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Research Article
Evaluation of the Anatomical Polymorphism of *Pistacia atlantica* Population in the Rechaiga Area, Tiaret, Algeria

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Abstract

Background and Objective: Algeria got a rich genetic patrimony unknown and unvalued. The Atlas pistachio tree, a vanishing species, which presents an ecological and a socio-economical interest. It is an important patrimony. In order to develop this specie an eventual variability in these stocks to valorize, rehabilitate and protect them. The aim of the study was evaluation of the genetic variability of anatomic parameters of the leaves in the stock of Rechaiga. **Materials and Methods:** Fifty two samples (folioles limb) were studied histologically, to characterize leaf structure and estimated the targeted population genetic variability. The data of different measurements in this study have been analyzed statistically (ANOVA one-way and dendrogram elaboration) by Statistica 8.0. **Results:** The results showed a high variability of the structural characters (thickness of the lacunae and palisade parenchyma, the thickness of the epiderm, the diameter of the ligneous ducts and the number of the ex-secretors channels) between males and females. **Conclusion:** This study noted that, the foliar structure of the atlas pistachio tree has an important variability at the individuals of the two studied sexes. It was noticed that this variability is rather marked at the individuals of the females compared to that of the males.

Key words: *Pistacia atlantica* Desf., genetic variability, anatomic parameters, folioles limb, epiderm

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Algerian ecologic area faces many obstacles, mainly climatic, which limits the conservation and preservation of vegetal genetic diversity[1]. Some species adapt to drought and high temperatures and can live longer[2]. The Atlas pistachio tree of is an imposed kind. A prospection and an evaluation of the genetic variability offered and allow its preservation and valorization on different ways. Complementary actions are to reach a strategy required for the protection of the existing genetic patrimony and its development through reforestation[3,4].

Pistacia atlantica tree is a dioecious tree of the anacardiaceous family 55 genus and about 500 species dispatched around the Mediterranean sea, in South West and East of central Asia and the American continent[5]. In Algeria it is recorded from the Mitidja in the North until the Southern region Monjauze[6] and Belhadj[7] shows that it exist around Djelfa (Senalba, Ain Ouessara, Messaâd), in the South of Laghouat and Guardaia (oued mzab). According to Ozenda[8], *Pistacia atlantica* of, time ago, existed between Laghouat and Biskra and in the Northern part of the country.

The Atlas pistachio tree is used as a rootstock for another pistachio tree (*Pistacia vera*), it is not a taproot and it is vigorous. It is used as a food (the seeds are eaten) and as a forage in summer and sometimes as a fuel[9,1]. Its rusticity allows the reforestation of arid zones it permits also the immobilization of the dunes in order to stop the desertification[10,4].

The genus pistacia which includes *Pistacia atlantica* belongs to xerophytes[11], this ecological clamp regroups dry places plants able to support aridity, due to anatomico-physiologic particularities[12,13]. Unfortunately its repartition area is decreasing[14]. The main cause of this regression is essentially anthropogenic[15].

The study of primary and secondary structures of vegetal organs and there remodeling is the aim of vegetal biology. Reduction of foliar area, the mesophyll thickness, the increasing of cellular veins and the diminution of chlorophyllien cells number are the aim of anatomic transformation[14,16,13]. These anatomic particularities help to maintain an optimal photosynthetic activity in a climate dominated aridity and high variations of temperature[17]. The estimation of the anatomic variability in the specie appears to be the fundamental and decisive mean during the operations of valorization and conservation. The present work treaty the characterization of leave anatomy of some individuals issued from a population around Rechaiga (South-East of Tiaret). This anatomic study is a way of estimation of the targeted population genetic variability. Use of structural parameters in genetic variability estimation is advantageous, for most of them are characterized by high heritability.

The most works about *Pistacia atlantica* were consecrated mainly to study of chemical composition of leaves[11]. Few works have been done on anatomical studies. The aim of the study was evaluation of the genetic variability of anatomic parameters of the leaves in the stock of Rechaiga.

