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Geographical patterns of morphological variation in *Pistacia lentiscus* L.

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Abstract

To study the morphological variability within and among *Pistacia lentiscus* L. populations, a comparative analysis was undertaken in twelve wild populations grown under different climatic conditions in Tunisia. This study addresses the morphological aspect of the leaves and the fruits. The morphological variability of leaves and fruits of *P. lentiscus* was studied based on the *Pistacia* descriptor developed by the International Plant Genetic Resources Institute. Statistical analysis of the morphological traits revealed a significant diversity within and among the populations. Samples from Chehid showed the longest leaves (7.92 cm) while those taken from Sidi Zid were shown the shortest with an average length of around 5.25 cm. The widest leaves are those from Azib (5.02 cm) and Tabouba (5.03 cm). Leaves from Jbel Orbata and Sidi Zid are the narrowest with an average of 3.85 and 3.88 cm, respectively.Tabouba provenance showed the most developed leaves and fruits and Feija site was characterized by small fruit size and low viability rate.

1. Introduction

Mastic tree, *Pistacia lentiscus* L., belongs to the family *Anacardiaceae*. This is an evergreen shrub growing in dry and rocky areas. This dioecious species can reach 3-4 m in height and grows in the Mediterranean countries. The most important component of *P. lentiscus* is resin. It has a great medicinal value and has already been used in traditional system of medicines for the treatment of some stomach diseases and as antiseptic for respiratory system [1].

The essential oil extracted from mastic is used to soothe rheumatism and stomach pains and to shrink tumors cells [2]. The essential oil of leaves of *P. lentiscus* has been reported to have high antioxidant property. It is commonly used as a decongestant and for varicose veins problems [3]. This essential oil, rich in phenolic functional compounds, showed remarkable in vitro antioxidant and anticancer effects and interesting antibacterial and antileishmanial activities [4].

In some countries, especially in Tunisia and Algeria, the oil extracted from mature fruits is commonly used in traditional medicine as an anti-ulcer, wound healing and antiseptic [5,6]. This oil showed neuroprotective, gastro-protective, therapeutic and anti-inflammatory activities [7,8]. The aerial part of

P. lentiscus has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties [9]. In Tunisian folk medicine, decoction of roots is used for the treatment of stomach ulcers and gastric and intestinal problems. Currently, exploitation of this plant is constantly increasing which requires suitable conservation measures to be developed .

To preserve and recover this species, there is a need to increase knowledge on its diversity and understand how it is adapted to its environmental conditions, especially, under climate change situation. The aim of the present study, which is carried out for the first time for *P. lentiscus* grown in Tunisia, is to determine the effect of growing area on morphological traits of fruits and leaves of mastic tree.

2. Methodology

2.1. *Plant material*

Leaves and fruits of *P. lentiscus* L. were collected from twelve different sites in Tunisia. The bioclimatic characterization of the sites is presented in Table 1. The plant was identified by Dr A. Khaldi from I.N.R.G.R.E.F-Tunisia and certified specimens (VS1-PL2009) were deposited at the Herbarium run by I.N.R.G.R.E.F.

Photo 1 : Leaves and fruits of *P. lentiscus* L.

Table1. Geographic and bioclimatic characterization of *Pistacia lentiscus* harvest sites

Site	Latitude (N)	Longitude (E)	Altitude (m)	Bioclimat
Ain Cristal	35.922725	9.4736111	489	Semi-arid
Azib	37.209918	9.97678983	39.14	Sub-humid
Chehid	36.5408333	9.32305555	707	Semi-arid
JbelAbderrahman	36.7833333	9.73333	218	Sub-humid
JbelBoukehil	36.30845467	9.11967517	551.47	Semi-arid
Jbel Mansour	36.248959	9.70930533	550.06	Semi-arid
Neber	36.32863283	8.78163967	424.5	Semi-arid
Oueslatia	35.8530556	9.59194444	475	Semi-arid
Feija	36.4800537	8.32923333	545.75	Humid
Tabouba	36.09299833	9.11211867	102.96	Humid
Kebouch	36.21061617	8.8881085	708.95	Semi-arid
Sidi Zid	36.7466667	10.3191666	676	Sub-humid

Sampling was done between mid-December and mid-January when fruits are fully ripe. Fifteen plants were chosen in each sampling site and 2-3 branches per plant were harvested at a mid-height to reduce

within individual variance. Leaves and fruits were removed in the field and immediately frozen until undergoing morphological analysis in the laboratory.

