melazem 1990). This site is subject to abundant grazing and strong competition. In the south, the site of Laghouat spreads out in Saharan climate. The soil is of light texture (Table 1).

2.2. Sampling and measurements

At each site (Batna, Tlemcen, Saida, Tissemssilt, Laghouat), thirty trees were randomly selected, followed by a topographic and edaphic description and the status of *Pistacia* population. On the sides of each tree, 30 leaves were collected in May and 30 nuts were collected in September: a total of 150 healthy and mature trees of

Table 1. Characteristics of study sites

Sites		Batna	Tissemssilt	Tlemcen	Saida	Laghouat
Climate		arid	subhumid	upper semi-arid	lower semi-arid	Saharan
Annual average temperature (min-max)		8.3-22.5	6.4-16.3	12-24.4	10.2-23.8	12.2-25.5
Geographic coordinate	X	5° 58' 31.92''	1° 58' 29.25''	0° 57' 54.37''	0° 44' 53.87''	2° 08' 51.24''
	Y	35° 17' 08.06''	35° 49' 32.92''	34° 55' 52.48''	34° 56' 44.04''	33° 56' 02.10''
Topographic and pedological description	Elevation [m]	1030	1243	749	869	1158
	Slope [%]	42	20	14	7	15
	Aspect	Northeast	Southeast	North	Northeast	East
	Soil type	limestone	clayey	fertiallitic limestone	alluvial	light clay
Grazing		heavy	heavy	absent	absent	low
Competition		moderate	strong	absent	moderate	absent

the same age class (>100 years), 4500 leaves and 4500 nuts were sampled.

Leaves and nuts were stored in the laboratory for biometric, quantitative, qualitative morphological measurements using the method described for the species of *Pistacia* genre by Zohary (1952): leaf length (L.L), leaf width (L.W), petiole length (P.L), basal leaflet length (B.L.L), basal leaflet width (B.L.W), terminal leaflet length (T.L.L), terminal leaflet width (T.L.W), leaflet number (L.N), leaf colour (L.C), cluster length (C.L), nut number (N.N), peduncle length (Pe.L), peduncle width (Pe.W), terminal nut length (T.N.L), terminal nut width (T.N.W), basal nut length (B.N.L), basal nut width (B.N.W).

2.3. Statistic analysis

Descriptive statistics for each morphological parameter of the tree in each site was calculated, and then one-way variance analysis (ANOVA1) was applied between means of morphological parameters in different sites. Duncan test was also used to analyze the variance just to compare the means. The test applied probability level of (p=0.05). Principal component analysis (PCA) was used to regroup the sites of study based on ecological parameters, then Pearson correlation analysis was employed to examine the relation between morphological parameters and study sites .

2.4. Anatomy of the leaf

Another harvest of leaves in the four sites (Saida, Laghouat, Tlemcen, Tissemssilt) was collected in 2019 to make a micro-morphological comparison of leaflets between previously mentioned sites employing scanning electron microscope (type Carl Zeiss EVO LS 10). The studied leaflets were carefully cut into portions of about 1 cm^2 taking into account the principal vein. Samples were cleaned with alcohol and then placed in the electron microscope to see the upper side using 500X magnification to observe the following organs: epidermal cells, trichomes, stomata, waxes, conductive devices.

3. Results

3.1. Principal component analysis (PCA)

The PCA was done along three axes (Fig. 2) which represented 96% of the total information, the first factorial axe representing 44.14%, the second – 28.74% and the third – 23.21%. The first axis consisted of the variables (aspect, competition, grazing), the second axis – (climate, elevation and density) and the third axis – of variables (slope and type of the soil) (Table 2).

PCA is used to explain the variance-covariance structure of a set of variables, in this case, multiple ecological parameters, through linear combinations. We noticed presence of the site of Batna, forcefully distant on the third axis and it represented the slope or the topographical action as compared to other sites. In contrast, Tissemssilt and Saida were negatively correlated with the third axis (low slope).

3.2. Morphological parameters of the leaf

Table 3 shows the analysis of leaf parameters in five studied sites, and Table 4 shows the correlation between leaf parameters and factorial axes which represented ecological factors of the sites.

The average leaf length of all the trees of the five sites was 7.63 cm. The minimum length was (2.47 cm) in Batna, and the maximum length was (12.50 cm) in Tlemcen. Variance analysis indicated a highly significant difference between the sites. Duncan homogeneity test showed existence of four distinct groups (G1: Batna, G2: Saida, G3: Laghouat, G4: Tlemcen and Tissemssilt). A negative correlation was observed between leaf length and the second and the third axes.

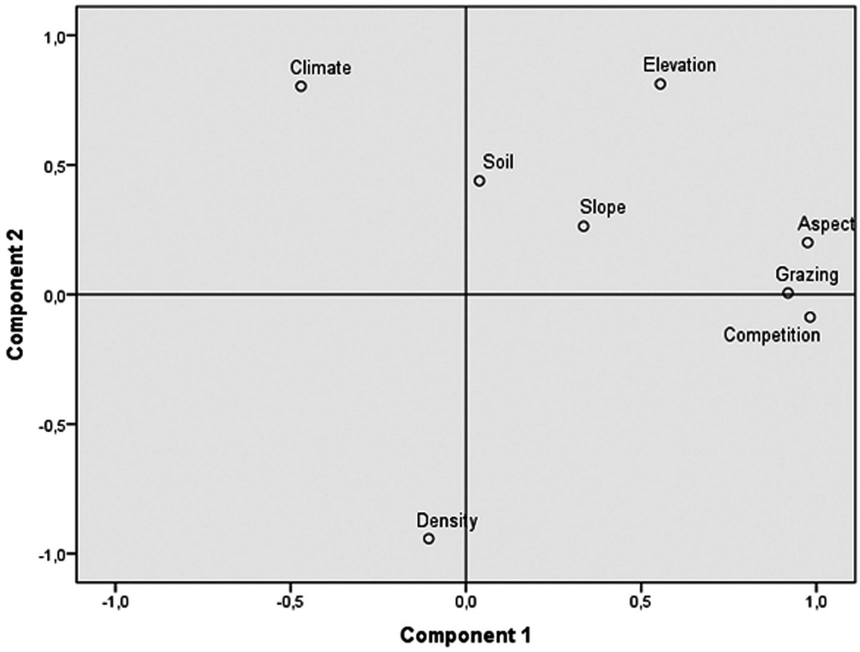

Fig. 2. Results of a principal component analysis

Table 2. Component matrix

	Component		
	1	2	3
Climate	-0.470	0.804	0.188
Slope	0.336	0.263	0.837
Elevation	0.555	0.812	0.071
Density	-0.106	-0.942	0.310
Grazing	0.919	0.006	0.365
Competition	0.982	-0.088	0.100
Soil	0.039	0.438	-0.869
Aspect	0.975	0.200	-0.038

