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The study of olive oil quality and morphological biodiversity of *Olea europaea* L. in the region of "Hbebsa"

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Abstract

As part of the protection of Olive biodiversity and enhance its role in protecting areas of high risk of erosion, we have study the genetic diversity of twenty two accessions of olive in the region of Hbebsa. The experimental approach was based on 29 morphological parameters as recommended by IOC (1997) and the study of oil (composition, content and quality) extracted from the 22 different accessions. We have noted a significant fluctuation of the flush percentage (from 40.62 to 88.49%), the fruit weight (from 0.28 to 4.48 g) and the endocarp weight (from 0.14 to 0.68g). The morphological study permitted a specific description of the characteristics for the tested accessions and their repartition into three groups. Olive oil content is high for all studied accessions, especially for the accession 3 with approximately 28.95%. The oil quality of the different studied accessions is classified as extra-virgin oils with high oleic acids and low palmitic and linolenic acids. In conclusion, the results have proved the wealth and the importance of the olive germplasm although the desertification of the studied site. These findings were of interest to protect the specimens studied accessions.

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Introduction

The olive tree was one of the earliest fruit crops to be domesticated. It spread from the Middle East towards the west of Europe approximately 6000 years ago (Zohary and Spiegel-Roy, 1975; Zohary and Hopft, 1994). Over time, a large number of cultivars have appeared due to events such as out crossing, mutation, clonal selection, and selective pressure (including grower requirements) on the original olive germplasm.

In Tunisia, the olive growing areas spread from the north to the south. About 60 million trees are distributed and spread on 1.6 million hectares, representing a third of the cultivated area. Tunisia occupies the fourth place on the world scale in terms of olive oil production (1997). However, this culture depends on two prevailing cultivars, Chetoui in the northern and Chemlali in central and southern parts of the country. These two cultivars covered 50% of the different olive trees plantation; conversely several minor varieties are maintained in restricted areas.

Many studies have been carried out about olive cultivars, and their olive oils in Tunisia: comparison of different cultivars and growing areas of autochthonous olive oils (Guerfel *et al.,* 2009; Kotti *et al.,* 2009) and comparison of Tunisian and European varieties cultivated in Tunisia (Aparicio and Luna, 2002; Dabbou *et al.,* 2009). However, there is not much information about the characterization and comparison of the Tunisian olive autochthonous varieties grown in their area of origin. The identification of this olive biodiversity has been traditionally carried out by morphological, agronomic and chemical traits (Ruby, 1916; Mehri and Hellali, 1995; Msallem and Mehri 2000; Trigui and Msallem, 2002).

The other hand, Tunisia is characterized by contrasts, both in geographical conditions and in agricultural practices, however in all these various agricultural systems; people conserved their local varieties which are considerate as a heritage. Olive tree is one such species. The farmers have selected some olive varieties adapted to the conditions, but this genetic diversity is threatened by modernization of production practices and by changes in agricultural and commercial policies. Thus, there is an urgent need to study and to inventory these traditional varieties before their lost. Specially, since both olive productivity and oil qualities are traits inherent to a variety (Abaza *et al.,* 2005; Baccouri *et al.,* 2007).

The aim of this work was to make a morpho-agronomic characterization of the Hbebsa olive germoplasm and to identify locally adapted accessions with a good aptitude for oil production. Quantitative traits were measured in pit, fruit and leave samples. Oil content and quality were also measured. This study will define for the first time in Tunisia the olive biodiversity in the region of Hbebsa which is characterized by a typical arid microclimate despite its presence in the North West of Tunisia.

Materials and Methods

Olives sampling

The olive grove under study is not irrigated, pruned each 2 years and subject to the traditionally cultural practices in the area. This olive grove was selected due to the regularity of the productions of the last years and because all the 22 accessions are presented, guaranteeing the homogeneity of the pedologic and climate conditions.

Manual pick up of the olive samples happened in December of 2011 in three trees previously marked of each accession to a total of 66. Three trees of each cultivar were selected because, besides biometric characterization, another objective of the global project was to extract from each tree mono-varietal olive oil for characterization. About 1 kg of olives was sampled. For the biometric study presented in this paper a subset of 40 olives and 40 leaves per tree were randomly selected.

Pomological characterization

The morphological analysis was carried out by using the methodology for primary characterization of olive varieties, proposed by the International Olive oil 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").