2.2.*Morphological analysis*

The morphological variability of leaves and fruits of *P. lentiscus* was studied based on the *Pistacia* descriptor developed by the International Plant Genetic Resources Institute [10].
In each population, 300 fully expanded leaves (from 15 trees, 20 leaves per tree) were taken and used to calculate mean leave area (LA), leave length (LL), leave width (LW), petiole length (PL) and number of leaflets (NL). Leave area was measured with a CI-202 Laser area Meter.
300 fruits from each population (from 15 trees, 20 fruits per tree) were sampled and characterized according to five traits; fruit length (FL), fruit width (FW), fruit length / width ratio (L/W), 100 seeds weight (W) and viability rate (V).

2.3. ***Statistical analysis***

The statistical significance between antioxidant activity values of the extracts was evaluated with the GLM procedure (General Linear Models) of the SAS (9.0) program. P values less than 0.05 were considered to be statistically significant. All values are the mean of three replications.

3. Results and Discussion

3.1. ***Morphological characterization of leaves***

To study the morphological variability of leaves, the following parameters were measured; leaf area, leaf length, leaf width, petiole length and number of leaflets.
The results of these parameters are summarized in Table 2.
ANOVA analyzes showed that the differences between the leaf area values were not significant. The leaf area parameter was not influenced by the sampling site. On the other hand, the other parameters: leaf length, leaf width, petiole length and the number of leaflets showed significant differences between studied sites ($p < 0.0001$).

Tableau 2. Morphological Characterization of *P. lentiscus* leaves harvested from different localities

Site	Mean leaf area (LA) (cm^2)	Leaf length (LL) (cm)	Leaf width (LW) (cm)	Petiole length (PL) (cm)	Number of leaflets (NL)
Ain Cristal	$12.22^{a} \pm 4.12$	$6.24^{g} \pm 1.0$	$4.56^{de} \pm 0.7$	$1.11^{g} \pm 0.23$	$8.68^{c} \pm 1.72$
Azib	$14.53^{a} \pm 4.16$	$6.59^{ef} \pm 0.9$	$5.02^{a} \pm 0.7$	$1.34^{c} \pm 0.35$	$7.41^{g} \pm 1.47$
Chehid	$18.88^{a} \pm 5.57$	$7.92^{a} \pm 1.4$	$4.8^{b} \pm 0.91$	$1.33^{c} \pm 0.31$	$10.2^{a} \pm 1.76$
JbelAbderrahman	$12.85^{a} \pm 3.83$	$6.72^{de} \pm 2.6$	$4.15^{g} \pm 0.7$	$1.33^{c} \pm 0.32$	$9.38^{b} \pm 6.7$
JbelBoukehil	$18.8^{a} \pm 8.1$	$6.82^{cde} \pm 1.02$	$4.69^{c} \pm 0.7$	$1.25^{de} \pm 0.27$	$8.16^{def} \pm 1.56$
Jbel Mansour	$13^{a} \pm 3.53$	$6.66^{ef} \pm 1.08$	$4.65^{cd} \pm 0.6$	$1.21^{ef} \pm 0.33$	$8.73^{c} \pm 1.66$
Neber	$14.97^{a} \pm 4.44$	$7.4^{b} \pm 1.22$	$4.88^{b} \pm 0.9$	$1.43^{b} \pm 0.34$	$8.99^{c} \pm 1.72$
Oueslatia	$11.47^{a} \pm 3.38$	$6.21^{g} \pm 1.13$	$4.16^{f} \pm 0.7$	$1.33^{c} \pm 0.31$	$7.91^{f} \pm 1.55$
Feija	$11.98^{a} \pm 4.26$	$6.63^{ef} \pm 1.06$	$4.56^{de} \pm 0.7$	$1.33^{c} \pm 0.28$	$8.1^{def} \pm 1.69$
Tabouba	$14.05^{a} \pm 3.53$	$6.48^{f} \pm 0.94$	$5.03^{a} \pm 0.8$	$1.32^{c} \pm 0.27$	$7.23^{g} \pm 1.46$
Kebouch	$15.87^{a} \pm 5.49$	$6.95^{c} \pm 1.15$	$4.22^{fg} \pm 0.7$	$1.32^{c} \pm 0.39$	$8.53^{cd} \pm 1.52$
Sidi Zid	$10.08^{a} \pm 3.38$	$5.25^{h} \pm 1.14$	$3.88^{h} \pm 0.68$	$1.54^{a} \pm 0.15$	$7.87^{f} \pm 4.33$