Average value of leaf width in all the studied sites was 6.71 cm with extreme values, minimum in Batna (2.72 cm), maximum in Tissemssilt (12 cm). The analysis of variance showed a highly significant difference between the sites. Duncan test for the variable L.W showed the existence of three distinct groups (G1: Batna, G2: Saida, Tlemcen and Laghouat, G3: Tissemssilt). The correlation coefficient confirmed existence of a negative correlation between leaf length and the third factorial axis which represented the slope factor.

Average petiole length was 2.40 cm in the studied sites. Minimum and maximum values were: 0.50 cm in Laghouat and 4.22 cm in Tlemcen, respectively. Variance analysis was highly significant showing very large variability between different sites for the PL parameters (p=0.000). The Duncan homogeneity test for the variable PL yielded two groups: (G1: Laghouat, Batna and Saida, G2: Tissemssilt and Tlemcen).

Correlation test showed that the petiole length and the factorial axis 2 were negatively correlated (Table 4).

Average petiole width was 0.3 cm in all the sites of the study ranging from a minimum length of (0.03 cm) in Saida and a maximum length of (0.2 cm) in Tissemssilt. The sites of Batna and Tlemcen revealed the same average length of (0.14 cm). Analysis of variance was highly significant explaining very large variability between the sites of Saida and Tissemssilt (p=0.000). Duncan test for the PW variable demonstrated existence of three groups (G1: Saida and Laghouat, G2: Batna and Tlemcen, G3: Tissemssilt). There was no link between the groups. The petiole width and the factorial axes 1 and 3 were positively correlated. By contrast, it was correlated negatively with axis 2.

Basal leaflet lengths in all the sites of the *Pistacia atlantica* exhibited average length of 3.13 cm. The longest leaflets reached 5.50 cm in Tissemssilt, while the shortest of 1.22 cm were observed in Batna. The variance analysis was very highly significant which explained very large variation between different studied sites for the BLL parameters (p=0.000). The test of Duncan for this variable demonstrated existence of three groups (G1: Batna, G2: Saida and Laghouat, G3: Tlemcen and Tissemssilt). The basal leaflet length was negatively correlated with axes 2 and 3.

Average basal leaflet width of the five sites was 1.12cm. The largest leaflets were 3 cm wide and were found in Saida, while the smallest ones – 0.42 cm were recorded in Batna. The variance analysis of the sites was highly significant (p=0.000). Duncan test for the BLW was formed by four groups (G1: Batna, G2: Laghouat, G3: Saida and Tlemcen, G4: Tissemssilt). There was a negative correlation between the width of the basal leaflet and axes 2 and 3.

Table 3. Morphological parameters of leaves originating from five studied localities

Parameters*	Sites				
	Batna	Tlemcen	Saida	Tissemssilt	Laghouat
Leaf length	4.32 (2.47-7.08)	8.55 (5.83-12.50)	7.80 (6.00-10.00)	9.39 (6.40-12.30)	8.08 (5.60-11.00)
Leaf width	4.36 (2.72-6.14)	7.07 (4.71-11.63)	6.10 (4.70-8.00)	8.65 (5.30-12.00)	7.37 (4.50-9.50)
Petiole length	2.06 (0.76-3.48)	2.74 (1.50-4.22)	2.68 (2.00-3.70)	2.51 (1.50-4.20)	2.04 (0.50-3.90)
Petiole width	0.14 (0.10-0.20)	0.14 (0.10-0.20)	0.09 (0.03-0.1)	0.17 (0.1-0.2)	0.1 (0.1-0.1)
Basal leaflet length	2.13 (1.22-3.36)	3.44 (2.20-5.08)	3.18 (2.00-4.20)	3.63 (2.20-5.50)	3.26 (1.80-4.50)
Basal leaflet width	0.70 (0.42-1.02)	1.27 (0.60-2.08)	1.18 (1.00-3.00)	1.36 (0.80-2.10)	1.08 (0.50-1.50)
Terminal leaflet length	1.98 (1.08-3.16)	3.59 (2.30-5.08)	3.76 (3.00-6.00)	4.01 (2.50-5.80)	3.36 (2.40-5.00)
Terminal leaflet width	0.58 (0.36-0.95)	1.29 (0.90-2.30)	1.08 (0.60-3.00)	1.31 (0.80-2.00)	0.98 (0.70-1.40)
Leaf color	1.57 (1-2)	1.40 (1-3)	1.00 (1-1)	1.00 (1-1)	1.63 (1-3)
Leaflet number	8.40 (5.60-10.26)	8.60 (7.00-11.00)	8.44 (6.60-9.80)	9.43 (7.00-11.00)	9.36 (7.00-11.00)

Explanation: * − significance level for all parameters: 0.000 at 0.05 level

Average length of terminal leaflets was 3.34 cm. This length varied between minimum value of 1.08 cm registered in Batna and maximum value of 6 cm observed in Saida. Analysis of variance was highly significant (p=0.000) accounting for very large variability between different sites. Duncan test for the variable TLL demonstrated existence of three different groups (G1: Batna, G2: Laghouat, G3: Tlemcen, Saida and Tissemssilt). A negative correlation between the terminal leaflet length and axes 2 and 3 was recorded.