Oil Content

For oil content determination, 40 g of olive fruits was dried in an oven at 80°C to constant weight. The dried olives were crushed and extracted with hexane using a Soxhlet apparatus (Bettach *et al.,* 1996). The results were expressed as percentage of dry matter (DM).

Fatty Acid Composition

The fatty acid composition of oil samples was determined as methyl esters by capillary gas chromatography analysis after alkaline treatment. The gas chromatograph (VARIAN CP-3800 Gas Chromatograph) was equipped with an autosampler (CP-8400), a capillary column HP Innowax (Agilent Technologies, USA) (30 m × 0.53 mm, 1 μm), a split–splitless injector and a flame ionization detector (FID). Alkaline treatment was carried out by mixing 0.1 g of oil dissolved in 3 ml of n-hexane with 0.5 ml of 0.2 N methanolic potassium hydroxide solution according to the method of Reg EC 2568/91. One microlitre of methyl esters was injected. Seven fatty acids including C16:0, C16:1, C18:0, C18:1, C18:2, C18:3 and C20:0 were identified from their retention times compared to those of standard compounds.

Data analysis

An average value for each trait and accession was calculated. The value of the quantitative and qualitative morphological traits was standardized and subject to a Principal Component Analysis (PCA). 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

Phenotypic characterization

The morphological study was based on 29 parameters as recommended by the International Olive Council in 1997.The information about the analyzed variables in the 22 accessions, including mean value, variability range, variation coefficient, and minimum significant difference among accessions, is reported in Table 1.

Table 1. Descriptive statistic analysis of the morpho-phenological parameters.

Trait	Maximum	Minimum	Average	CV%
V1	66.850	40.323	54.679	11.90***
V2	15.050	7.470	11.344	14.35***
V3	6.490	4.044	4.947	15.46***
V4	24.110	10.340	16.048	26.26***
V5	18.970	6.500	11.361	32.11 ***
V6	1.680	1.161	1.437	10.57 ***
V7	4.480	0.280	1.596	82.74 ***
V8	16.630	8.399	12.959	20.28 ***
V9	9.370	5.090	6.522	18.94 ***
V10	2.490	1.450	2.005	14.88 ***
V11	0.680	0.140	0.324	53.78 ***

P-value: ** significant ($P < 0.05$); *** Highly significant ($p < 0.01$)

CV% Variation coefficient expressed in percentage

The morphological traits showed considerable variability among the accessions, especially the fruit parameters V4, V5 and V7, as well those that were measured in the endocarp V8, V9 and V11. The fruit weight varied from 0.28 to 4.48 g, the endocarp weight ranged from 0.14 to 0.68g and the flush percentage from 40.62 to 88.49%.

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 fruit and endocarp parameters to discriminate between the olive varieties (Zaher *et al.,* 2011; Paula *et al.,* 2005).

Table 2. Methyl ester fatty acid composition and their levels in the analyzed oils according to the norm of the IOC, (1997).

Norm	C16 :0	C16 :1	C18 :0	C18 :1	C18 :2	C18 :3	C20 :0
	7,5–20	0,3–3,5	0,5–5	55–83	3,5–21	<0,9	≥0,6
A1	17.92	3.4	1.68	58.02	17.98	0.50	0.23
A2	9.17	0.74	1.49	77.27	10.56	0.49	0.43
A3	8.08	0.31	1.52	78.01	10.98	0.36	0.35
A4	9.98	0.74	2.1	76.18	9.2	0.29	0.31
A5	13.9	0.4	3.5	71.8	9.79	0.21	0.25
A8	8.01	0.21	2.1	75.49	12.9	0.42	0.28
A9	10.88	0.33	2.01	73.31	12.28	0.39	0.41
A10	8.89	0.83	2.02	74.82	11.12	0.59	0.27
A11	15.72	2.68	2.67	68.62	11.82	0.44	0.33
A12	18.9	2.3	3.09	59.77	15.14	0.7	0.4
A13	15.81	2.28	3.12	67.8	12.07	0.56	0.46
A14	27.85	2.33	1.53	48.78	20.34	0.52	0.43
A15	16.99	2.45	2.62	66.01	10.18	0.53	0.5
A16	21.72	3.4	1.68	49.72	22.42	0.48	0.41
A17	13.53	0.22	0.98	61.43	22.89	0.45	0.35
A18	14.67	1.24	1.09	69.16	12.64	0,48	0.39
A19	10.77	0.74	3.65	75.01	8.64	0.46	0.43
A20	29.21	2.68	3.21	66.25	10.17	0.51	0.31
A21	14.39	2.75	1.7	71.76	9.18	1.12	0.35
A22	9.48	0.23	1.92	76.59	10.49	0.33	0.40

