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Agronomic and morphological study of two autochthones Tunisian olive varieties "Neb Jmel" and "El Hor"

Mnasri Rahmani Sameh[1*], Saddoud Debbabi Olfa[1], Ben Saleh[3] and Ferchichi Ali[2]

[1]*National Gene Bank of Tunisia, Street Yesser Arafet, 1080, Tunis, Tunisia*

[2]*National Institute of Agronomy of Tunisia, 43 Street Charles Nicolle, 1082-Tunis Mahrajène Tunisia*

[3]*Institute of Arid Regions of Gabes, 6051 Nahal Gabes Tunisia*

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Abstract

In Tunisia, little is known about the existing olive patrimony. The number of local cultivars is probably underestimated because of the scarce information on minor local varieties. In this present study, we performed a morphological and technological characterization of two autochthones olive varieties "Neb Jmel" and "El Hor" cultivated in three different geographical locations Béjà, Siliana and Bizerte, with the aim to obtain further information on the diversity of the cultivars in various geographical sites. The varieties "Neb Jmel" and "El Hor" showed the highest fruit weight (2.06 and 1.68g) in the station of Béja, while Siliana revealed the lowest values (1.28 and 0.75g). The cultivar "El Hor" revealed a significant fluctuation of the fleshpercentage according to its culture location (varied from 66. 66 to 75%). The oil quality of the studied varieties is classified as extra-virgin oils and varies widely depending on the cultivar and on the geographical location. The oleic acid varied in the individuals of "Neb Jmel" (from 76.59 to 61.99%) and the highest percentage was found in the region of Siliana. Concerning linolenic (C18:3) and arachidic (C20:0) acids the cultivars of the two studied varieties revealed a high significant difference values between the three studied sites. In this case, we can recommend studying the potentialities of each cultivar in various geographical locations, according to various culture techniques, in order to explore all their potentialities in diversity expression.

***Corresponding Author:** Mnasri Rahmani Sameh ✉ mnasrisameh@yahoo.fr

Introduction

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. However, this culture depends on two prevailing cultivars, "Chetoui" in the northern and "Chemlali" in central and southern parts of the country. Conversely, several minor varieties are maintained in restricted areas. The olive germoplasm is estimated to include about 56 cultivars (Trigui and Msallem, 2002), most of them landraces vegetatively propagated at a farm level since ancient times. The number is probably underestimated because of the scarce information on minor local varieties widespread in the different olive growing areas. The study of these less-common cultivars is important because they may have traits not considered important in the past but necessary to meet the challenges of modern olive growing. In this context we have study two main autochthones varieties "Neb Jmel" and "El Hor" which are characterized by a small geographic dispersion in the North West of Tunisia. These two cultivars were first described by Kamoun (2007) and Hanachi (2008) but little morphological and biochemical information is available and the precise locality remains unknown.

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. Thus, we can recommend studying the potentialities of each cultivar in various geographical locations, according to various culture techniques, in order to explore all the potentialities in diversity expression. Mainly, that climatic and pedologic factors affect the morphological parameters of olives, and the analytical characteristics of oil (Angerosa *et al.,* 1989).

The aim of this work was to make a morphological and technological characterization of two autochthones varieties "Neb Jmel" and "El Hor" cultivated in three different geographical locations (Béjà, Siliana and Bizerte) and to obtain further information on the diversity of these varieties according to climatic and pedologic factors, as well to identify locally adapted cultivars with a good aptitude for oil production.

Materials and methods

Plant Material

The morphological traits were measured during the 2011–2012 and 2012–2013 periods. The plant specimens were collected randomly from "Neb Jmel", and "El Hor" adult trees in three regions with different ecological conditions (Table 1) in the North West of Tunisia (Siliana, Béja and Bizerte). The methodology used in this characterization is based in the recommendations of the International Olive Council (IOC, 1997) which refer the analysis of 32 different characteristics including the tree, leaves, flowering, fruits and endocarps. This work focuses only in the leaf, fruit and endocarp characterization (total of 29 parameters shown in Table 2). Three trees of each cultivar were selected because, besides biometric characterization, another objective of the global project was to extract from each tree monovarietal olive oil for characterization—about 2 kg of olives was sampled. Forty-organ samples from the South-facing sides of trees were characterized for each parameter 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) according to the method prepared by the EU RESGEN CT 96/97 project.

