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RESEARCH ARTICLE

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ANCIENT OLIVE TREES IN BSHAALEH LEBANON

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ABSTRACT

Since Lebanon does not have a comprehensive reference on national olive (*Oleaeuropaea* L.) germplasm, concerning especially the oldest olive trees growing the Bshaaleh area, in northern Lebanon, this study was conducted to characterize these trees using a morphological and biochemical approach and to evaluate the level of their similarity. Samples were taken from leaves, flowers, fruits and stones from the 15 olives accessions and oil was extracted from fruits. Notable all morphological characteristics considered were similar to many different cultivars identified in the Mediterranean area including: tree canopy, leaves, inflorescence and fruit characteristics. According to the PCA and the cluster analysis, morphological characterization of the ancient olive trees of Bshaaleh showed that the olive trees were clustered into three groups, with difference in morphology between B1, B2, B3 and B5 (group 1), B4, B7, B8, B9, B10, B12, B13 and B15 (group 2) and B6, B11 and B14 (group 3).Concerning the biochemical characteristics of the 15 accessions oils, the acidity, peroxide number and the spectrophotometer absorbencies in ultra-violet were low and iodine value high. All of the biochemical values (acidity, peroxide number, absorbencies in ultra-violet, refractive index, iodine value and saponification values) used to evaluate oil quality were within the IOC trade standards for extra virgin olive oil.

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INTRODUCTION

The cultivated olive (*Oleaeuropea L.)* is a long-lived evergreen tree native to the Mediterranean basin (Poljuha*et al*., 2008).It is of great importance to evaluate and characterize the existing genetic diversity of the crop species, mainly for those, such as the case of olive, which still have well preserved a great cultivar patrimony, in spite of the disturbance of the environment where they are cultivated. This issue is of particular importance in areas where a number of varieties shows adaptation to the local difficult environmental conditions like Bshaaleh-Lebanon. A report published 44 years ago indicated the presence in Lebanon of four varieties.The characterization of these varieties was based on purpose of use, sensitivity to climatic factors, oil content, and easiness of grafting (Kassab, 1973).

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In 2005, Owen *et al.* have conducted a study on the genetic diversity within cultivated olive germplasm from the Eastern Mediterraneanthat has included two cultivars from Lebanon:Ayrouni and Souri. Given the absence of a comprehensive national reference on Lebanese olive germplasm, a study was conducted by Chamoun*et al.* (2008); to assess *in situ* diversity, in established Lebanese olive groves, by using molecular techniques. The only known studies conducted for morphological characterization of Lebanese cultivars were performed by the Lebanese Agricultural Research Institute (LARI) (Chalak, 2008). Moreover, for the cultivar Souri, a morphological description is also reported in the world catalogue of olive varieties published by IOC in 2000 (Barranco*et al.*, 2000). The importance of old autochthonous olive varieties has recently increased due to their adaptation to local conditions, high oil quality and consumers 'preference for typical olive oils. Accordingly, the need and validity for the protection of those oils are arising.

One of the crucial prerequisites for its realization is forming the complete database of identified autochthonous olive varieties with respective oil characteristics (Poljuha*et al.*, 2008). The potential amount of oil accumulating in the fruit at maturation, the quality of oil and evenits aroma compounds accumulation are determined by the cultivar (Manai*et al.*, 2008; Rjiba*et al.*, 2010), but they vary greatly depending on climate, growing conditions, age and, to a lesser extent, fruit load (Lavee, 1977).The pattern of oil accumulation in olive fruits has been studied with many cultivars and in many different locations. These studies, however, were performed for most cultivars separately and under various growing conditions(Lavee and Wodner, 1991). The cultivar is also one of the most important factors in determining oil quality. However, the chemical composition of olive oil, also depend on a complex combination of factors such as environment conditions (climate and soil) and cultural practices (Dhifi, 2004; Inglese *et al.*, 2009). Until now, Bshaaleh olive fruits and oil composition have never been investigated, although they have a great historical and biological importance, that goes back to 1350 years (Bou Yazbeck *et al.*, 2018b). The aim of this study is to characterize these trees using a morphological and biochemical approach and to evaluate the level of their similarity by comparingtheiroils with those of known local and world olive varieties and providing a first characterization of such oils.