The principal component analysis performed on the morphological descriptors of the fruit, endocarp, and leaf (ACP) is presented in Fig 1. The eigenvalues of the first, second, third, and fourth axis of the principal components, accounted the 55.14%, 17.93%, 14.37% and 7.22% of the total variance, respectively. The relative magnitude of the first PC eigenvectors showed that weight, length, and maximum diameter of fruit and endocarp, as well the qualitative parameters of the fruit (symmetry in position A and nipple presence) and the endocarp (number and distribution of grooves, surface in position B and base in position A) were important attributes for the classification of accessions in cluster.

The inertia accounted for the second PC was due to the contribution of the fruit (shape, position of maximum transversal diameter, apex in position A and dimension of small lens) , as well with the endocarp (shape, symmetry in position A, symmetry in position B and apex in position A).

The leaf traits (Length, shape and the longitudinal curvature of the blade) had relatively high eigenvectors in the third PC, while V16 and V 20 showed a high contribution to inertia accounted for the fourth axis. V18 and V22 were not used to distinguish accessions due to their low contribution to the total inertia.

The projection of individuals in the plane generated by the axis 1, 2, 3 and 4 showed the distribution of the tested accessions in three main groups. The cluster 1 grouped the accessions (6, 7, 9, 17 and 18) characterized by the highest fruit and endocarp weight. These cultivars were classified in the olive categories of high to very high weight fruit and they can be used with a double aptitude (Barranco *et al.,* 2000). In turn, the cluster 2 which grouped accessions 3, 4, 5, 8, 10 , 14 and 22 is characterized by elongated and low weight fruit asymmetric in position A and a sharp-pointed apex, as well by elongated and mean weight endocarp. The rest of the accessions were grouped in cluster 3 and characterized by around and low weight fruit with an around apex in position A and an oval and low weight endocarp with rounding apex and base.

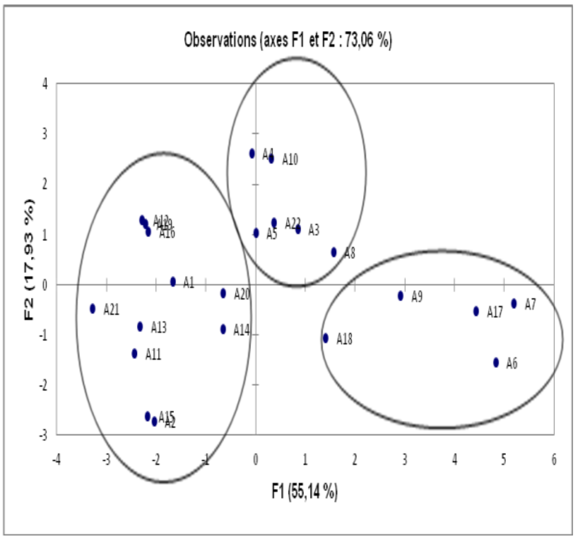

Fig. 1. Projection of the twenty two accessions in the plane generated by the first two principal components based on leaf, fruit and endocarp traits.

Biochemical Characterization

Oil yield of olives

As reported in fig 2, all the studied accessions are characterized by a high oil yield according to the classification of (Tous and Romeo, 1993). Expressed as percentage of dry matter, the oil yield presented significant differences between the 22 olive accessions; the content of oil in the samples ranged from 24.89% (accession 15) to 77. 3% (accession 3). Therefore following classification of Tous and Romero, we can qualify the tested cultivars except accessions (11, 15, 20 and 21) by cultivars with high oil yield (> 46%). The analysis shows that the study of these less-common cultivars appears of particular interest because they may have agronomic characteristics which can influence the quantity and the quality of the olive oil in Tunisia. These findings are in good agreement with those of other authors working on Tunisian olive oil varieties (Hanachi *et al.,* 2008; Taamalli *et al.,* 2010).