MATERIALS AND METHODS

The study was carried out during the period of August 2015 and December 2016, the samples used in this research come from the district houassi, forest of Rechaiga (latitude 35°18'09" N and 35°22' 50" N, Longitude: 02°06' 37"and 02°08' 45"), in an area of 2539 ha including about 2000 *Pistacia atlantica* trees. Each of the 52 samples is divided into male and female half. Histological section of leaves folioles were used for anatomic study.

The folioles limb median part is extracted and immediately fixed with a mixture of ethanol [C_2H_6O] and acetic acid [CH_3COOH] (15: 3) during 24 h. After that, the samples were washed with tap water for another 24 h. The samples were dehydrated in ethanol with increasing concentrations (70, 100%) the samples are after that impregnated and included in blocs of paraffin and cut horizontally with a microtome (LEICA RM 2145) 7 µm thick. After paraffin taking off and coloration of the sections, the measures concerned structural parameters of parenchyma's, of the epidermis and the conducting ducts. The parenchymatous parameters considered are linked to the lacunous and palyssadic strata and the number of cell veins in the two parenchyma. The number of excreting ducts was determined. The measurements on the epidermis structure concern the thickness the external wall of revetment cells. The central nervure number of metaxylem ligneous veins and there diameters were also determined. Finally the ratios palyssadic parenchyma/leave thickness and lacunous parenchyma/leave thickness were determined.

Statistic analysis: The data of different measurements in this study have been analyzed statistically (ANOVA one-way at 5% probability level and dendrogram elaboration) by Statistica 8.0.

RESULTS

Characterization of the leaf mesophyll: The thickness of the palisade parenchyma presents important variations after study of males and females cases. The variation is higher in male population ($p<0.01$) than female ($p<0.001$). For female cases the thickness results are included between 44.07 µm and 310.72 µm with a variation coefficient equal to 46%. This values fluctuation means an important variability in the female cases. There is a variation too in the male cases. The results reported are between the extrems 44.25 and 144.93 µm with variation degree equal to 25% (Table 1).

The lacunous parenchyma recorded thicknesses present themselves an important variation between the cases of the same sex. In the female cases the values of this parameter (Fig. 1) are between 15.19 and 208.77 µm and present a variation rate about 49%. In male individuals, the extreme values are included between 25.75 and 115.77 µm with a variation rate of 28%.

The results obtained from the two parenchyma show an accentuated polymorphism in the male cases ($p>0.05$).

The index obtained from the ratio of palisade parenchyma and leaf thickness express partly the structural variability in all individuals ($p<0.001$). This variability is important and is justified by the values fluctuation ratios (between 0.19 and 2.21 µm) for female (between 0.21 and 0.58 µm) and male, individuals.

These results differentiate significatively, the variation rate of this parameter expression between the two sexes. Therefore the variation is more important in female sex than male these variations are between 63.70 and 22.12%. The leaf thickness gives a high polymorphism for both sexes ($p<0.01$ for females and $p<0.001$ for males). For female individuals, the results are included between 38.35 and 722.48 µm with a variation ratio of 50.31%. The males' results are included between 147.64 and 276.91 µm with a variation ratio of 12.91%.

Table 1: The mean square, the F test and the degree of significance of the influence of the individuals nature studied on the variations of the anatomical parameters

	CM		F-test		Coefficient of variation (%)	
Parameters	Male (ddl=25)	Female (ddl=24)	Male	Famele	Male	Female
TPP (µm)	757.3	28.51325	3.300***	14.241**	25.1998	46.3638
TL (µm)	2176	50683	18.14**	89.87**	12.9119	50.3103
R1	0.01372	0.22809	1.697***	4.173*	22.1240	63.7023
TLP (µm)	427.6	2232.0	2.949ns	5.638*	28.5132	48.8226
R2	0.3436	1.0233	1.3935***	1.7742**	38.1398	43.6085
TE (µm)	27.56	479.55	3.784***	60.719**	17.90057	45.70978
DLD (µm)	266.6	237.3	2.884**	2.558	28.9762	28.3356