MATERIALS AND METHODS

Plant material: Morpho-agronomic analyses were performed on all the 15 olive accessions of Bshaaleh that are very ancient and have not a clear origin and denomination. These olives are considered as the oldest trees, still productive, in Lebanon. Tree, leaf, inflorescence, fruit and endocarp were studied. For each organ, 40 replicates collected from the south-facing side of each tree were used (Cantini *et al.*, 1999;Wazaz*et al.*, 2007).For each tree, the average for each characterized trait was used in statistical analysis.

Morphological characterization of olive accessions: The morphological characteristics were evaluated by using the “methodology for primary characterization of olive varieties” adopted by the International Olive Council (IOC) (Ebiad and Abu-Qaoud, 2014, Chehade *et al.*, 2016). All characters of the IOC methodology and related to the tree, the fruiting shoot, the leaf, the inflorescence, the fruit and the endocarp were used to perform the morphological characterization. They were 32 and, among them, 15, marked by an asterisk (*), were considered the most heritable and so the most important in characterization studies. In addition, 2 other characters were taken into consideration: the position of maximum transverse diameter in the leaf and the inflorescence structure as they are also reported as useful in morphological studies (Barranco and Rallo, 1985).

Assessed characters

Tree

Three qualitative characters (vigor, growth habit and canopy density) were evaluated.

Vigor refers to both the tree size and the intrinsic ability of the scaffold branches and shoots to grow in length and width.

Growth habit describes the natural distribution of the scaffold branches and shoots before there is interference from the training system and when vigor exerts little influence.

Canopy density indicates the extent of canopy vegetation and can be measured on the basis of light penetration. It is the result of the interaction between the internodes length, the shoots number and vigor and the leaves size.

Fruiting Shoot

Length of the internodes was measured in 8-10 fruiting shoots located around the tree at shoulder level.

Leaf

This was done in samples of approximately 40 adult leaves taken from the middle section of 8-10 one-year-old shoots chosen from the most representative shoots on the south-facing side of the tree at shoulder level.

Length (L) of the leaf.

Width (W) of the leaf.

Shape (*) was determined by the L/W ratio.

Longitudinal curvature of the blade was evaluated by observing the longitudinal axe of the leaf.

Inflorescence: The inflorescence length and number of flowers/inflorescence were evaluated in samples of 40 inflorescences at the white stage, taken from the middle section of 8-10 fruiting shoots (grown the previous year), chosen from the most representative ones on the south-facing side of the tree.

Length of the inflorescence

Number of flowers/inflorescence

Fruit: Observations were carried out on a sample of 40 fruits collected, roughly upon completion of color change which characterizes the start of ripening, from the middle part of several fruiting shoots chosen from the most representative ones on the south-facing side of the tree. Very small or very large fruits were discarded from the sample. Some of the considered characters refer to two positions: position "A", that is the position in which the fruit generally displays the greatest asymmetry when held by either end between the index finger and thumb (Figure 1); position "B", that is reached by turning 90° from position "A" in such a way as to present the most developed part to the observer. Four of the 11 fruit characters are marked with an (*), because they are considered to have a great capacity in discriminating varieties.

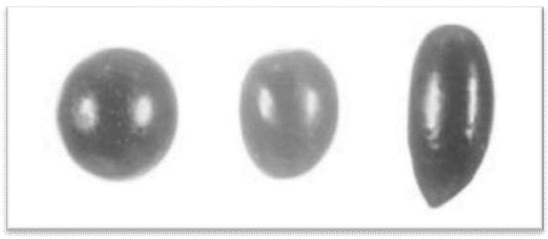

Figure 1. Different fruit shape in position A.
(*http://www.internationaloliveoil.org/resgen/eng/rg-fruto.html*)

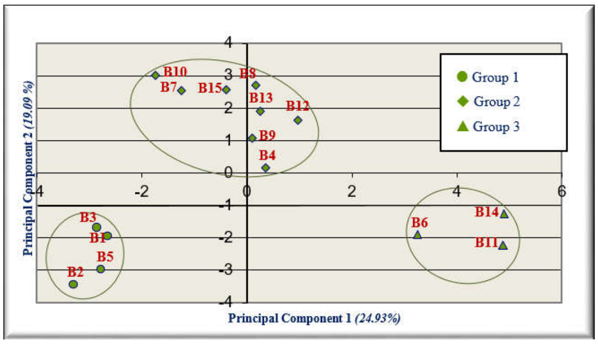

Figure 2. Scatterplot of principal component (PC) 1 versus 2 for *Oleaeuropaea* from Bshaaleh showing a clustering of 3 groups