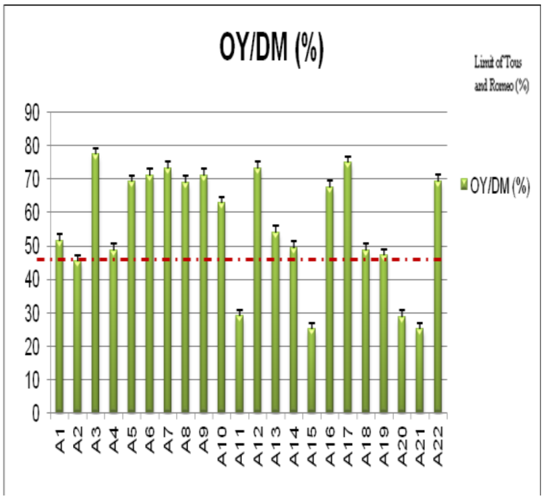

Fig. 2. Comparison of the oil yield rate expressed as percentage of dry matter of the 22 olive accessions with the method of Tous & Romeo (1993).

Fatty acid composition

Methyl ester fatty acid composition and their levels in the analyzed oils are shown in Table 2. As it can be observed, palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids are the major fatty acids present in the studied samples. The fatty acid composition of olive oils varies widely depending on the cultivar.

These findings are in good agreement with those of other authors working on Tunisian olive oil varieties (Baccouri *et al.,* 2007a; Haddada *et al.,* 2007). Among the studied samples, nineteen accessions showed a percentage of oleic acid (C18:1) > 55%, a palmitic acid rate which did not exceed 20 % and a low amounts of a linoleic acid (C18:2) varies from 8.64 to 22.89 %.

Other interesting points for the chemical characterization of studied oils are the proportions of some classes of free fatty acids. The monounsaturated fatty acids have great importance because of their nutritional implication and effect on oxidative stability of oils (Martinez de Victoria and Manas, 2001). Fig 3 show that the proportion of monounsaturated fatty acids changed according to the cultivar. It varies from 78.11% to 53.18%. The C18:1/C18:2 ratio has the most marked relationship with stability, and it is said that oil presents a good stability index if this value is over 7. Nevertheless, Tunisian olive oils are described in bibliography to present lower C18:1/C18:2 ratios compared to most of the European ones (Baccouri *et al.,* 2007b; Zarrouk *et al.,* 2009). We found that seven accessions among the studied olive samples (2, 3,4,5,19,21 and 22) present C18:1/C18:2 ratios ranged from 7.1% to 8.68%. And eight accessions (8, 9, 10, 11,13,15,18 and 20) present C18:1/C18:2 ratios ranged from 5.47 to 6.72%.

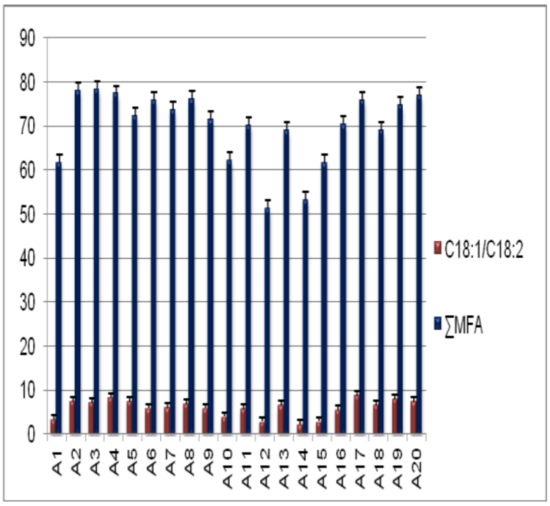

Fig. 3. Rate variation of the monounsaturated fatty acids and the C18:1/C18:2 ratio of the tested oils according to the norm of IOC, (1997).

Conclusion

In order to group the genetic material and evaluate the phenotypic variability, descriptive statistics, and principal components analysis were used. The 22 accessions were grouped in 3 clusters based on the multivariate analysis of 29 traits. The accessions featured phenotypic variability for all the studied traits, especially for the fruit and endocarp parameters. Eighteen accessions suitable for oil production were selected, four of which could also be used for table. The olive oil content is high for all studied accessions, especially for the accession 3 with approximately 28.95%. The oil quality of the different studied accessions is classified as extra-virgin oils with high oleic acids and low palmitic and linolenic acids for the majority of the tested olive oils.

The phenotypic and biochemical diversity observed among the olive accessions suggests a high genetic potential, which could be used from the agronomic point of view to substantially improve the production of Hbebsa, specifically, since both olive productivity and oil qualities are traits inherent to the cultivars (Gómez-Rico *et al.,* 2008 ; Hannachi *et al.,* 2008 ; Pinheiro *et al.,* 2005). The use of molecular markers (AFLP and SSR) in future studies will be essential to verify the denomination of each accession and increase the knowledge about the diversity of this species.

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