Table 1. Pedo-climatic Characteristics of the different studied geographical sites

Cultivar	Site	Latitude/ longitude (grade)	Altitude (m)	Soil type	Average annual precipitation (mm)	*Average annual temperature (C°)*	Bioclimatic stage
Neb Jmel 1 El Hor 1	Béja	3700/900	375	*Red Mediterranean Soil*	720	17.8	Subhumid with warm winter
Neb Jmel 2 El Hor 2	Siliana	3548/ 942	360	*Sandy brown* semi-desert *soil*	257	16.5	Semi-arid
Neb Jmel 3 El Hor 3	Bizerte	3713/986	48	*Red Mediterranean Soil*	411	18	Subhumid to semi-arid

Table 2. List of morphological traits and their codes, used in the multivariate analysis

Trait	Code
Leaf Length (cm)	LL
Leaf Width (cm)	LW
Leaf Length/width	LL/LW
Longitudinal curvature of the leaf blade	L.cb
Fruit Length	FL
Fruit Width (cm)	FW
Fruit Length/width	FL/FW
Fruit Fresh weight (g)	F.we
Fruit Symmetry (position A)	F.symA
Fruit Position of maximum transversal diameter (position B)	F.pmtd
Fruit Apex (position A)	F.apex
Fruit Base (position A)	F.base
Fruit Nipple presence	F.nipple
Fruit Presence of small lens: (when the fruit is green)	F.Plens
Fruit Dimension of small lens	F.Dlens
Fruit Localization of initial turning from the base of the fruit	F.Lturn
Fruit Color of mature fruit	F.Cmat
Endocarp Length	EL
Endocarp Width (cm)	EW
Endocarp Length/width	EL/EW
Endocarp Weight (g)	E.we
Endocarp Symmetry (position A)	E.sym A
Endocarp Position of maximum transversal diameter (position B)	E.pmtd
Endocarp Apex (position A)	E.apex
Endocarp Base (position A)	E.base
Endocarp Surface (position B)	E.surf
Endocarp Number of grooves	E.No.gro
Endocarp Distribution of grooves	E.D.gro
Endocarp Presence of the mucro	E.P.mucro

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 auto-sampler (CP-8400), a capillary column HP Innowax (Agilent Technologies, USA) (30 m, 0.53 mm, 1 µm), a split–split-less 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 solutions according to the method of European Union Commission Regulation 2568/1.7.91.. 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). 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 (Table 2). The descriptive statistics of the quantitative traits measured on leaf, fruit and endocarp are shown in Table 3. The morphological attributes except the endocarp weight (E.we) for the variety "Neb Jmel", showed a high significant variability between the two varieties and a significant variability ($p < 0.01$) among the individuals of the same cultivar. Previous studies explained that Traits that come from the fruit and endocarp, not only did feature high variability among cultivars, but also among the regions (Zaher *et al.,* 2011; Hanachi *et al.,* 2007; Ozkaya *et al.,* 2006). The varieties "Neb Jmel" and "El Hor" showed the highest fruit weight (2.06 and 1.68g) in the region of Béja, while Siliana revealed the lowest values (1.28 and 0.75g). The cultivar "El Hor" revealed a significant fluctuation of the flesh percentage according to its culture location (varied from 66. 66 to 75%). Similar study was demonstrated that "Chaibi", "Gerboui", and "Chetoui" olive cultivars in Tunisia and "Arbequina" in Argentina can adapt well to the environmental conditions (Hanachi *et al.,* 2007; Aybar *et al.,* 2008; Torres *et al.,* 2009). The favorable climate for olive production in the region of Béja can be the principal cause to obtain high performance for the tow studied varieties where plant-water needs are satisfied through the annual precipitation (720 mm).