Table 1. Principal Component Analysis of Bshaaleh olive trees (Values for first three PCs shown)

Eigen vectors	Proportion	Cumulative
Principal Component 1	0.2493	0.2493
Principal Component 2	0.1909	0.4401
Principal Component 3	0.1358	0.576

Table 2. COPH Matrix correlation MXCOMP for Bshaaleh accessions morphological clustering analysis generated by NTSYS.PC v 2.11Q 2000-2003

2-way Mantel test -- Mantel (1967) method	Tests for association: Matrix correlation: r = 0.88893
N = 105 points	(= normalized Mantel statistic Z)
Mean X = 0.5165 SSx = 3.4854 Mean Y = 0.5165 SSy = 4.4108	ApproximateMantel t-test: t = 6.9914 Prob. random Z < obs. Z: p = 1.0000

Weight

Shape (position A) (*) was determined by the Length/Width ratio (Figure 1).

Symmetry (position A) was determined by the extent to which the two longitudinal halves match.

Position of maximum transverse diameter (position B) (*).
Apex shape (position A).

Base shape (position A).

Presence of Nipple(*) that is an extrusion of the fruit apex.

Presence of lenticels was evaluated when the fruit was still green.

Size of lenticels was evaluated when the fruit was still green.

Location of start of color change (*) was evaluated by observing where the first violet blotches appeared on the epidermis of the fruit.

Color at full maturity was evaluated at the end of the ripening process, when the fruit had attained its definitive color.

Endocarp (stone): Observations were carried out on stones extracted from the sample of 40 fruits used for fruit characterization. As in the case of the fruit, some characters also refer to two positions as described above.Almost all the characters taken into account for the stone morphological evaluation were marked with an (*) and so were very important for variety identification/characterization.

Weight.

Shape (position A) (*) was determined by the Length/Width ratio.

Symmetry (position A) (*) was determined from the extent to which the two longitudinal halves match.

Symmetry (position B) (*).

Position of maximum transverse diameter (position B) (*).

Apex shape (position A) (*).

Base shape (position A) (*).

Surface (position B) (*) was evaluated according to the depth and abundance of the tracks of fibro vascular bundles.

Number of grooves (*) counting the grooves that can be seen from the stalk insertion point.

Distribution of the grooves (*).

Termination of the apex (position A) (*).

Biochemical analysis: The major parameters taken into account to assess olive oil includedoil acidity, peroxide value and spectrophotometric absorbency in ultraviolet, that are necessary to grade the olive oil as extra virgin or other categories according to the IOC trade standard for olive oils (COI/T.15/NC no 3/Rev. 3-November 2008), the laws in force in the European Union (EC regulation no 702/2007), the *codex alimentarius* standard for olive oils and olive pomace oils (CODEX STAN 33-1981(Rev.2-2003) and the Lebanese standard (NL 756:2005). Other parameters that contribute to characterize the oil were also studied, such as refractive index, iodine and saponification values.

Samples preparation: Three composite mixture samples were prepared; each one in 2 replicates. The first sample group included the first 6 trees of Bshaaleh B1-B6, the second sample group included 3 trees B7-B9 and the third sample group included 4 trees B10-B13. This selection was based only on geographical criteria where trees close one to the other were put together. From each group, 2 kg of healthy olive fruits were picked by hands at the end of October and at the same stage of ripening (70 % of olives just turned dark-colored and the remaining were still green).

The olives were crushed with a lab hammer mill, and then the mash was malaxed for 30 minutes. The malaxed olive paste mixture was transferred to a laboratory centrifuge in order to extract the oil and the plant water; the oil was then separated from the water using a funnel-shaped separator and filtered through a paper filter. The oils obtained were stored at 4°C in filled up and sealed dark-colored glass bottles until analysis were performed (Poljuha *et al.*, 2008).

Oil quality characterization: A total of 36 characteristics were determined on the oil, according to the IOC, reported in the EEC No 2568/91 (2008). The tested parameters were: free acidity (expressed as % of free oleic acid), peroxide value (expressed as meq of O_2 / kg of oil), spectrophotometric absorbency in Ultra-Violet (K232, K270 and ΔK), refractive index, iodine value and saponification values.