TPP: Thickness of the palisade parenchyma, TLP: Thickness of the lacunary parenchyma, TL: Thickness of leaf, R1: Relationship between the thickness of the palisade parenchyma and the thickness of the leaf, R2: Relationship between the thickness of the palisade parenchyma and the lacunary parenchyma, TE: Thickness of epiderm, DLD: Diameter of the ligneous ducts *Significant, **and ***Highly significant

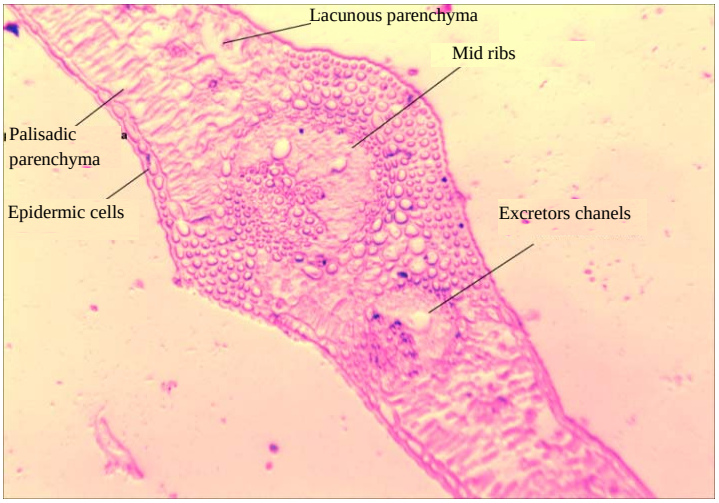

Fig. 1: Anatomic section showing the structure of the pistachio tree of Atlas

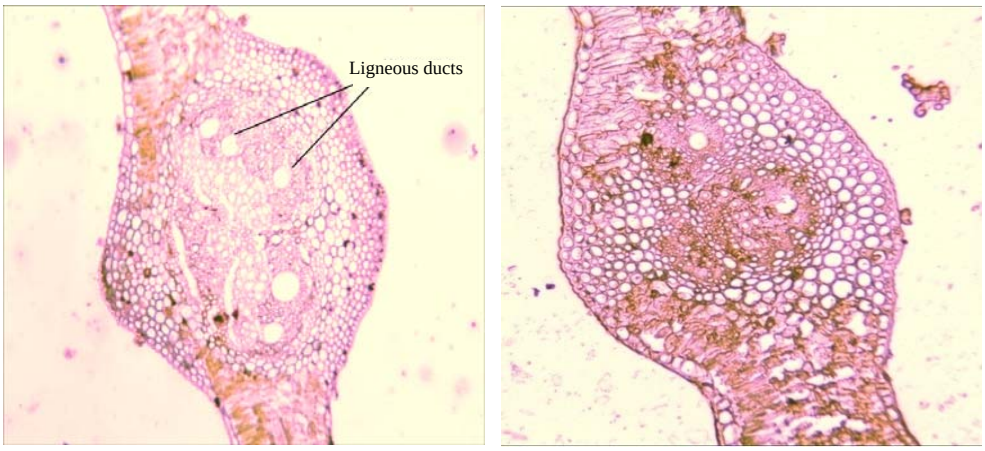

Fig. 2: Anatomic sections showing the variability of the ligneous ducts

Fig. 3: Anatomic section showing the excreting duct

The thickness of the epiderm is studied includes the epidemic cells with thickness due to modifications of external walls. The values given by the different individuals studied and belonging to the two sexes are variables (Table 1). For female cases, the values are between 8.46 and 83.95 µm with a variation equal to 45.70%, for male cases the values are included between 12.22 and 29.61 µm with a variation ratio of 17.90%.

The obtained number of parenchymatous palisades cells fibers shows the existence of two values 2 or 3 strata by foliate structure. The repartition of these values does not depend on the sex.