Average terminal leaflet width in all studied stands was 1.05 cm. It varied between 0.36 cm in Batna and 3 cm in Tissemssilt. The variance analysis was highly significant (p=0.000) accounting for inter-populating variability. Duncan test for variable BLW demonstrated existence of three groups (G1: Batna, G2: Laghouat and Saida, G3: Tlemcen and Tissemssilt). There was a negative correlation between the the terminal leaflet length and factorial axes 2 and 3.

The colour was quite difficult to define and colour overlapping was observed. This is why three more dominant colours were considered, namely: green, yellow and red. This parameter was considered as a qualitative variable which was viewed through the average of the green colour that was dominant in all the studied sites. The variance analysis was highly significant (p=0.000) explaining very large variability between sites. Duncan test for the variable leaf colour showed existence of two groups (G1: Saida and Tissemssilt, G2: Tlemcen, Batna and Laghouat). A negative correlation was observed between leaf colour and axis 1 and a positive correlation with axes 2 and 3.

Average number of leaflets per leaf in all the studied sites was 8.83 with the same average of maximum number (11 leaflets per leaf) in the three sites: Tlemcen, Tissemssilt and Laghouat, and an average of minimum number (5.60 leaflets per leaf) in Batna. Variance analysis was highly significant between the studied sites (p = 0.000). Duncan test for the LN variable showed existence of two groups (G1: Batna, G2: Tlemcen, Saida, Laghouat and Tissemssilt). A negative correlation existed between this parameter and axis 3.

Table 4. Correlation between morphological parameters of leaves and factorial axes

Parameters	Axe 1	Axe 2	Axe 3
Leaf length	0.06	-0.28**	-0.69**
Leaf width	0.09	-0.12	-0.59**
Petiole length	0.00	-0.38**	-0.14
Petiole width	0.31**	-0.24**	0.18*
Basal leaflet length	-0.01	-0.22**	-0.52**
Basal leaflet width	0.04	-0.30**	-0.46**
Terminal leaflet length	0.07	-0.29**	-0.63**
Terminal leaflet width	0.00	-0.42**	-0.49**
Leaf color	-0.27**	0.22**	0.19*
Leaflet number	0.07	0.14	-0.22**

Explanations: **, * − significant at the 0.01 and 0.05 levels, respectively

Table 5. Morphological parameters of nuts originating from five studied localities

Parameters	Sites					Significance level
	Batna	Tlemcen	Saida	Tissemssilt	Laghouat	
Cluster length	7.92 (6.30-9.60)	8.14 (6.20-11.20)	7.73 (6.30-9.60)	7.93 (5.50-11.00)	6.69 (5.20-12.00)	0.326
Nut number	22.41 (13-35)	35.88 (16-62)	21.60 (11-35)	41.16 (17-66)	15.70 (9-23)	0.000
Peduncle length	2.60 (1.00-5.00)	1.71 (0.50-3.50)	2.54 (1.00-5.00)	1.51 (0.50-2.20)	1.86 (0.80-3.00)	0.101
Peduncle width	0.40 (0.30-0.50)	0.31 (0.20-0.40)	0.40 (0.30-0.50)	0.35 (0.20-0.50)	0.34 (0.20-0.50)	0.102
Terminal nut length	0.55 (0.50-0.60)	0.66 (0.50-0.80)	1.23 (0.60-0.65)	0.78 (0.70-0.90)	0.59 (0.40-0.80)	0.396
Terminal nut width	0.5 (0.50-0.50)	0.61 (0.50-0.80)	0.51 (0.50-0.60)	0.56 (0.40-0.70)	0.47 (0.40-0.60)	0.000
Basal nut length	0.55 (0.50-0.60)	0.57 (0.50-0.60)	0.65 (0.60-0.70)	0.63 (0.50-0.70)	0.50 (0.40-0.60)	0.000
Basal nut width	0.48 (0.40-0.50)	0.55 (0.50-0.70)	0.51 (0.40-0.60)	0.48 (0.40-0.60)	0.44 (0.30-0.50)	0.008

3.3. Morphological parameters of the nut

Table 5 shows the analysis of variance of nut morphological parameters in five studied sites, and Table 6 shows the correlation between the parameters of the nut and the three factorial axes which represented ecological factors of the sites.

Average cluster length in the five sites was 7.66 cm; minimum length – 5.20 cm and maximum length – 12 cm in the same site of Laghouat. Variance analysis (p=0.3) showed that there was no difference between sites accounting for absence of inter-populating variability of this variable. Correlation coefficient analysis revealed no environmental impact on this parameter.

Average number of nuts was 25.72 in the five studied sites and it varied between 9 in Laghouat and 66 in Tissemssilt. Variance analysis was highly significant (p=0.000) showing very large variability in the number of nuts per cluster between the sites. Duncan test for LN variable showed existence of two groups (G1: Laghouat, Saida and Batna, G2: Tlemcen and Tissemssilt). Negative correlation was observed between the number of nuts per cluster and axis 2.

Our measurements showed that average peduncle length of the nut was 2.12 cm, with minimum of 0.5 cm in Tlemcen and maximum of 5 cm in Batna and Saida. Variance analysis was not significant (p=0.1). Besides, there was no correlation between peduncle length and environment factors.

Average peduncle width of nuts was 0.36 cm with minimum of 0.20 cm registered in three sites (Tlemcen, Tissemssilt and Laghouat) and maximum – 0.5 cm registered in the remaining sites (Batna and Saida). Variance analysis was not significant; there were no differences between sites and no correlation was found between peduncle width of nuts and environmental factors.

Average length of the nut terminal was 0.75 cm and ranged from 0.40 cm in Laghouat to 0.9 cm in Tissemssilt. The analysis of variance was not significant: no difference was observed and no correlation was recorded between the length of the nut terminals and the environmental factors.

Average width of the nut terminal in the five sites was 0.52 cm and varied between 0.4 cm in Tissemssilt and Laghouat and 0.8 cm in Tlemcen. Variance analysis was highly significant (p=0.000) explaining large variability between the sites. Duncan test for TNW variable showed existence of three groups (G1: Laghouat, G2: Batna, Saida and Tissemssilt, G3: Tlemcen). A negative correlation occurred between the length of the nut terminals and axis 2.