Data analysis: The standardized traits mean values (mean of each trait was subtracted from the data values and the result divided by the standard deviation) were used to perform Principal Component Analysis (PCA) using the software SAS Version 9.1 / PROC PRINCOMP module (SAS, 1985) and cluster analysis using the program NTSYS.PC version 2.11Q 2003 (Rohlf, 2003).Cluster analysis conducted on the Euclidean distance matrix generated with Dice coefficient using UPGMA clustering methods, a hierarchical method of clustering (SAHN module), was performed selecting only the variables accounting for high loadings on the first two eigenvectors (Rotondi *et al.*, 2003;Abdelhamid *et al*., 2013). Morphological data were binary coded as 1 for the presence of a character option and 0 for its absence (Moussavi *et al*., 2014). In order to assess the fit goodness, the cophenetic correlation assessment between the dendrogram and the similarity matrix was performed using the COPH module and the MXCOMP module in the NTSYSpc version 2.11Q software. For biochemical characterization of oil, all analyses were carried out in triplicate and the results presented as means of 3 repetitions. Standard deviation values were calculated. In order to check for the difference among the three mixtures,the individual result was compared with the mean and standard deviation interval.

RESULTS

Morphological characterization: Considering that the 15 olive trees of Bshaaleh should belong to the same variety according to the grove history, the average for each characterized quantitative trait used and the most frequent qualitative characterized trait were recorded for this primary characterization. These accessions should belong to a variety named Baladi or Souri according to the local farmers. Ancient olive accessions in Bshaaleh are characterized by a strong growth, marked trunk and canopy development, in terms of both height and volume, and vigorous, long branches. Its growth habit (spreading) is the natural growth habit of the species. The canopy density is medium, which means that the vegetation is abundant but internodes length and growth always leave internal spaces. Flowers are borne axially along the shoot in inflorescences called panicles. The panicles of Bshaaleh old trees carried an average of 20 to 25 flowers. The leaf charactersare similar to many different cultivars identified in the Mediterranean area (Barranco*et al.*, 2000). The fruit of these trees is characterized by a high relative weight and a shape that varies between ovoid and elongated. The fruit tip has a tenuous style and the start of color change has been noticed to be in most cases from the apex. Knowing that most of the endocarp characters are highly discriminatory in identifying varieties (Barranco *et al.*, 2000), the characterization of the endocarps was executed in details. The collected endocarps are characterized by an elliptic to elongated shape and a not perfect symmetry. The apex and the base in position A are almost pointed. It has been noticed that the surface of the endocarps varies between smooth and rugose, reflecting a medium to low depth and abundance of the fibro vascular bundles. The grooves are distributed in a regular way and a mucro has been identified at the termination of the apex in position A.

Phenotypic variability: The eigenvalues obtained by PCA on the morpho-agronomic data indicate that two to three components provide a good summary of the data.PCA showed that two principal components (PC) accounted for a quite high percentage of the total variance (44.01%) among the different olive trees of Bshaaleh. With the third principal component, they account cumulatively for 57.6% of the total variance between groups (Table 1).

PC 1 (24.93% of the total variance) was predominately influenced by the length of the inflorescence (Eigenvector Coefficient (EC)=-0.248), shape of the fruit (EC=-0.237), position of maximum transverse diameter (position B) of the fruit (EC=-0.236), presence of nipple (EC=+0.295) and endocarp apex form in position A (EC=+0.313); this means that these descriptors are very important in differentiating between the selections. PC 2 (19.09 % of the total variance) was mainly influenced by the shape of the endocarp base (position A) (EC=-0.297), fruit apex form (position A) (EC=-0.207), length of the internodes (EC=-0.204), fruit weight (EC=+0.264), canopy density (EC=+0.275) and endocarp shape (position A) (EC=+0.348). Therefore, based on the principal component analysis, traits such as canopy density, internodes length, inflorescence length, some fruit characters, including shape, apex form (position A), weight, nipple presence and position of maximum transverse diameter (position B), and some endocarp characters, including endocarp shape (position A), base shape and apex form in position A were the most effective characters for distinguishing among the 15 selected ancient olive trees of Bshaaleh.

It should be evidenced that fruit shape, maximum position of transverse fruit diameter (position B),nipples presence, endocarp shape, apex and the base form (position A) are considered among the characters having a great ability to discriminate the different varieties according to the IOC. Figure 2 showed a scatter plot of PC 1 versus PC 2 for olive trees of Bshaaleh. In this scatter plot, a clear separation of the three groups can be seen. The scatterplot of principal component analysis showed that there was difference in morphology between B1,B2,B3 and B5 (group 1), B4, B7, B8, B9, B10, B12, B13 and B15 (group 2) and B6, B11 and B14 (group 3).