Structure of ligneous ducts of xylem: The study of the results concerning the number of ligneous ducts of the chief nerve (Table 1) shows an important variation in there development between males and females. The medium data shows a repartition of this number in an interval between 2 and 6 ducts by nerve (Fig. 2).

The ligneous duct diameter present an important variation in male individuals ($p<0.01$) and females ($p<0.001$). The medium results got from males are between 18.46 and 75.41 µm with an variation rate about 28.95%. Concerning the female individuals, the diameters recorded are between 15.51 and 76.15 µm with a variation rate of 28.33%.

Structure of excreting ducts: The foliar structure of this specie got resin excreting ducts. The sections show that there number is variable. For female individuals, the number varies between 1 and 6, though 2 is preponderant. For male individuals, the variation is more important, it is between 1 and 9 ducts per foliar structure (Fig. 3).

The dendrogram for the 26 male individuals studied (Fig. 4) permit the distinction of 3 different groups. The first one is composed of 18 different individuals, the second six and the third two individuals.

The dendrogram for the 26 female individuals (Fig. 5) permit the distinction of 03 different groups. The

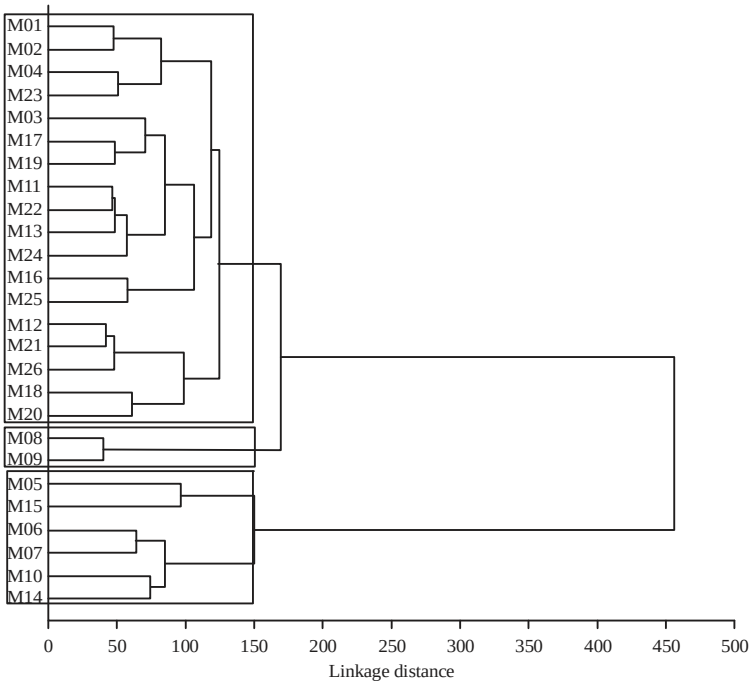

Fig. 4: Dendrogram showing a classification of male individuals according to anatomic parameters

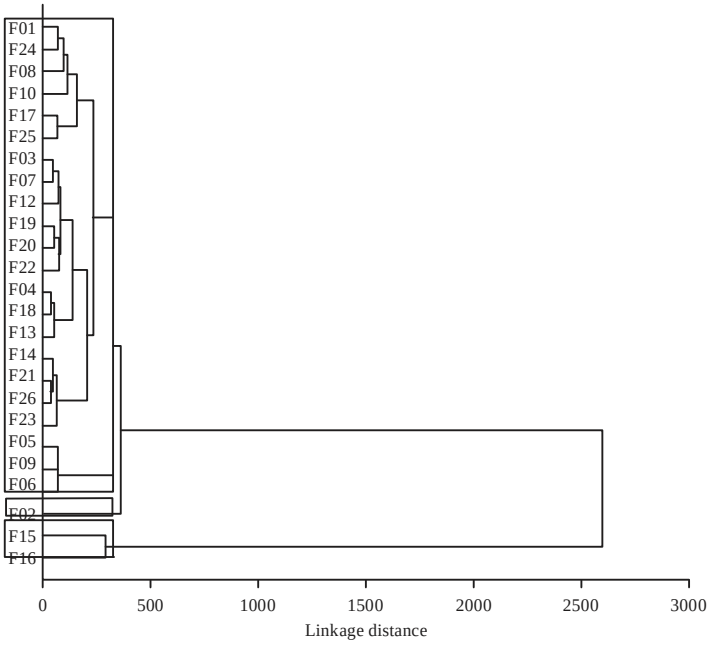

Fig. 5: Dendrogram showing a classification of female individuals according to anatomic parameters

first one is composed of 22 different individuals, the second tow and the third one individual.