Table 3. Descriptive statistic analysis of the morpho-phenological parameters

	Variety « Neb Jmel »			Variety « El Hor »		
Trait	**Max**	**Min**	**CV%**	**Max**	**Min**	**CV%**
V1	47,330	66,850	0,17***	46,620	60,809	0,13***
V2	10,350	16,625	0,25***	7,470	12,640	0,27***
V3	3,533	6,490	0,33***	4,290	6,792	0,22***
V4	17,390	19,795	0,066***	12,170	15,726	0,12***
V5	10,750	13,096	0,098***	8,550	13,253	0,21***
V6	1,460	1,680	0,074*	1,180	1,420	0,096***
V7	1,280	2,063	0,23*	0,750	1,682	0,40***
V8	15,150	15,863	0,024***	10,412	11,500	0,055***
V9	6,060	6,477	0,033**	5,800	6,270	0,04***
V10	2,450	2,490	0,008**	1,796	1,960	0,04***
V11	0,300	0,329	$0,04^{NS}$	0,220	0,250	0,06***

P-value: NS: no significant ** significant ($P < 0.05$); *** highly significant ($p < 0.01$)

CV% Variation coefficient expressed in percentage

A principal-component analysis (PCA) was carried out on the morphological descriptors of the leaf, fruit and endocarp (Figure 1). The eigenvalues of the first, secondand third, axis of the principal components, accounted the 55.72%, 21.45% and 14.2% of the total variance, respectively. The relative magnitude of the first PC eigenvectors showed that the fruit traits (weight, length, symmetry in position A and apex in position A) and particularly the endocarp traits (length, width, shape, weight, symmetry in position A, apex in position A and the distribution of fibrovasculars sulcus) were important attributes for the classification of accessions in cluster, which proved the results find by Paula *et al.* (2005) on three Portuguese cultivars. The inertia accounted for the second PC was due to the contribution of the variables FW and E.surf, while the leaf traits (width,

shape and longitudinal curvature of the blade) had relatively high eigenvectors in the third PC.

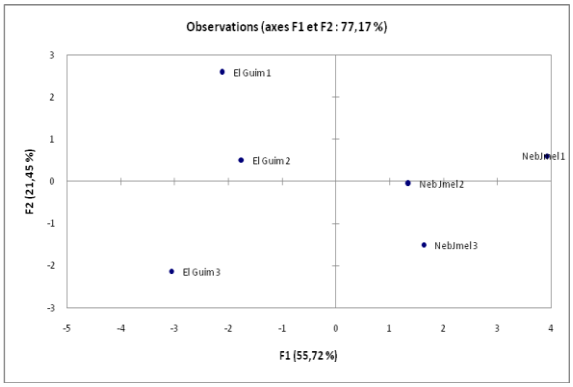

Figure 1. Projection of "Neb Jmel" and "El Horr" individuals in the plane generated by the first two principal components based on leaf, fruit and endocarp traits.

The projection of individuals in the plane generated by the axis 1, 2 and 3 illustrated the significant difference between the two studied varieties as well among the individuals of the same cultivars, especially for the variety "El Hor". As a result the PCA showed variability as regards several quantitative and qualitative traits, which can be due to three principal components, which are genetic, environmental and ontogenetic, respectively. Therefore it is necessary to characterize individuals from a same cultivar in different culture sites, to determine the limit of the best agronomical potentialities, especially that little is known about performance of varieties outside their traditional growing regions.

Oil yield of olives

The information about the analyzed cultivars, as reported in Figure 2, showed that all the studied individuals of the tow varieties "Neb Jmel" and "El Hor" are characterized by a high oil yield according to the classification of Tous and Romero (1993) and showed variation according to the cultivar and to the culture locations. Expressed as percentage of dry matter, this parameter presented significant differences between the two varieties and the individuals of the same cultivars; the content of oil in the cultivars of "Neb Jmel" was in the range of 69.3% in the region of Siliana and 71.9% in the region of Béja, While the individuals of the variety "El Hor" presented the lowest content of oil in the three studied regions which are respectively 49.3% , 51.52 and 57.91%.

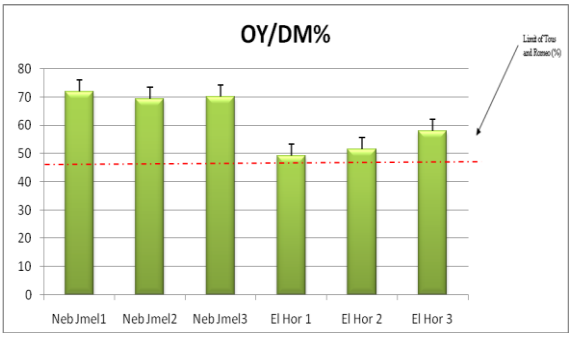

Figure 2. Comparison of the oil yield rate expressed as percentage of dry matter of the studied olive cultivars with the method of Tous and Romeo (1993).