Clustering analysis: Eleven of 34 variables were thus selected for clustering analysis. It includes the variables accounting for high loadings on the first two eigenvectors, i.e. canopy density, internodes length, inflorescence length, five fruit characters, including shape, apex form (position A), weight, nipple presence and position of maximum transverse diameter (position B) and three endocarp characters, including endocarp shape (position A), base shape and apex form in position A. Cluster analysis was performed on the selected standardized variables by Clustering procedure. The TREE plotting was then used to produce a tree diagram (dendrogram) able to identify disjoint clusters at a specified level in the tree (Rotondi *et al.*, 2003) using the NTSYS.PC version 2.11Q 2000-2003.The dendrogram obtained by clustering procedure using the 11 variables showed the accessions grouped in clusters with their respective distances (Figure 3).

Table 3. Detailed morphological characterization results for the 15 olive trees from Bshaaleh by cluster. Each result was presented with a numeric code of the option (Details about each option are not shown)

	Cluster 1					Cluster 2							Cluster 3		
	B1	B2	B3	B4	B5	B7	B8	B9	B10	B12	B13	B15	B6	B11	B14
Tree															
Vigour	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Growth habit	2	2	2	2	2	2	2	2	2	2	2	1	2	2	1
Canopy density	1	1	1	1	1	2	2	2	2	2	2	1	1	2	1
Length of the internodes	3	2	2	2	2	1	1	2	2	2	2	1	2	2	1
Leaf															
Shape	3	2	2	1	3	2	1	2	2	1	2	2	2	2	1
Length	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Width	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Longitudinal curvature of the blade	2	2	2	3	2	2	3	2	2	3	2	2	2	2	3
Maximum diameter	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inflorescence															
Length	2	2	2	2	2	2	2	2	2	2	1	2	2	1	1
Number of flowers	3	3	2	2	2	2	3	2	2	2	3	3	2	2	2
Structure	3	3	2	2	2	4	3	1	4	3	3	4	2	1	1
Fruit															
Weight	3	2	2	3	2	3	3	3	3	3	3	3	3	3	3
Shape (p. A)	3	3	3	3	3	3	3	3	3	3	3	3	2	2	3
Symmetry (p. A)	3	2	2	2	1	3	3	3	3	3	3	3	3	3	3
Position of maximum transverse diameter (p. B)	2	2	2	2	1	2	2	2	2	1	2	2	1	1	1
Apex (p. A)	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
Base (p. A)	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
Nipple	2	2	2	2	2	2	2	3	2	3	2	2	3	3	3
Presence of lenticelles	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Size of lenticelles	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Location of start of colour change	3	1	3	2	2	1	2	2	1	2	3	2	3	1	2
Colour at full maturity	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Endocarp															
Weight	3	2	2	3	2	3	3	3	3	3	3	3	3	3	3
Shape (p.A)	3	3	3	3	3	4	4	4	4	4	4	4	2	2	2
Symmetry (p. A)	2	2	2	2	2	2	2	2	3	3	2	2	2	1	1
Symmetry (p. B)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Position of maximum transverse diameter (p.B)	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Apex (p.A)	1	1	1	1	1	1	1	1	1	2	1	1	2	2	2
Base (p.A)	3	3	2	2	2	2	2	2	2	2	2	2	3	3	3
Surface (p.B)	1	2	1	3	2	1	2	2	1	2	2	2	3	3	2
Number of grooves	1	2	1	2	1	1	1	1	1	1	2	1	1	2	2
Distribution of the grooves	1	1	1	1	2	1	1	1	1	2	1	1	1	1	1
Termination of the apex	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2

Table 4. Chemical and physical characteristics of the three olive oil mixtures

	Mix 1	Mix 2	Mix 3	Average Value	Standard Deviation
Free acidity (%)	0.230	0.260	0.315	0.268	0.044
Peroxide number	4.40	5.25	7.00	5.55	1.285
Ultra-Violet K232	1.855	1.900	1.870	1.875	0.061
Ultra-Violet K270	0.175	0.150	0.170	0.165	0.024
Ultra-Violet / ΔK	0.0045	0.0035	0.0045	0.0042	0.0008
Iodine Value (%)	92.55	89.15	86.40	89.37	3.1411
Refractive Index	1.46910	1.46905	1.46905	1.46910	0.0004
Saponification value	197.90	195.35	193.45	195.57	2.8493

The UPGMA cluster analysis revealed two main groups. Group 1 comprised two subgroups. One of them comprised 5 accessions including the oldest olive tree present: B1 that is characterized by the highest length of internodes and a rounded apex of the fruit in position A. The second subgroup comprised 7 accessions (B7, B8, B9, B10, B12, B13 and B15). This group is characterized by medium canopy density and elongated shape of the endocarp. The three accessions B6, B11 and B14 were clustered together in the second group. An ovoid shape of the fruit in position A and a symmetric endocarp in position A characterized the accessions in this group. The cophenetic correlation between the dendrogram and the similarity matrix revealed a good degree of fit (r=0.88).

