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Research article

Genetic diversity analysis using morphological parameters in Tunisian Pistachio (*Pistacia vera* L.)

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ABSTRACT
Pistachio (*Pistacia vera* L.) is an interesting crop for arid areas, well adapted to marginal lands and to drought conditions. Little attention has been directed towards the evaluation of the *Pistacia vera* L. genetic resources in Tunisia. Therefore, the genetic diversity of twenty ecotypes of pistachio collected from the south of Tunisia was analyzed using leaves morphological parameters. Morphological analysis helped to highlight a fairly high level of diversity and estimate phylogenetic relationships. In addition, the principal component analysis and UPGMA (Unweighted Pair Group Method with Arithmetic Average) tree allowed difference among several ecotypes over the entire samples studied. This variability of leaves traits between varieties which were cultivated in a relatively homogeneous environment may be attributed to different genetic architectures developed. This can be a consequence of adaptation to varied environmental conditions exiting in the distributional area of *Pistacia vera* L.
The evaluation of germplasm in Tunisia revealed promising landraces. Additional biochemical and molecular studies will provide the necessary complementary information that could result in potentially valuable landrace selection.

Key word: *Pistacia vera* L., Morphological traits, genetic resources.

1. INTRODUCTION

The *Pistacia vera* L. species is a member of the Anacardiaceae family, which including 83 genus with about 860 known species (Zohary, 1952). The *Pistacia* genus consists of 11 species (Zohary, 1952). Within the *Pistacia* genus, *Pistacia vera* (also called "Green Gold Tree") is the only edible and worldwide marketable species (Fares et al., 2009).

Pistachio trees are diploid (2n = 30) and dioecious, meaning that there are separate male and female trees (Zohary, 1996). The pistachio, native to the Asia Minor area, has been propagated from the islands of the Mediterranean in the west to India in the east (Zohary and Hopf, 1994). It may be grown in desert areas since it needs long, hot summers for fruit maturation and high winter chilling. The pistachio has been considered a delicacy since the create of recorded history, and has been cultivated for centuries throughout its native range and cultivated over large areas around the world. Main producers comprise Iran, United States, Turkey and China (FAO, 2014). In Tunisia, The most important pistachio producing zones are Gafsa, Sidi Bouzid, Kasserine, Sfax, and Kairouan. Leaves are extremely important organs of a tree, sensitive to growth conditions, especially during a leaf expansion phase (Masarovicova, 1988, Bayramzadeh et al., 2008). Consequently, they can effectively adapt to the environment from which the plants originate (Bayramzadeh et al., 2011; Amjad Ali et al., 2011) by making appropriate modifications in their morphology and anatomy (Bussotti et al., 2000, Gratani et al., 2003).

The analysis of morphological leaf traits supplies deep insight into the taxonomy, genetics, biogeography, and evolution, which are the parts of the major classification of scientific areas related to a successful conservation of natural ecosystems (Main, 1966). Therefore, such kinds of studies can be very helpful in species with wide geographical ranges, for which the little information is available. *In situ*, traditional region of pistachio cultivation was prospected in the South of Tunisia (Gafsa: El- Guetar region) (Fig.1). In the herein study, we analyzed the diversity observed in local pistachio germplasm based on morphological leaf parameters Using appropriate statistical analyzes.

2- MATERIALS AND METHODS

2-1 Plant Material

Investigation of local pistachio germplasm was assumed in the regions of "El-Guetar" (fig. 1). This region is well-known historical pistachio production area and is differentiated by harsh climatic conditions. The "El-Guetar" has a continental Mediterranean climate, and an annual rainfall is 140 mm with high-temperature amplitudes between day and night and between seasons (Ghrab et al., 2012). For our survey, twenty ecotypes of *Pistacia vera* L. were collected and it composed of fifteen females trees and five pollinators (Table 1). The plant material consists of mature leaves taken from each ecotype of four different orientations.

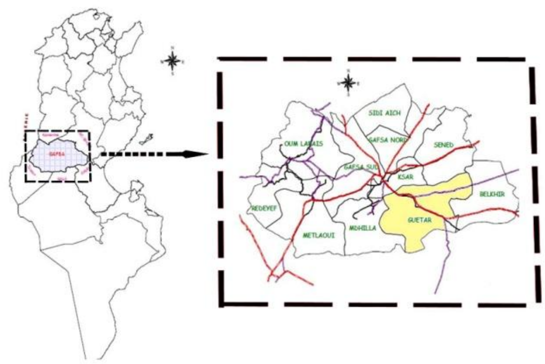

Fig.1 Geographical distribution of the analyzed pistachio cultivars.

Code	Cultivars Sexe	Code	Cultivars Sexe
P1	♀	P11	♀
P2	♀	P12	♀
P3	♂	P13	♀
P4	♂	P14	♀
P5	♀	P15	♂
P6	♂	P16	♀
P7	♀	P17	♀
P8	♀	P18	♀
P9	♂	P19	♀
P10	♀	P20	♀

Table 1: Codes and sex of studied cultivars.