Average length of nut basal of trees was 0.57 cm for all studied populations. It varied between 0.4 cm in

Table 6. Correlation between morphological parameters of nuts and factorial axes

Parameters	Axe 1	Axe 2	Axe 3
Cluster length	0.10	-0.26	0.15
Nut number	0.17	-0.51**	-0.01
Peduncle length	0.10	0.07	0.21
Peduncle width	0.24	0.10	0.16
Terminal nut length	0.09	-0.11	-0.15
Terminal nut width	-0.06	-0.55**	-0.01
Basal nut length	0.41**	-0.49**	-0.17
Basal nut width	-0.09	-0.50**	0.06

Explanations: **, * – significant at the 0.01 and 0.05 levels, respectively

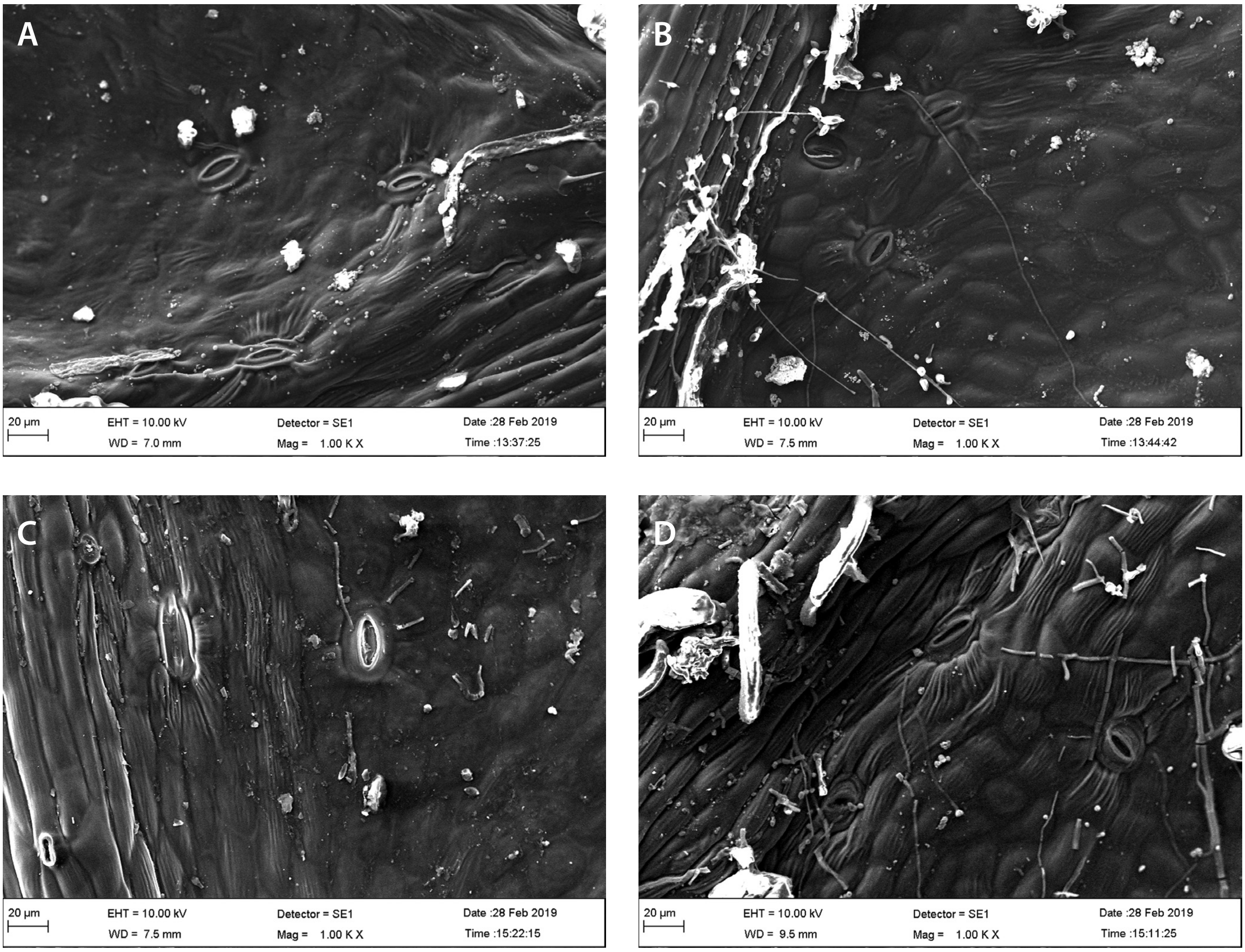

Fig. 3. SEM images of leaflets originating from four studied localities showing epidermal cells, stomata, and stripes of cuticle
Explanations: A – Saida, B – Laghouat, C – Tlemcen, D – Tissemssilt

Laghouat and 0.7 cm in Saida and Tissemssilt. Variance analysis was highly significant (p=0.000) for BNL variable. Duncan test showed existence of three groups (G1: Laghouat and Batna, G2: Tlemcen, G3: Tissemssilt and Saida). A correlation existed between the length of the nut basal and environment factors. It was positively correlated with axis 1 and negatively – with axis 2.

Average width of the nut basal in the studied populations of *Pistacia atlantica* trees was 0.49 cm with minimum width of 0.30 cm in Laghouat and maximum of 0.7 cm in Tlemcen. Variance analysis was highly significant (p=0.008). Duncan test for BLW variable showed existence of three groups (G1: Laghouat, Tissemssilt and Batna, G2: Saida, G3: Tlemcen). A negative correlation occurred between widths of the nut basal and axis 2.

3.4. Anatomy of the leaf

Pictures obtained from electron microscope scanning (Carl Zeiss EVO LS 10) made it possible to compare leaf parameters (epidermal cells, stomata, trichome, wax, and vessel element) in the four sites (Fig. 3)

The upper side of the leaflet was generally smooth in Saida, Laghouat and Tlemcen sites; however, in Tissemssilt it was corrugated. The shape of epidermal cells was different in four sites. In Saida, cells were elongated flat, in Laghouat, cells formed groups of striated cells with rays oriented towards the centre, in Tlemcen, they had irregular shapes although majority were rectangular. However, cells in Tissemssilt were regularly ordered in a convex, isodiametric and were cross-linked forming rounded ridges.