The means of the same column with different letters are significantly different ($p<0.05$)

The leaf area of *P. lentiscus* varied from 9.59 to 18.88 cm^2, respectively, for Jbel Orbata and Chehid. The maximum area value is in agreement with that reported in theliterature. Gratani et al. [11] studied the morphology of Italian lentisk leaves and have shown that the average area value was around 19 cm^2. Samples from Chehid showed the longest leaves (7.92 cm) while those taken from Sidi Zid showed the shortest ones with an average length of around 5.25 cm.

The widest leaves are those from Azib (5.02 cm) and Tabouba (5.03 cm). Leaves from Jbel Orbata and Sidi Zid are the narrowest with an average of 3.85 and 3.88 cm, respectively. The low values of the dimensions (length and width) of the leaves in Sidi Zid could be linked to the strategy developed by the species to contain the difficult climatic conditions at this site, which is characterized by poorly developed soils.

The leaves collected at the Sidi Zid site have the longest petioles (1.54cm) while those of Ain Cristal have the shortest petioles (1.11 cm).

Furthermore, the results of the present study showed that the measured leaves of *P. lentiscus* contain an average of 7-10 leaflets per leaf. Minimum value of 7 leaflets per leaf was observed in Tabouba and Azib, while the highest number of leaflets (10 leaflets) was observed for Chehid locality.

The variability in morphology of *P. lentiscus* leaves, depending on the harvest site can be explained by several factors such as environmental conditions and genetic factors.

In the literature it has been mentioned that the form and size of the leaves as well as their arrangement on the stem helps plants to photosynthesize.

Under genetic control, the form and structure of the leaves reflect adaptations that have allowed plants to survive in different environments [12]. The morphology of the leaves can give an idea on the nature of the environment were the plant is developed. Generally, broad leaves with a thin, smooth blade characterize plants that live in humid environments, where temperatures are low, the level of light is weak and the wind absent. The large surface is related to the low light [12].

According to Alyafi [13], the number and color of the leaflets are very affected by ecological factors. Other authors have mentioned that the number of leaflets can be affected by leaf age and longitudinal gradient [14]. Work developed by Karimi et al. [15] on three species of pistachio: *Pistacia vera*, *Pistacia khinjuk* and *Pistacia atlantica* have demonstrated the existence of morphological variability between different harvest sites. This morphological variability has been explained by genetic diversity.

The effect of gender on the leaf morphological diversity in *Pistacia lentiscus* was studied by Doghbage et al. [16]. They showed significant differences for the measured variables between male and female plants.

3.2. Morphological characterization of leaves

To characterize the fruits of *P. lentiscus*, five descriptors were measured: fruit length, fruit width, length / width ratio, 100-seed mass and viability rate. The results of this characterization are summarized in Table 3. ANOVA analyzes showed the existence of significant differences between the sites of harvesting on the one hand and between individuals on the same site on the other, this being valid forall measured parameters ($p < 0.001$). The fruits from Tabouba showed the highest mass with an average of the order of 7.27g / 100 seeds. The highest viability rate was recorded for Tabouba fruits (52.8%) so that the lowest rate was determined for the fruits of Jbel Abderrahmen (12.33%). The fruits of *P. lentiscus* are, on average, 4 to 5 mm long. The minimum and maximum values are respectively, 4.34 mm in Jbel Boukehil and 5.31 mm in Tabouba. The minimum and maximum values of fruit width were recorded for the same populations with a width of 3.71 mm for Jbel Boukehil and 5.02 mm for Tabouba.

These results allow us to conclude that the fruits of the Tabouba site are the largest and are the most viable. This important seed viability is the result of a high fertility rate that occurred after pollination of female flowers.