2-2 Morphological study

Interesting morphological traits were identified using the pistachio descriptor list (IPGRI, 1997). In this study, we were interested in parameters relative to leave. On this subject, nine characters were retained which represent most of the morphological and production-related variability observed within the pistachio germplasm (Table 2). The morphological description of the leaves was based on: leaf length (Ll), leaf width (Lw), leaflet number (NL), terminal leaflet length (TLl), terminal leaflet width (TLw), Leaf ratio (LWR), Size Terminal Leaflet (STL), Petiole length (PL) and Petiole diameter (DP).

With the aim of studying the genetic diversity and looking for discriminating morphological parameters, we realized an analysis of the variance (ANOVA) and an analysis in main component (PCA) and Discriminating Canonical Analysis (CAN-DISC).

On another hand, MAHALANOBIS distance was calculated based on morphological parameters with the Statistical Analysis System (SAS) (SAS, 1990). These genetic distances between varieties are all the

smaller as the measurements made are close and vice versa.
Using this information, a dendrogram was constructed via the Unweighted Pair Group Method with Arithmetic averages (UPGMA). In addition, multivariate analyzes were carried out, namely; analysis of the variance (ANOVA) and the Principal Component Analyses (PCA) using the (SAS) software (SAS, 1990).

Parameters	Code
Leaf length	Ll
Leaf width	Lw
Leaflet number	LN
Terminal leaflet length	TLl
Terminal leaflet width	TLw
Length/width ratio	LWR
Size Terminal Leaflet	STL
Petiole length	PL
Petiole diameter	PD

Table 2. Code of morphological parameters used in this study.

3- RESULTS

3-1 Analysis of the variance (ANOVA)

The analysis of variance (table 3) demonstrated that, overall, the characters used were very successful for the estimation of genetic variability in studied samples. Indeed, with the exception of morphological Petiole diameter (PD) parameter, which showed a non-significant difference between ecotypes, all the others one showed highly significant differences. This result reflected the good choice of morphological parameters used in this study.

parameters	Sum of squares	Square average	Test F	PR > F
Ll	35770.19000	1882.64158	3.82	0.0001
Lw	62108.94750	3268.89197	7.21	0.0001
LN	39.27187500	2.06694079	4.22	0.0001
TLl	32114.96250	1690.26118	5.37	0.0001
TLw	7656.25687	402.96089	4.25	0.0001
LWR	3.97121091	0.20901110	8.18	0.0001
STL	8.47500000	0.44605263	3.60	0.0001
PL	3831.536875	201.659836	2.72	0.0010
PD	3.95171875	0.20798520	1.68 NS	0.0568

Table 3. Variance analysis (ANOVA).

3-2 Principal component analysis (PCA)

Principal Component Analysis was achieved in order to recognize the main characters responsible for El Guetar pistachio landraces differentiation (Table 4).
Data analysis using PCA revealed the main principal components (PCs). The first three components absorbed 97.23 % of the total variability observed between specimens from "El-Guetar". The first component (PC1) explained 86.95 % of the total diversity and it was positively correlated to the parameters Ll, Lw, and TL1. PC2, which absorbs 7.3 % of the total variability, was positively related to TL1 and negatively with Lw essentially. Whereas PC3 was positively correlated to the parameter Lw and LTw. It was also defined by negative correlations with the Ll variable.
The distribution of studied cultivars on the plane defined by the two first components (axis one and two (Fig.2) showed that, the majority of varieties was grouped in the meeting place of both axes with the exception of the ecotypes P2, P5, P6, P7, P10, and P18. P2 and P10 got loose positively by the second component, thus they were positively distinguished with the parameters which defined this axis (Lw and TLl).
P5, P6, and P18 were removed negatively from the other groups, they essentially correlated well with the variable Lw. The cultivar P7 was detached positively with respect to the first component so it was distinguished by the Lw parameter.

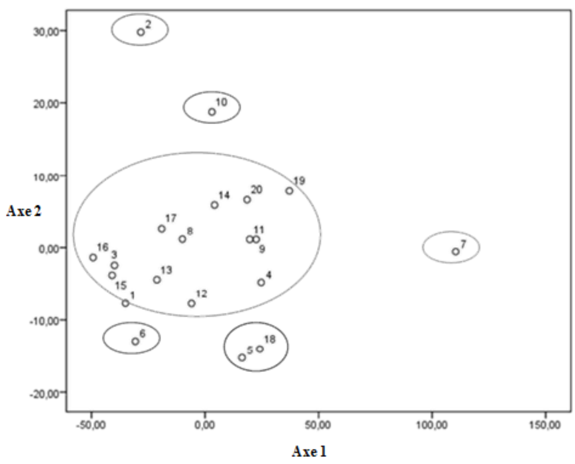

Fig. 2 Dispersion of pistachio cultivars in the 1-2 plane based on morphological study.