Stomata occurred in various numbers and shapes. The main types of stomata observed were: anomocytic, actinocytic, which were most abundant, especially in Tissemssilt; these two types were found in all sites in a variety of forms: oval, oblong, circular. However, they were rectangular Tlemcen. In the remaining three sites (Saida, Laghouat, Tlemcen), stomata were placed near the central vein, elliptical. Hairs or rays around stomata were observed in Saida and Tissemssilt sites.

Trichomes were epidermal extracts on the leaflet in various forms. Saida site contained two types: glandular and arched but they were situated above the main vein.

Leaflets in Laghouat site rarely had trichomes, whereas they were very abundant in Tlemcen, their extracts were present on the whole surface of the leaflet in two different forms elongated unicellular and the other was glandular. Leaflets in Tissemssilt site contained abundance of trichomes in various forms: glandular, elongated, and arched.

Microscopic examination of leaflets showed wax in the form of crystals, balls and, sometimes, as a flow near stomata. Wax was found in all sites, but it was more notable in Tlemcen and Tissemssilt. In Laghouat, wax flows were observed particularly in and around the main vein.

Microscopic examinations distinguished differences in the form of the vessel element. In Saida, it appeared intertwined, in Laghouat and Tlemcen – elongated and regular, whereas in Tissemssilt, it was elongated, intertwined, eminent and swollen.

4. Discussion

4.1. Morphological parameters

Intra and inter-populating variability observations of specific morphological parameters are sometimes sufficient to distinguish different species. In some cases, one observed feature allows to identify a species or a group of species. However, in other cases, a set of features had to be considered to characterize a species (Lesins & Lesins 1979). In this study, variability evaluation of specific features, like leaf or fruit morphology, was a good evaluation tool. The obtained data clearly showed that the studied populations of *Pistacia atlantica* are characterised by a high intra and inter-population morphological variability. Such a high phenotypic variability resulted from multiple indicators related to the diversity of edaphic, topographic, climatic and anthropic factors. It may result from a strong genotypic heterogeneity and influence of environmental factors (Bonny & Dje 2011), or environmental degradation due to overgrazing and global climatic changes leading to increasing length and severity of drought periods that compromise *P. atlantica* natural regeneration (Zaafouri & Chaieb 1999). In Turkey, Vargas *et al.* (1997) and Kafkas and Kaska (1998) demonstrated the occurrence of a significant genetic variability of this species.

According to Monjauze (1980), leaf length in *Pistacia atlantica* trees rarely exceeds 12 cm. Doghbage *et al.* (2018) claim that leaf length and width vary, depending on stations, between (10.69- 11.04 cm) length and (7.14-7.44 cm) width, it has between 3 to 11 leaflets, the terminal leaflet is 3.61 cm long and 1.12 cm wide, the petiole is 2.22 cm. According to Berrichi *et al.* (2017), average leaf length is 12.6 cm and width – 9.25 cm with a petiole 2.73 cm long. The big leaflet is 5.16 cm long and 1.72 cm wide, while the small leaflet is 3.41 cm long and 1.30 cm wide. Quézel and Médail (2003); Ait Said *et al.* (2011); Benabdallah (2012) and Benfoddil (2015) mentioned that the parameters of the leaf, leaflet, and petiole of *Pistacia atlantica* tree in the region of Tlemcen showed more elevated vegetative values than those in the Saharan and arid zones.

As far as the leaf colour is concerned, it is green or dark green (Belhadj 2008; Kafkas *et al.* 2002; Yaltirik 1967). Thus, our study explained this heterogeneity by differences in environmental factors: soil, density, climate and slope, for all the parameters of the leaf. According to Royer *et al.* (2008), dimensions and morphology of leaves are affected by the climate which leads to variations in the same species, the reduction in the leaf size is correlated with the decrease of perspiration Fahn (1967). Barboni *et al.* (2004) noted that dimensions of the leaf may vary under the impact of certain ecophysiological phenomena in relation with chlorophyll pigments on the one hand, and on the other hand, with chlorophyll contents tending to change its function depending on the availability of water because water stress can cause oxidation of chlorophyll pigments resulting in a fair green colour of leaves. The number of pairs as well as the colour of the leaflets are the most affected features by ecological factors and can also vary due to leafage and longitudinal gradient (Zohary 1952; Alyafi 1979). Small leaves tend towards being very abundant in highlands and cold regions (Barboni *et al.* 2004). Nevertheless, part of the influence of the genetic factor on the population under the influence of the environment and which is implied in the adaptation with environmental changes cannot be omitted (Maxted *et al.* 1997).

Hence, nut features are used to differentiate the four species of Pistacia (Alyafi 1979; Behboodi 2004). In this study, the length of the nut was almost the same, whereas the width varied. According to Yaltirik (1967); Belhadj *et al.* (2008), the width of the fruit is between 0.5 and 0.6 cm. This difference is explained by the effect of climate, soil type and elevation factors. The cross fecundity (allogamy) may explain differences observed at individual levels of fruits and grains (Holaly *et al.* 2015), so this difference allows for a better adaptation to the environment (Hamrick & Godt 1990; Legg *et al.* 1992).

Morphological and anatomical variability allows the species to adapt and survive in different environments, these variations include: decrease in size, winding of leaves and density of stomata (Bosabalidis & Kofidis 2002). As mentioned by Martins and Zeini (2003), anatomical features can be used as indicators of tolerance during drought. So the epidermal surface, the trichome and wax can modify gas exchange flow.

