



Preliminary characterization and morph-agronomic evaluation of millennium olive varieties in Tunisia

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Abstract

Tunisia is one of the oldest agricultural settlements in history. Evidences revealed by archeological excavations indicated that olives were cultivated before about 3000 years in Tunisia. Although the importance of millennium olives, studies about characterization and evaluation are scarce. The aims of this work were to make a morph-agronomic characterization of eighteen millennium olive cultivars collected from eight different archeological sites. This work was conducted in the framework of the activities of the fruit tree network in the Tunisian National Gene Bank. Quantitative and qualitative traits were measured in pit, fruit and leaf samples. In order to group the genetic material and evaluate the phenotypic variability, descriptive statistics, cluster analysis, factorial and principal components analysis were used. The 18 accessions were grouped in 2 clusters based on the multivariate analysis of 18 traits. The collection featured phenotypic variability for all the studied traits, especially for the fruit and endocarp parameters. Principal components analysis conducted on quantitative and qualitative traits showed an important degree of variability of about 81.41% for the two first principal components and the factorial analysis was conducted for quantitative data and revealed 79.25% of the total diversity for the two first factors. The phenotypic diversity observed among the millennium cultivars suggests a high genetic potential of this heritage and confirmed the urgency to protect the specimens studied cultivars.

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Introduction

Olive (*Olea europaea L.*) is one of the oldest agricultural tree crops worldwide and is an important source of oil with beneficial properties for human health. This emblematic tree has coexisted with humans for about 5000 to 6000 years, going back to the early Bronze Age (3150 to 1200 BC). Its origin can be traced to areas along the eastern Mediterranean Coast (Vossen, 2007) and, then extended to the west and north of the Mediterranean basin. The civilizations of the eastern and western Mediterranean such as the Phoenicians, Greeks and Romans, have spread this culture throughout the Mediterranean Basin (Brown, 2004). By 1200 BC, the population growth in the Mediterranean basin led to the establishment of numerous colonies by the Phoenicians in North Africa (Carthage) where they brought their olive growing and developed its business. The distribution of *Olea* varieties in the Mediterranean basin gave rise to a very complex and highly articulated structure of olive culture which was marked by the existence of a considerable number of different olive cultivars (Bartolini *et al.*, 2005). The ancient olive varieties, estimated at 2000 in the Mediterranean basin, are gradually disappearing due to the abandonment of olive orchards and this is due to their low fertility, urbanization and replacement by modern cultivars (Bartolini *et al.*, 1998). Currently, there are few data about the genetic biodiversity of millennium olive varieties in Tunisia and the newly introduced varieties of olive trees, have significantly increased the loss of genetic authenticity of the millennium olive varieties and reduced the benefit of few varieties of high reputation. This implies the introduction of potential cultivars that are neither interesting nor adapted to local biodiversity. On the urgency to reduce the loss of genetic authenticity of Tunisian varieties and to preserve the local genetic resources of olive (*Olea europaea L.*), this work aimed the conservation of the eldest trees, known for its tolerance to drought and good productivity, by a morphological, biochemical and molecular characterization.

Material and methods

Plant material

The morphological traits were measured during the 2010–2011 and 2011–2012 periods. The plant specimens were collected randomly from eighteen millennium olive trees in eight different archeological sites with different ecological conditions (Table 1).

The methodology used in this characterization is based in the recommendations of the International Olive Council (IOC, 1997). This investigation include the analysis of 29 distinct characters: four related to the leaf (length “V1”, width “V2”, shape “V3” and Longitudinal curvature of the blade “V12”), 12 related with the fruit (length “V4”, maximum diameter “V5”, shape “V6”, weight “V7”, symmetry in position (A) “V13”, position of maximum transversal diameter “V14”, apex “V15”, base “V16”, nipple presence “V17”, presence of small lens “V18”, dimension of small lens “V19” and the localization of initial turning “V20”), and 13 related to the endocarp (length “V8”, maximum diameter “V9”, shape “V10”, weight “V11”, symmetry in position (A) “V21”, symmetry in position (B) “V22”, position of maximum transversal diameter “V23”, apex “V24”, base “V25”, surface “V26”, number of fibrovasculars “V27”, distribution of fibrovascular sulcus “V28” and the mucro presence “V29”).

Samples were collected from the mid-shoot portion of the current year’s growth from the most representative shoots at shoulder level (approximately 1.5 m from the ground). Forty-organ samples from the South-facing sides of trees were characterized for each parameter and the average for each characterized trait was used in the statistical analysis.

Data analysis

An average value for each trait and accession was calculated. Factorial Analysis was conducted for quantitative data. The value of the quantitative and qualitative morphological traits was standardized and subject to a Principal Component Analysis (PCA). Hierarchical analysis based on Ward’s method has

been applied to establish a group structure between cultivars to minimize the internal variance within group. A dispersion and central tendency descriptive analysis was applied to estimate the variability existing in the collection. Each trait was also subject of one-way analysis of variance (ANOVA) at a significant level of $P < 0.05$. All calculations were done by the using of XLSTAT software (2010).

Results and discussion

The first objective of this work was to study the biodiversity of millennium olive varieties in Tunisia and to assess if the leaf, fruit and endocarp data sets have enough information to allow the discrimination about the studied varieties. The information about the analyzed variables in the eighteen accessions, including mean value, variability, range, variation coefficient and minimum significant difference among accessions is reported in table 2. The quantitative parameters showed considerable variability among the accessions, especially the endocarp traits (width (V8), length (V9) and weight (V11)). Previous studies explained that the description of the morphological characteristics is the usual methodology accepted from a legal point of view for patenting and registration of varieties (Badenes, 1991), especially the importance of the endocarp traits to discriminate between the olive varieties (Zaher *et al.*, 2011; Paula *et al.*, 2005 and Mnasri *et al.*, 2013). The endocarp weight varied from 0.1 to 0.72 g, the fruit weight ranged from 0.35 to 5.61 g and the flush percentage from 71.42 % to 87.16%. These informations are important because the higher the weight of the fruit and the lower the weight of the endocarp, the olive yield is higher (Mehri and Mehri Kamoun, 2007).