Detailed results are presented in table 2. According to the PCA analysis and the cluster analysis, morphological characterization of the ancient olive trees of Bshaaleh showed that the olive trees are clustered into three groups. The detailed results of the primary characterization for each tree are present in table 3, taking into account the clustering result in the presentation of the accessions' order. The morphological features of the ancient olive fruit of Bshaaleh are shown in figure 4.

Biochemical characteristics of oil: The acidity and peroxide number of the oils of all mixtures were very low (Table 4), and this reflected a low degradation of triglycerides and a low level

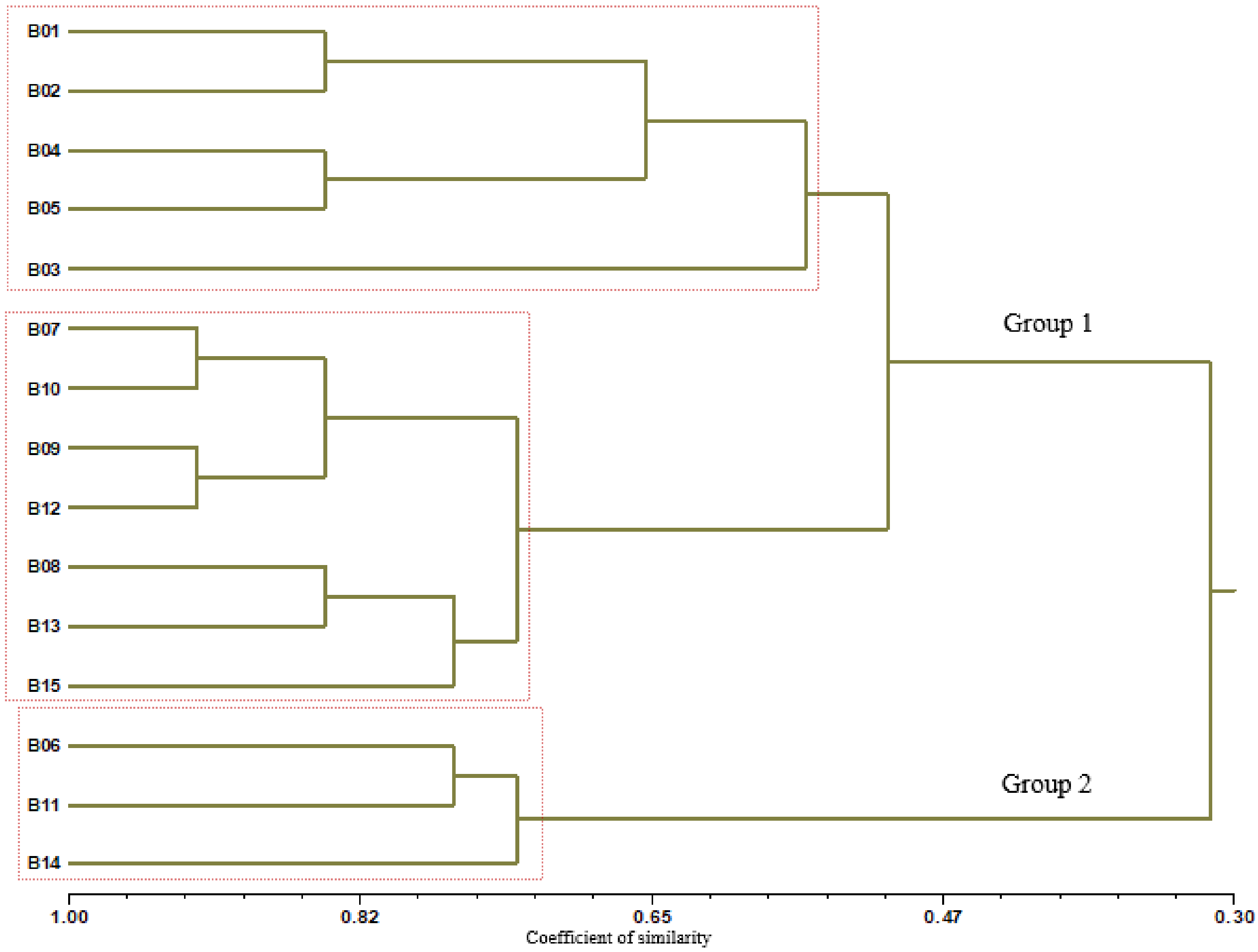

Figure 3. Dendrogram of Bshaaleh olive accessions derived by clustering UPGMA analysis from the dissimilarity matrix of the morphological data. Distances between accessions are shown by the length of the horizontal connecting segments

Figure 4. Morphological features of the ancient olive fruit of Bshaaleh

of oxidation of fatty acids, respectively (EEC, 2008). Also thespectrophotometric absorbencies in ultra-violet were relatively low, showing good quality levels. It should be noticed that the greater the value of K232 the greater the concentration of conjugated dienes, whereas K270 is proportional to the concentration of conjugated trienes and therefore, these parameters reflected the freshness of the oil(EEC, 2008).The 3 mixtures had an iodine value between 86% and 93%: this relatively high iodine value means that the individual fatty acid that comprise the oil contained a higher average number of double bands (and therefore a higher proportion of unsaturated fatty acids). Therefore, a high iodine value suggests better healthfulness. The refractive index was very similar in the three mixtures, showing an average value of 1.47. This index is directly related to the chain length and degree of fatty acid unsaturation (EEC, 2008). The saponification mean value was at the higher limit value established by the *codex alimentarius* standard for olive oils and olive pomace oils (CODEX STAN 33-1981 (Rev.2-2003)), reflecting relatively high average molecular weight (or chain length) of all the fatty acids present. A high number of ester bonds suggest that the fat molecule is intact and that the oil has been properly processed from sound fruit (EEC, 2008).

DISCUSSION

The morphological characterization of the ancient olive trees indicated a considerable level of variation within the ancient olive trees in Bshaaleh. This variation was recognized through the PCA analysis and the clustering analysis. A certain level of results' overlapping was noticed between the two methods. Several studies agree that after an ancestral spreading of few olive varieties along the Mediterranean basin, a majority of modern cultivars were derived from the crossing of these ancient cultivars among themselves, or by their breeding with wild plants, followed by local selection (Angiolillo *et al.