4.2. Anatomic parameters of the leaf

The microscopic study has specified the examination of the leaf epidermis which can be used in the identification of species and types of species (Navarro & El Oualidi 1999; Newton 1972; Stant 1973) and their aridity plays a role in survival and growth of the *Pistacia atlantica* tree (Ait Said *et al.* 2004), the trichome is considered as a taxonomic trait (Salmaki *et al.* 2009; Shaheen *et al.* 2009; Krak & Mraz 2008), it has an adaptive and a defensive role (Jeffree 1986) against mollusc, insects and herbivores (Duffey 1986), also the type of glandular trichome has an important role in the production and storage of essential oils (Polichuk *et al.* 2007; Giuliani & Bini 2008; Biswas *et al.* 2009). Stomata play a significant role in gas and water exchanges between the leaf and the atmosphere (Miyashita *et al.* 2005), they open and close depending on environmental factors (light, the CO2 concentration of water), and even some cations (potassium, calcium), anions (chloride) are actively involved in opening and closing the stromata, wax reduces wasting water after closing the stomata delaying severe water stress (Kozlowski & Pallardy 1979).

Results of electron microscope scanning of micro-morphology of the leaflets made it possible to distinguish large micro-morphological variability in the four studied sites due to environmental conditions where stomata variability was observed. This mechanism of stomatic control is very efficient in saving intracellular water of the leaf (Flexas & Medrano 2002; Gallé *et al.* 2007).

According to Ait Said *et al.* (2004), presence of half-open stomata in the most arid sites is due to huge resistance to aridity, In our study, two different types of stomata (anomocytic and actinocytic) were found (Belhadj 2008). Also a high variability of trichomes was observed. Elevation and ow temperature play a key role in the in the occurrence of trichomes and their distribution (Belhadj 2008). Although the stomata are declared by Metcalfe and Chalk (1950) to be anomocytic, Lin *et al.* (1984) pointed out the presence of actinocytic ones. The differences in the amount of wax on the surface of the leaflets noticed in our sites, can be explained by environmental differences (Carpenter *et al.* 2005; Holmes & Keiller 2002; St-Laurent *et al.* 2000). In certain sites, stomata close and are covered by trichomes or wax; according to (Fahn 1967), this mechanism is notable in xeromorphic plants to prevent water waste.

5. Conclusion

The presence of *Pistacia atlantica* tree in Algeria and its adaptation to different environments encouraged us to investigate whether morphological and micro-morphological variability of leaves of this species occurs in all sites under different environmental conditions (Batna, Tlemcen, Saida, Tissemssilt and Laghouat). In five studied populations, average measurements were: leaf – 7.63 cm length and 6.71 cm width, petiole – length 2.40 cm and width 0.3 cm. the basal leaflet was 3.13 cm long and 1.12 cm wide, terminal leaflet was 3.34 cm long and 1.05 cm wide. The number of leaflets per leaf was 8.83 with three main colours (green, yellow and red). Each cluster held 25.72 nuts with average length of 7.66 cm, its terminal nut was 0.75 cm long and 0.52 cm wide, whereas its basal nut was 0.57 cm long and 0.49 cm wide. Length and width of the peduncle were 2.12 cm and 0.36 cm, respectively.

Environmental factors were found to exert a remarkable impact on heterogeneity of almost all studied features where differences in all leaf parameters were caused by soil change, climate, density and slope. The nut length variability was explained by climate, soil and elevation effect.

Pictures obtained from electron microscope scanning showed differences in leaf anatomy between four studied sites. Majority of epidermal surface was smooth. The observed stomata were anomocytic and actinocytic. Different types of trichomes: glandular, arched and elongated were observed in studied sites. Wax was found to occur in all sites and there were differences in the architecture of vessel elements.

This phenotypic variability of natural populations offers choice possibilities to select a vegetative material adaptable to different regions.

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References

Ait Said S. 2011. Strategies adaptatives de deux especes du genre pistacia (p. lentiscus l. et p. atlentica desf.) aux conditions d'altitude, de salinite et d'aridite:[texte imprimé]: approches morpho-anatomiques, phytochimiques et ecophysiologiques (Doctoral dissertation, Universite Mouloud Mammeri). https://www.doc-developpement-durable.org/file/Huiles-vegetales-noix/Fiches_plantes/pistachier%20-%20pistache/STRATEGIES%20ADAPTATIVES%20DE%20DEUX%20ESPECES%20DU%20GENRE%20Pistacia_These.pdf

Alyafi J. 1979. Approches systématique et écologique du genre Pistacia L.'dans la région méditerranéenne (Doctoral dissertation, Université de droit d'économie et des sciences d'Aix-Marseille).

B.N.D.E.R. 2008. Etude du développement agricole dans la wilaya de Saida. 297 pp. Rapport final et documents annexes.

Baba Aissa F. 2000. Encyclopédie des plantes utiles, flore d'Algérie et du Maghreb, substances végétales d'Afrique, d'Orient et d'Occident. Ed Librairie moderne Rouiba, 46.

Barboni D., Harrison S. P., Bartlein P. J., Jalut G. New M., Prentice I. C., Sanchez-Goni M. F., Spessa A., Davis B. & Stevenson A. C. 2004. Relationship between plant traits and climate in the Mediterranean region: A pollen data analysis. J Veg Sci 15: 635-646.

Behboodi B. S. 2004. *Pistacia atlantica* Desf. 1800 in Iran. FAO-CIHEAM–NUCIS-Newsletter 12: 27- 29.

Belhadj S. 1999. Les pistacherais algériennes. Etat actuel et dégradation. Cahiers Options MED 56: 107-109.

Belhadj S., Derridj A., Aigouy T., Gers C., Gauquelin T., & Mevy J. P. 2007. Comparative morphology of leaf epidermis in eight populations of Atlas pistachio (*Pistacia atlantica* Desf., Anacardiaceae). Microscopy research and technique. 70(10): 837-846. https://doi.org/10.1002/jemt.20483.

Belhadj S., Derridj A., Auda Y., Gers C., & Gauquelin T. 2008. Analyse de la variabilité morphologique chez huit populations spontanées de *Pistacia atlantica* en Algérie. Botany 86(5): 520-532. https://doi.org/10.1139/B08-008.