These results confirm the existence of variability for the studied characters. These variability explain variations at the lever of the anatomic structure for the individuals and their genetic operation.

DISCUSSION

The survival of spontaneous plants in dry land areas depends on their developed adaptation faculty to tolerate the imposed pression by the abiotic stress. The responsible mechanisms adaptation functions are of multiple orders. Their

Table 2: Relations between the various anatomical parameters within the individuals of the female sex

Variable	TPP	TL	R1	TLP	R2	R3	TE	DLD	NLD	NTCPP
TL (µm)	0.8298**									
R1	0.0571	-0.3469*								
TLP (µm)	0.5853**	0.6321**	-0.0879							
R2	0.2510*	0.0838	0.1099	-0.5242**						
R3	-0.1202	-0.3781**	0.7845**	0.2639	-0.4378**					
TE (µm)	0.8151**	0.8604**	0.0119	0.6171**	0.0974	-0.0844				
DLD (µm)	-0.0436	-0.1875	0.3422**	-0.1739	0.1040	0.1979	-0.0152			
NLD	-0.1613	-0.0392	-0.1814	0.1112	-0.2862*	0.0400	-0.1519	-0.1003		
NTCPP	0.2864**	0.3638**	-0.1126	0.1820	0.1343	-0.1752	0.3084**	0.0567	0.0255	
NED	-0.2581*	-0.1276	-0.2067*	-0.0400	-0.1610	-0.0586	-0.2076*	-0.1770	0.2214*	0.1573

TPP: Thickness of the palisade parenchyma, TL: Thickness of the leaf, TLP: Thickness of the lacunary parenchyma, R1: Relationship between the thickness of the palisade parenchyma and the thickness of the leaf, R2: Relationship between the thickness of the palisade parenchyma and the lacunary parenchyma, R3: Relationship between the thickness of the lacunary parenchyma and the thickness of the leaf, TE: Thickness of the epiderm, DLD: Diameter of the ligneous ducts, NLD: Number of the ligneous ducts, NTCPP: Number of the threads cells of palisade parenchyma, NED: Number of the excreting ducts. *,**Significant with the threshold of 0.1%

effectiveness to combat drought depends on their high heritability and their speed appearance which appears in the reaction process to these difficult conditions. The anatomic parameters defining the plant structure constitute an effective group of characteristics to combat the abiotic stress (Oppenheimer)[12,13]. These parameters are classified in three main categories, following there implication in the use of hydric resources necessary and rare.

The protection ducts of vegetative organs and specially the leaf rule the hydric exchanges with the immediate plant environment[18,19,20]. The cholorophyllian parenchyma structure of this organ and the resulting modifications give a better efficiency in using the hydric resources for different processes to reach the carbonate plant nutrition[21,22,23]. Indeed, many researches[17,12], showed the efficiency of the conducting ducts transformations to facilitate the crud sap circulation in water deficit conditions.

The results obtained showed that the Atlas pistachios trees situated in Rechaiga area is characterized by a high variability related to the anatomic parameters studied on the leaf limb. This variability level concerns both populations male and female. Among the features concerned with these variations one retains the transformations of the epidermic protective ducts concerned by thickening of the external wall of revetment cells. The wall transformations at this level of the foliar structure constitute one of the strategies developed by the plants to effectively limit the water losses by the cuticular way[22]. This transformations result from a simple cellulosic thickening of the wall and the incrustations of chemical substances reinforcing the parietal impermeability, such as cutin and waxes.