3-3 Mahalanobis distances and UPGMA method

Phenotypic distances (MAHALANOBIS distances) were calculated using the obtained morphological data. The distances values ranged from 1.035 to 6.656. The shortest distance was recorded between P19 and P20 cultivars, while the highest was observed between P3 and P7. These results show that cultivars P19 and

P20 presented the maximum of similarities, contrary to cultivars P3 and P7 which presented the maximum of difference in the tested parameters.

The landraces of each prospected area were classified using hierarchical clustering based on all the measured characters by UPGMA method (Fig. 3). Cluster dendrogram showed the existence of two major groups: The first is composed exclusively by the ecotype p7 which is significantly detached from the rest of the trees. The second group included all the other cultivars with a significant detachment of P6 which can make a third group. These results were consistent with our previous interpretations at the PCA concerning the divergence of P6 and P7 from all other cultivars in this study.

	Axe 1		Axe 2		Axe 3	
Eigen value	1295,42		108,70		44,41396	
Proportion	0,8695		0,0730		0,0298	
%variation	86,95		94,25		97,23	
	Characters	**Contribution**	**Characters**	**Contribution**	**Characters**	**Contribution**
PCs (Axes)	Ll	+0,515	Ll	+0.056	Ll	-0.836
	Lw	+0,685	Lw	-0,597	Lw	+0,374
	LN	-0,006	LN	-0,041	LN	-0,044
	TLl	+0,456	TLl	+0,752	TLl	+0,255
	LTw	+0,197	LTw	+0,250	LTw	+0,303
	LWR	+0,001	LWR	+0,005	LWR	-0,005
	STL	-0,004	STL	+0,009	STL	-0,015
	PL	+0,131	PL	-0,097	PL	-0,012
	PD	+0,003	DP	-0,0003	DP	-0,001

Table 4. Eigen values, proportion of variation and contribution associated with the axes of the Principal component analysis in pistachio ecotypes.

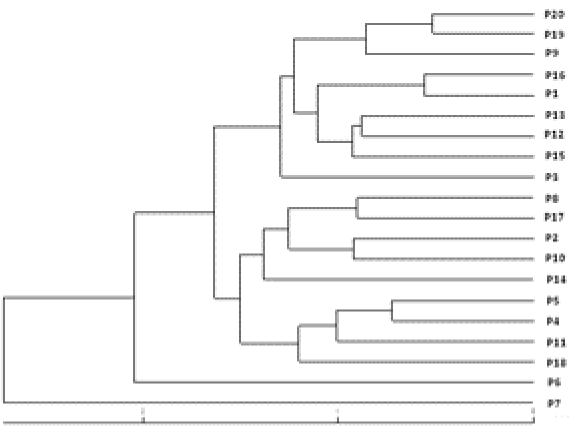

Fig. 3 Cluster dendrogram of pistachio germplasm from El-Guetar oasis, using UPGMA method, based on morphological study.

4- DISCUSSION

The major goal of this survey was to characterize the local landraces and to identify those with higher adaptability to specific conditions. Genetic diversity in *Pistacia vera* L. was considered to be very narrow. In comparison with other fruit trees having the same ancient histories, only a very small number of pistachio cultivars have been reported (Vargas and Romero 1998). The reason for this paucity of cultivars was thought to be the long juvenility of pistachio, longer life duration of the trees and the hybridization phenomenon that was very common among different species within the genus *Pistacia* (Maggs, 1973).

Furthermore, many causes of genetic erosion, such as replacement of landraces, environmental degradation, and monoculture population pressure, involved to the lessening of the cultivar database (Caruso et al., 1998). However, a significant degree of variation still exists among pistachio germplasm.

A high level of morphological variability was found among the population of *Pistacia vera* in "El-Guetar". Indeed, with the exception of morphological parameter Petiole diameter (PD), which showed a non-significant difference between ecotypes, all the others showed a highly significant difference, which reflecting the good choice of morphological parameters.

Principal component analysis also indicated that there were several multivariate directions of variation in the morphological leaf traits in the investigated population. Variation in all input variables was adequately explained by 3 principal components (accumulated variables, PCs). Nevertheless, there are relationships among the majority of the studied variables, and these almost represent that similar environmental or genetic factors control the studied leaf traits in *Pistacia vera* L.

Our sample covers latitude and a longitude of 34.20 N-8.57 E respectively and presents a mean annual temperature of 28.15°C. These parameters are similar in the studied locations. However, this important dissimilarity observed, by AMOVA and UPGMA analysis, in the morphological parameters indicated a considerable phenotypic polymorphism between cultivars. This variability of leaves traits between varieties which were cultivated in a relatively homogeneous environment may be attributed to different genetic architectures developed as a consequence of adaptation to varied environmental conditions exiting in the distributional area of *Pistacia vera*.

In our study, we observed a high level of morphological variability in the population of *Pistacia vera* in "El-Guetar" province. Nevertheless, we recognize that the limited geographical and phylogenetic scope in our research allows only a preliminary assessment of this expectation. However, another study would be needed in order to completely separate environmental and genetic factors explaining the discovered level of natural variability.

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