Benabdallah F. Z. 2012. Etude morphologique des feuilles et des fruits du pistachier de l'atlas (*Pistacia atlantica* Desf.) et valorisation des huiles essentielles des feuilles et de l'oléorésine. Magister en biologie :Université Miloud Mammeri, Tizi Ouzou, Algérie.

Benabid A. 1986. Grands écosystèmes naturels marocains, équilibre de fonctionnement, perturbation, préservation et restauration. Grande encyclopédie du Maroc. Flore et Végétation, Rabat, pp. 117-190.

Benfoddil O. 2015. Inventaire des champignons endophytes des feuilles de *Pistacia atlantica* Desf. De la dayate El Gouffa (Laghout, Algérie). Magister en biologie. Université Miloud Mammeri, Tizi Ouzou, Algérie.

Benhssaini H. & Belkhodja M. 2004. Le pistachier de l'Atlas en Algérie entre la survie et disparition. La feuille et l'aiguille 54: 1-2.

Berrichi M., Chikh M., Haddad A., Allam H., Gueffar M. & Belkhodja Y. 2017. quelques Aspects Histo-morphologiques du Pistachier de l'atlas (*Pistacia atlantica* DESF.) dans le nord occidental de l'atlas tellien (Tlemcen-Algerie). Algerian Journal of Arid Environment 7(1) :111-121.

Biswas K. K., Foster A. J., Aung T., & Mahmoud S. S. 2009. Essential oil production. Relationship with abundance of glandular trichomes in aerial surface of plants. Acta Physiologiae Plantarum 31(1): 13-19. https://doi.org/10.1007/s11738-008-0214-y

Bonny S. B. & Djè Y. 2011. Variabilité morphologique et agronomique des variétés traditionnelles de voandzou [*Vigna subterranea* (L.) Verdc. (Fabaceae)] de Côte d'Ivoire. J Appl Biosci 41: 2820-2835.

Bosabalidis A. M. & Kofidis G. 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant science 163(2): 375-379. doi:org/10.1016/S0168-9452(02)00135-8

Carpenter K. J. 2005. Stomatal architecture and evolution in basal angiosperms. Am J Bot 92(10): 1595-1615. https://doi.org/10.3732/ajb.92.10.1595

Doghbage A, Boukerker H. & Belhadj S. 2018. Analyse eco-botanique comparative de deux populations du *Pistacia atlantica* en Algerie par le biais de marqueurs morphologiques foliaires. Courrier Du Savoir. (25) :119-126.

Duffey S. S. 1986. Plant glandular trichomes: their partial role in defence against insects. Insects and the plant surface. Edward Arnold, London, pp. 151-172.

El Yaaqoubi N. L. & Abba G. 2009. Identification of physical parameters including ground model parameters of walking robot rabbit. In Humanoids pp. 269-276. https://doi.org/10.1109/ICHR.2009.5379565.

Fahn A. 1967. Plant anatomy. Pergamon Press New York.

Flexas J. & Medrano H. 2002. Drought-inhibition of photosynthesis in C3 plants, stomatal and non-stomatal limitations revisited. Ann Bot 89(2):183-189. https://doi.org/10.1093/aob/mcf027

Gallé A., Haldimann P. & Feller U. 2007. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. New Phytologist 174(4): 799-810. https://doi.org/10.1111/j.1469-8137.2007.02047.x.

Giuliani C. & Bini L. M. 2008. Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae. Plant Syst Evol 276(3-4): 199.

Halitim A. 1988. Sols des régions arides d'Algérie. Ed. OPU, Alger, Algérie.

Hamrick J. L. & Godt M. W. 1990. Allozyme diversity in plant species. Plant population genetics, breeding, and genetic resources., 43-63.

Holaly G. E., Simplice K. D., Charlemagne G., Kodjovi A., Kokou A., Tchadjobo T. & Jacques S. 2015. Étude ethnobotanique des plantes utilisées dans le traitement du diabète dans la médecine traditionnelle de la région Maritime du Togo. The Pan African Medical

Journal (20): 437. https://dx.doi.org/10.11604%2Fpamj.2015.20.437.5660.

Holmes M. G. & Keiller D. R. (2002) : Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. Plant, Cell & Environment 25(1): 85-93. https://doi.org/10.1046/j.1365-3040.2002.00779.x.

Jeffree C. E. 1986. The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. Insects on the plant surface, pp. 23-64.

Kafkas S. & Kaska N. 1998. Suitability of some selected and fast growing *Pistacia Atlantica* Desf. types as pistachio nut rootstock. Cahiers Options Méditerranéennes 33: 185-190.

Kafkas S. & Perl-Treves R. 2002. Interspecific relationships in Pistacia based on RAPD fingerprinting. HortScience 37(1): 168-171. https://doi.org/10.21273/HORTSCI.37.1.168.

Khaldi A. & Khoudja M. K. 1996. Atlas pistachio (*Pistacia atlantica* Desf.) in North Africa: taxonomy, geographical distribution, utilization and conservation. In: S. Padulosi, T. Caruso & E. Barone (eds.). Taxonomy, distribution, conservation and uses of *Pistacia* genetic resources. Proceedings of the IPGRI Workshop 29-30 Jun 1995. Palermo, Italy, pp.57-62.

Kozlowski T. T. & Pallardy S. G. 1996. Physiology of woody plants. Academic press. Printed in the United States of America.

Krak K. & Mráz P. 2008. Trichomes in the tribe *Lactuceae* (Asteraceae) – taxonomic implications. Biologia 63(5): 616-630. https://doi.org/10.2478/s11756-008-0106-z

Larouci A. 1987. Etude biochimique et physiologique des essences du pistachier de l'Atlas. Mémoire ingéniorat. Université de USTHB Alger, Algérie.

Legg C. J., Maltby E. & Proctor M. C. F. 1992. The ecology of severe moorland fire on the North York Moors: seed distribution and seedling establishment of *Calluna vulgaris*. J Ecol 80(4): 737-752. http://dx.doi.org/10.2307/2260863

Lesins K. A. & Lesins I. 1979. Genus *Medicago* (Leguminosae). A Taxogenetic Study.