Like all the species dicotyledonous, the Atlas pistachio tree presents a heterogeneous mesophyll made up of the superposition of two types of parenchyma, palisade and lacunary. The results indicate that the organization of the latter is very variable through the individuals within each one of the sexes, male and female. Thus, the palisade parenchyma is constituted by a number of cellular threads variable. Its importance conditions the thickness of the leaf and the number of the photosynthetic cells/foliar unit of area. The leaf surface reduction under the dry conditions is compensated by its thickening in order to ensure an optimal photosynthetic activity[22]. The conditions of drought also force the parenchymatous cells reduction in order to better preserve a state of optimal hydration[24]. The transformations which relate to conducting ducts, in particular the xylem constitute mechanisms strongly implied in the adaptation of the plants to the dry conditions. Boukhari and Wenbeldou[25] shows that the reduction amongst woody vessels and their diameters contribute to a better circulation of the sap by increasing hydraulic resistance. In the study, there parameters exteriorizes an important variability within the male and female individuals. The relations established between the whole of the parameters studied at the individuals of the two sexes show the distinction of the structural models. Thus, at the females individual, the thickening of the leaf is supported by the increase thicknesses of the two parenchyma's, palisade (r=0.829**) and lacunary (0.585**). These increases thicknesses are also accompanied by the reinforcement by the leaf skin. It is noted that the thickening of the palisade parenchyma is conditioned by the increase amongst cellular threads which constitute it (r=0.286**) (Table 2). The same relations were noted on the level of the male individuals population leaf structure (Table 3).

Table 3: Relations between the various anatomical parameters within the individuals of the male sex

Variable		TPP	TL	R1	TLP	R2	R3	TE	DLD	NLD	NTCPP
TL	0.4344**										
R1	0.8399**	-0.1093									
TLP	0.0057**	0.0240	-0.0256								
R2	0.6263*	0.2688**	0.5420**	-0.6984**							
R3	-0.1751	-0.3799**	0.0164	0.9076**	-0.7532**						
TE	0.0332	0.3638**	0.1851	0.0797	-0.0111	-0.0641					
DLD	0.0192	-0.1704	0.1359	0.0155	-0.0388	0.0616	-0.2854**				
NLD	-0.0517	-0.0405	-0.032	0.0559	-0.0445	0.0622	0.0896	0.1646			
N TCPP		0.0056	0.0680	-0.047	0.0365	-0.0189	-0.0235	-0.1974	0.0486	0.0375	
NED	0.1214	0.3697*	-0.1040	-0.0681	0.14246	-0.1913	0.10756	0.0248	0.1022	0.0996	

TPP: Thickness of the palisade parenchyma, TL: Thickness of the leaf, TLP: Thickness of the lacunary parenchyma, R1: Relationship between the thickness of the palisade parenchyma and the thickness of the leaf, R2: Relationship between the thickness of the palisade parenchyma and the lacunary parenchyma, R3: Relationship between the thickness of the lacunary parenchyma and the thickness of the leaf, TE: Thickness of the epiderm, DLD: Diameter of the ligneous ducts, NLD: Number of the ligneous ducts, NTCPP: Number of the threads cells of palisade parenchyma ,NED: Number of the excreting ducts, *,**Significant with the threshold of 0.1%

CONCLUSION

This study noted that, the foliar structure of the Atlas pistachio tree has an important variability at the individuals of the two studied sexes, this variability relates to the structure of the mesophyll, the epiderm, the conducting vessels (xylem) as well as the channels excretors. It was noticed that this variability is rather marked at the individuals of the females compared to that of the males.

SIGNIFICANCE STATEMENTS

This study searching for the characterization of leave anatomic of some individuals issued from a population around Rechaiga (South East of Tiaret). This anatomic study is a way of estimation of the targeted population genetic variability. The present work will contribute to the definition of the leaves anatomy of this species and its variations likely to deepen the mechanisms of adaptation to dry climatic conditions.

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