*, 1999, Besnard *et al.*, 2001a). These findings indicate how a great variability in olive germplasm originated and have important implications concerning both the adaptation of the cultivars to their local environment and their agronomic performance under specific conditions. This latter consideration implies that every initiative to promote olive cultivation has to take into consideration the local varieties and that every region should preserve its own plant material to safeguard olive adaptation and productivity. The olive germplasm classification is not only complicated by the richness of olive genetic patrimony, but also by the absence of reference standards and confusion on the cultivar names, with numerous cases of homonymy and synonymy (Bartolini *et al.*, 1998) and, in this regard, the morphological characterization of these ancient olive trees could be used as reference to identify other varieties in the country.

This should be linked to a massive morphological characterization of the different olive genotypes in Lebanon that should lead to a comprehensive national reference collection, useful also for a safer, in terms of genetic and sanitary certainty, olive propagation. The introduction of computerized systems applied to morphological characterization protocols, allowing the analysis of a very large number of samples and reducing handling and instrument errors, has dramatically increased the reliability of the morphological characterization method (Hagidimitriou *et al.*, 2005). By comparing some morphological traits of Bshaaleh accessions with other traits defined for Souri varietyin previous studies(Abdine*et al.*, 2007; Ebiad and Abou Qaoud*,* 2014), it is possible to detect a very high correspondence, indicating that, probably, the accessions of Bshaaleh may be accessions from the Lebanese Souri variety. A study within the RESGEN project took into consideration a total of 40 olive varieties or accessions located in 25 stands (Chalak, 2008). Both primary (morphological) and secondary (bio-agronomic) characterizations were performed according to the procedures set by the IOC (Barranco*et al.*, 2000). The results showed a high level of morphological diversity in the Lebanese olive germplasm, since 39 accessions were clearly distinguishable at the Euclidian distance 0.1 among the 40 accessions studied (Chalak, 2008). The good effectiveness of morphological characterization to distinguish different olive cultivars has also been seen in several other researches carried out in other countries (Barranco and Rallo, 1985; Barranco*et al.*, 2000; Taamalli*el al.,* 2006; Wazaz*et al.*, 2007; Ebiad and Abu-Qaoud, 2014). Moreover, the biochemical results of this study provide a first, preliminary characterization of the oils produced by the ancient olive trees in the Bshaaleh area.