Mahmoud B., Kourosh S., Mohsen A., Ahmad A., Shirin S., Mahmoud R., Fariba B. & Ehsan B. 2015. The effects of nutritional and medicinal mastic herb (*Pistacia atlantica*). Journal of Chemical and Pharmaceutical Research 7(1): 646-653.

Martins M. B. G. & Zieri R. 2003. Leaf anatomy of rubber-tree clones. Scientia Agricola 60(4): 709-713. https://doi.org/10.1590/S0103-90162003000400015

Maxted N., Hawkes J. G., Guarino L. & Sawkins M. 1997. Towards the selection of taxa for plant genetic conservation. Genet Resour Crop Ev 44(4): 337-348.

Melazem B. 1990. Etude des facteurs limitant de la régénération naturelle du cèdre de l'Atlas Cedrus atlantica Manetti dans le parc national de Theniet.El.Had. Mémoire, Ingéniorat, Université USTHB Bab Ezzouar-Alger, Algérie.

Metcalfe C. R. & Chalk L. 1950. Anatomy of the Dicotyledons: leaves, stem, and wood, in relation to taxonomy, with notes on economic uses. Anatomy of the Dicotyledons: leaves, stem, and wood, in relation to taxonomy, with notes on economic uses 1-2.

Miyashita K., Tanakamaru S., Maitani T. & Kimura K. 2005.: Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ Exp Bot 53(2): 205-214. https://doi.org/10.1016/j.envexpbot.2004.03.015

Monjauze A. 1967. Note sur la régénération du Bétoum par semis naturels dans la place d'essais de Kef Lefaa. Bull. Soc. Hist. Afr. du Nord. 57: 59-65.

Monjauze A. 1980. Connaissance du bétoum *Pistacia atlantica* Desf. http://documents.irevues.inist.fr/bitstream/handle/2042/21418/RFF_1980_4_356.pdf?s

Navarro T. & El Oualidi J. 1999. Trichome morphology in *Teucrium* L.(Labiatae). A taxonomic review. In Anales del Jardín Botánico de Madrid 57(2): 277-297. https://doi.org/10.3989/ajbm.1999.v57.i2.203

Newton L. 1972. Taxonomic use of the cuticular surface features in the genus *Aloe* (Liliaceae). Bot J Linn Soc 65(3): 335-339.

Polichuk D. R., Zhang Y., Reed D. W., Schmidt J. S. & Covello C. S. 2010. A glandular trichome-specific monoterpene alcohol dehydrogenase from *Artemisia annua*. Phytochemistry. 71(11-12): 1264-1269. https://doi.org/10.1016/j.phytochem.2010.04.026

Pietracarprina P. 1988. Morphologie et classification des sols CIHEAM (Bari).

Quézel P. & Médail F. 2003. Ecologie et biogéographie des forêts du bassin méditerranéen. 572. Paris, Elsevier.

Rezaei P. F., Fouladdel S., Hassani S., Yousefbeyk F., Ghaffari S. M. & Amin G. 2012. Food and Chemical Toxicology 50(3): 1054-1059. https://doi.org/10.1016/j.fct.2011.11.012

Royer D. L., Mcelwain J. C., Adams J. M. & Wilf P. 2008. Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*. New Phytol 179: 808-817. https://doi.org/10.1111/j.1469-8137.2008.02496.x

S.A.T.E.C. 1976. Etude développement intégré de la daïra de Saida. Rapport technique, 93 pp.

Said S. A., Fernandez C., Greff S., Derridj A., Gauquelin T. & Mevy J. P. 2011. Inter-population variability of leaf morpho-anatomical and terpenoid patterns of *Pistacia atlantica* Desf. ssp. atlantica growing along an aridity gradient in Algeria. Flora 206(4): 397-405. https://doi.org/10.1016/j.flora.2010.08.002

Salmaki Y., Zarre S., Jamzad Z. & Bräuchler C. 2009. Trichome micromorphology of Iranian *Stachys* (Lamiaceae) with emphasis on its systematic implication. Flora 204(5): 371-381. https://doi.org/10.1016/j.flora.2008.11.001.

Shaheen N., Ajab M., Yasmin G. & Hayat M. Q. 2009. Diversity of foliar trichomes and their systematic relevance in the genus *Hibiscus* (Malvaceae). Int J Agric Biol 11: 279-284

Stant M. Y. 1973. Scanning electron microscopy of silica bodies and other epidermal features in *Gibasis* (*Tradescantia*) leaf. Bot J Linn Soc 66(3): 233-243. https://doi.org/10.1111/j.1095-8339.1973.tb02172.x

St-Laurent L., Baum B. R., Akpagana K. & Arnason J. T. 2000. Leaf tri-chome morphology and density in West AfricanTremaspp. (Ulma-ceae: Celtidoideae). Can J Bot 78: 34-39.

Taran M., Mohebali M. & Esmaeli J. 2010. In Vivo Efficacy of Gum Obtained *Pistacia atlantica* in Experimental Treatment of Cutaneous Leishmaniasi. Iran J Public Health 39(1): 36.

Vargas F. J., Romero M. A., Monastra F., Rouskas D. & Mendes Gaspar A. 1997. Sélection de variétés de pistachier adaptées à l'aire nord méditerranéenne. Options Méditerranéennes, Serie B, Etudes et Recherches. 16: 93-119.

Yaltirik F. 1967. *Pistacia* L. Dans Flora of Turkey and the east Aegean Islands. Sous la direction de P.H. Davis. Edinburgh University Press, Edinburgh, R.-U.(2): 542-548.

Zaafouri M. S. & Chaïeb M. 1999. Arbres et arbustes de la Tunisie méridionale menacés de disparition. Acta Bot Gallica 146(4): 361-373. https://doi.org/10.1080/12538078.1999.10515823

Zohary D. 1996. The mode of domestication of founder crops of Southwest Asian agriculture. In: D. R. Harris (ed.). The origins and spread of agriculture and pastoralism in Eurasia, pp. 142-158. University College London Press, London.

Zohary M. 1952. A monographical study of the genus *Pistacia*. Palestine Journal of Botany (Jerusalem Series) 5(4): 187-228.