Factorial analysis

With due attention to the complex relations of the morphological traits with each other the final judgment cannot be done on the basis of simple correlation coefficients and it is necessary to use multivariable statistical methods in order to perceive deeply the reactions among the traits. In the

meantime factor analysis is an effective statistical method in decreasing the volume of quantitative data and getting results of the data which showed a high correlation among the primary variables (Cooper, 1984). Factorial analysis showed that the first 2 axis comprise about 79.25% of the total variation (Fig 1). The relative magnitude of the first FA eigenvectors showed that weight, length, and maximum diameter of the fruit and the endocarp were important attributes for the classification of accessions in cluster which confirm the previous results of one-way analysis of variance (ANOVA). The inertia accounted for the second FA was due to the contribution of the quantitative parameter of the leave (length and shape) and to the form of the fruit and the endocarp.

The projection of individuals in the plane generated by the axis 1 and 2 exhibits no particular aggregation as reported in Figure 1. This suggests that quantitative traits alone cannot clearly discriminate between the studied accessions. The cultivar 'M4' from the region of Makthar seems to diverge significantly from the other cultivars. This result is expected since it has presented the highest average value for weight (5.61 g), length (2.58 cm) and fruit diameter (2.01 cm).

Principal components analyses

PCA is a common statistical technique used for finding patterns in quantitative and qualitative data of high dimension and it is generally used before the cluster analysis in order to determine the relative importance of the classification variables (Berdahl *et al.* 2002). PCA analyses show that 81.41 % of the total variability was accounted the first two principal components. The first PCA accounted for 50.45 % of variability and was correlated positively with weight, length, and maximum diameter of fruit and endocarp, as well the qualitative parameters of the fruit (symmetry in position A, nipple presence and presence of small lens) and the qualitative traits of the endocarp (symmetry in position A, position of maximum transversal diameter and apex in position A). Whereas the second PCA accounted of 30, 96% of

the total variability and is correlated with the quantitative and qualitative parameters of the leaf (Length, shape and the longitudinal curvature of the

blade), the fruit (shape and color in maturity) and the endocarp (shape, and distribution of grooves).

Table 1. Pedo-Climatic Characteristics of the different studied archeological sites.

Cultivar	Site	Latitude/longitude (grade)	Altitude (m)	Soil type	Average annual precipitation (mm)	Average annual temperature (C°)	Bioclimatic stage
B 1 B 2	Baja	3700/900	375	Red Mediterranean Soil	720	17.8	Sub-humid with warm winter
M 1 M 2 M 3 M 4 M 5	Makthar	3586/ 915	1059	Brown calcareous soil	1000	440-560	Semi-arid with cold winter
K1 K2 K3	Kesra	3580/938	878	Sandy brown semi desert soil	411	18	Semi-arid
S	Sbitla	3526/906	626	clay and sandy loam soil	350	17	Arid with cold winter
AL1 AL2	El Ala	3566/1010	67	Brown calcareous alluvial soil	290	20	Semi-arid with cold winter
Mo	Monastir	3578/1083	10	Calcimagnesian Soil	282	18.7	Semi-arid
J	Djerba	3380/1090	34	sandy soil	231	19.8	Arid
T	Tataouine	3293/1045	246	sandy loam soil	118	20	Arid

The projection of cultivars in the 1-2 plot exhibits the aggregation of the 18 accessions in three main groups. The cluster 1 grouped only the accession M4 localized in the region of Makthar (Maktharis), locally named “Etkoubri” and characterized by the highest fruit and endocarp weight. This cultivar presents a fruit weight higher than 5g and is essentially a table olive, also valued for its high oil content, despite an erratic production and alternator. In turn, the cluster 2 which grouped accessions M1, M2, M5, K1, K2, Ala1 and Ala 2 is characterized by ovoid to around fruit. These cultivars were classified in the olive categories of medium to low weight fruit and they can be used with a double aptitude (Barranco *et al.*, 2000). The rest of the accessions were grouped in cluster 3 and characterized by around and low weight fruit and endocarp.

As a result the combinations of both qualitative and quantitative data allow a better analysis of the studied traits. Previous studies have demonstrated the

importance of the principal components analyses to study the morphological descriptors of fruit tree (Mnasri *et al.*, 2013 and Saddoud *et al.*, 2012).

Cluster analysis

The dendrogram obtained from the Ward cluster analysis carried out on the 18 phenotypic traits selected in the PCA is shown in Fig 3. Two clusters were distinguished. Cluster 1 showed essentially table cultivars, ‘M4’ and ‘M5’ from the region of Makthar and ‘B1’ and ‘B2’ from the region of Beja. The accession ‘M4’ featured crops with elongated shape and the highest fruit and endocarp weight. Whereas, accessions ‘M5’, ‘B1’ and ‘B2’ are essentially cultivars with medium fruit and endocarp weight with symmetric position of maximum transversal diameter. The cluster 2 included accessions with medium to low fruit weight, which are essentially used for oil production. Archaeological studies show that olive oil was already extracted in Tunisia during

living archives, and although we were not successful in extracting dendroclimatological information from them, it is likely that in future we can extract valuable information on the history of local weather, or on the history of the cultivation of olive trees in Tunisia.

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