Tableau 3. Morphological Characterization of *P. lentiscus* fruits harvested from different localities

Site	100 seeds weight (W) (mg)	Fruit length (FL) (mm)	Fruit width (FW) (mm)	Fruit length / width ratio (L/W)	Viability rate (V) (%)
Ain Cristal	5.26^{e}± 0.13	4.53^{e}±0.5	3.7^{g}±0.58	1.2^{b}	24.86^{e}±2.39
Azib	5.99^{cd}±0.13	4.7^{d}±0.54	4.34^{d}±0.5	1.08^{f}	43.86^{c}±18.96
Chehid	4.18^{fg}±0.06	4.51^{e}±0.43	4.09^{e}±0.4	1.1^{e}	16.46^{f}±1.7
JbelAbderrahman	4.01^{g}±0.09	4.57^{e}±0.52	4.07^{e}±0.4	1.13^{e}	12.33^{g}±1.6
JbelBoukehil	4.54^{f}±0.1	4.34^{f}±0.57	3.71^{g}±0.5	1.17^{d}	29.53^{d}±2.02
Jbel Mansour	5.51^{de}±0.15	4.84^{c}±0.57	4.48^{c}±0.5	1.08^{f}	31.33^{d}±2.5
Neber	6.08^{bc}±0.15	4.85^{c}±0.54	3.84^{f}±0.6	1.26^{a}	28.6^{d}±2.3
Oueslatia	5.24^{e}±0.13	4.71^{d}±0.62	4.04^{e}±0.7	1.15^{d}	6.13^{h}±0.8
Feija	6.5^{b}±0.14	4.96^{b}±0.49	4.62^{b}±0.5	1.07^{f}	48.46^{b}±2.5
Tabouba	7.27^{a}±0.15	5.31^{a} ±0.43	5.02^{a} ±0.42	1.06^{f}	52.8^{a}±2.4
Kebouch	5.3^{e}±0.2	4.58^{e} ±0.88	3.86^{f} ±0.87	1.19^{c}	30.4^{d}±2.6
Sidi Zid	5.83^{cde}±0.42	4.65^{d} ±0.39	4.34^{d} ±0.37	1.07^{f}	41.46^{c}±0.23

The means of the same column with different letters are significantly different ($p<0.05$)

In the case of the mastic tree, which is a dioeciously species, pollination is carried out by pollinator agents which are either animals, such as insects and birds, or the wind. The availability of these agents in the site where the mastic shrubs exist influences the pollination phenomenon and consequently the seed viability rate [17]. The ratio between the number of male and female shrubs, or sex ratio, is also a determinant factor of pollination in dioeciously species [18].

The Tabouba site, with the highest viability rate, probably contains more pollinator agents and / or a greater number of male shrubs compared to other sites of harvest.

The length / width ratio was between 1.06 and 1.26 suggesting variability at the level the form of the fruit harvested in different sites in Tunisia. The maximum value was recorded for Neber fruits (1.26) indicating an ovoid form of the fruits. The highly significant differences observed for the dimensions of the fruits are probably linked to the different climatic and edaphic conditions between the harvest sites [19]. The effect of water availability on fruit size has long been studied. Huguet [20] showed the existence of variations in the dimensions of fruits which could be related to the water supply of the plant. More focused studies on the development process of several fruits have allowed establishing a relationship between the variability in fruit shape and size and the age of the tree, position of the fruit on the fruiting branch and / or environmental conditions [21,22]. Several studies carried out on other species, including tomato, have shown that the variation of the size of the fruit is genetically controlled. Mutations that can occur at the level of this gene are responsible for the variation in fruit size [23,24,25].

Conclusions

Morphological characterization was carried out on the leaves and fruits of *P. lentiscus* collected in different sites in Tunisia. This study concerned 4 morphological characters of the fruits and 5 morphological parameters of the leaves. The effect of harvest site on the morphology of the individuals studied was highlighted.

Tabouba provenance showed the most developed leaves and fruits, on the other hand Feija site was characterized by small fruit size and low viability rate.

Since this morphological diversity could be attributed to environmental conditions and / or the existence of genetic diversity, a study of the genetic diversity between these populations of *P. lentiscus* is recommended.

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