The mean values of acidity, peroxide number and spectrophotometric absorbencies in ultra-violet allow to classify all the extracted oils as extra virgin according to the trade standards taken into consideration and mentioned above, showing that the oils produced by the very old trees of the Bshaaleh area can reach very high qualitative levels. According to Criado *et al.* (2004), the olive oil phenolic fraction, the hydroxytyrosol and tyrosol contents were significantly higher in olive oils from cultivars grown at higher altitude and precipitation rate. Characterization of olive oil can be an extremely difficult task because of the variation in composition caused by extraction methods and environmental and storage conditions and can be further complicated by the presence of adulterants. The quality of olive oil is a result of both chemical and sensorial properties and therefore, is ultimately determined by different parameters. Chehade *et al.,* (2016) evaluated the fruit and oil characteristics of the main Lebanese olive varieties (Aayrouni, Abou Chawkeh, Baladi, Del and Soury). He found that for all varieties, the values of free fatty acids, peroxide values, absorbance in ultraviolet, fatty acid composition, sterol content and composition and erythodiol+ uvaol of the oils were within the requirements of the IOC Trade Standard for extra virgin olive oil. Olive oil production and quality relies on appropriate cultivar selection as well as good orchard management. Production based only on superior cultivars result in improved yield, oil quality, and production management (Sanz-Cortés, 2003). By assessing the quality of oils obtained from the ancient olives growing in the Bshaaleh area, we concluded that the resulted oil can be classified as extra virgin olive oil in reference to the international standards.

However, we have to consider the reported results as preliminary ones since only one year of observation of olive oil samples was considered. Therefore, more completed database of chemical characteristics based on some extra years of observation is needed. Moreover, further oil parameters to be tested are needed for a better oil characterization, including the fatty acid composition and the polyphenol, tocopherol and sterol content and composition. The characterization should also regard the sensorial profile, that could be performed by a panel test according to the procedure described in the EEC/Reg. 2568/91, the EC/ Reg. 796/02 and EC Reg. 640/2008, as reference. The execution of such analysis could also help to discriminate possible difference among the ancient trees growing in the Bshaaleh area. The evaluation of the oil quality in different times during the olive ripening period could also be useful to characterize the oil quality obtained from these trees at several times and also to establish the best harvest period. While this study showed that the accessions of Bshaaleh were clustered into three main groups according to the morphological characterization, a previous study conducted by Bou Yazbek *et al*. (2018 a), showed that all the ancient olive trees of Bshaaleh where clustered into the same genetic pool according to the molecular characterization. Differences between the two methods of characterization has also been reported in several other studies, where discrepancies were explained by taking into account the possible interferences of environmental factors (Ercisli *et al.*, 2008). The apparent unique nature of Bshaaleh olive germplasm revealed by our results supports the case for the implementation of more intense characterization and conservation strategies. Studies like the one presented here are useful to provide new insights that can help to recognize the different genotypes, to perform programs for the development of new varieties and for a better choice of the variety to use in relation to the successful productive objective and environmental conditions.

Conclusion

Our results indicate that ancient olive accessions in Bshaaleh are similar to many different cultivars identified in the Mediterranean. There is a considerable level of morphological variability within these accessions. This variability was recognized through both PCA analysis and clustering analysis. The PCA analysis and the cluster analysis applied to morphological characters showed that the ancient olive trees of Bshaaleh are clustered into three groups. The PCA analysis was able to identify the most powerful characters in discriminating the considered trees. By assessing the quality of oils obtained from the ancient olives growing in the Bshaaleh area, we concluded that the resulted oil can be classified as extra virgin olive oil in reference to the international standards. After the characterization of Bshaaleh olive old trees, it could be interesting to develop a database on local national olive varieties which may be used to indicate adequate time to harvest the olives and the variability of individual cultivars in relation to the environment (site) and conditions of cultivation, by measuring quality parameters, such as fatty acids, polyphenols, etc., with particular regard to those taken into account by the IOC trade standards and other national and international trade standards.

Conflict of interest: The authors declare that they have no conflict of interest

List of abbreviations

PCA Principal Component Analysis

IOC International Olive Council

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