Fatty acid composition

Methyl ester fatty acid composition and their levels in the analyzed oils are shown in Table 4. As it can be observed, oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids are the major fatty acids present in the studied samples (Borchani *et al.,* 2010). The comparison of the fatty acids content with the market standards showed that the majority of the studied sample was in agreement with these limits. The individuals of the studied varieties matched perfectly and reflect the high quality of the tested oils. The fatty acid composition of olive oils varies widely depending on the cultivar and on the geographical location, especially the individuals of the variety "Neb Jmel". These findings are in good agreement with those of other authors working on Tunisian olive oil varieties (Baccouri *et al.,* 2007; Haddada *et al.,* 2007). The oleic acid varied in the individuals of "Neb Jmel" according to their culture site (from 76.59 to 61.99%) and the highest percentage was found in the region of Siliana, which is characterized by a semi-arid climate,while a moderated variation of this fatty acid was noted between the individuals from "El Hor" (66.25 to 63.64%). Concerning linolenic (C18:3) and arachidic (C20:0) acids the cultivars of the two studied varieties revealed a high significant difference according to the variety and the geographical site. The

proportion of monounsaturated fatty acids also changed according to the cultivar and the location. It reached a maximum value of 76.82% for the Neb Jmel olive oil in the region of Siliana, which was characterized, among the studied oils, by the highest C18:1/C18:2 ratios (7.3). Oil presents a good stability index if the value of this parameter is over 7. Consequently "Neb Jmel" variety present oil with high stability index, whereas 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.,* 2007 ; Zarrouk *et al.,* 2009).

Table 4. Methyl ester fatty acid composition and their levels in the analyzed oils according to the norm of the IOC, (1997)

	C16:0	C16 :1	C18:0	C18 :1	C18 :2	C18 :3	C20:0
Norm	**7.5–20**	**0.3–3.5**	**0.5–5**	**55–83**	**3.5–21**	**<0,9**	**≥0.6**
Neb Jmel 1	13.34	0.23	3.13	61.99	19.87	0.72	0.47
Neb Jmel 2	9.48	0.23	1.92	76.59	10.49	0.33	0.4
Neb Jmel 3	9.68	0.4	2.56	68.89	16.99	0.38	0.19
El Hor 1	16.34	2.12	4,16	65.86	10.55	0.82	0.19
El Hor 2	16.87	2.68	3.21	66.25	10.17	0.51	0.31
El Hor 3	16.3	2.44	1.69	63.64	15.33	0.4	0.2
Average	13.67	1.35	2.78	67.2	13.9	0.52	0.29
Min	9.48	0.23	1.69	61.99	10.17	0.33	0.19
Max	16.87	2.68	4.16	76.5	19.87	0.82	0.47
CV%	24.87^{***}	86.66^{NS}	32.73^{***}	7.67^{***}	29.42^{***}	38.46^{***}	41.37^{***}

Conclusion

In order to evaluate the phenotypic and technological variability among the varieties "Neb Jmel" and "El Hor", descriptive statistics and principal components analysis were used. Morphological traits that come from fruit and endocarp not only did feature high variability among the studied varieties, but also among individuals within each cultivar according to their cultural sites. This variability can be due to two principal components, which are genetic and environmental, respectively. The olive oil content is high for the studied accessions, especially in the region of Béja, which is characterized by a sub-humid climate and a red Mediterranean soil. . The acidic composition of the tested oils varies widely depending on the cultivar and on the geographical location of the different studied cultivars, and they are classified as oils with high oleic acids and low palmitic and linolenic acids . The studied locations, with their different climate and their different soil composition seemed to influence the content in oleic (C18:1), linolenic (C18:3) and arachidic (C20:0) acids. Consequently, the study of agronomic potentialities of local olive varieties in different culture sites is well important to resolve the performance of varieties outside their traditional growing regions and to improve the technological standards of the cultivar by choosing the most adequate geographical sites that enabled valuable technological characteristics, particularly that little is known about performance of varieties outside their local areas. The use of molecular markers (AFLP and SSR) in future studies will be essential to increase the knowledge about the diversity of the studied species.

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