Journal of Fundamental and Applied Sciences

ISSN 1112-9867

Available online at http://www.jfas.info

Research Article

CHARACTERIZATION OF FIVE NEW OLIVE (*OLEA EUROPAEA* L.) CULTIVARS OBTAINED IN A CROSSBREEDING PROGRAM IN TUNISIA

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Received: 20 February 2019 / Accepted: 30 August 2019 / Published online: 01 September 2019

ABSTRACT

Five new olives (Olea europaea L.) variety issued from a Tunisian breeding program were released in 2017. The objective of this program is to enhancethe quality of the oil of local variety 'Chemlali Sfax', its oil has a moderate rate of oleic acid (55-64 %) and is relatively rich in palmitic acid (17-22 %). A wide genetic diversity was observed within the new cultivars which differ from the typical cultivar. The results of the morphological evaluation of the leaf, fruit and stone showed mainly the appearance of new morphological states for the fruit and the stone and a significant increase of the fruit weight (medium).The fatty acid composition was better than that of Chemlali Sfax varity. In November, the palmitic and linoleic acids concentrations decreased significantly for the different olive oils, while the oleic acid concentrations were higher than the original cultivar. From November to January, the different fatty acid levels were always better than those of Chemlali Sfax.

Keywords: Chemlali Sfax; hybridization; morphology; oil quality.

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doi: http://dx.doi.org/10.4314/jfas.v11i3.19

1. INTRODUCTION

Olive genetic improvement by the hybridization method has been adopted in several countries such as Israel [1], Spain [2], Italy [3] and Morocco [4]. Accordingly, the most significant

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results come from Israel where several varieties have been characterized and released as ‘Kadesh’, ‘Barnea’, ‘Maalot’, ‘Askal’, ‘Kadeshon’, ‘Sepoka’ and ‘Masepo’ [5-9]. In Spain, a hybridization program has been carried out since 1991. Whither, reciprocal crosses between cultivars have been carried out with the aim of reducing the juvenile period and improving olive production and oil yield. Recently, a new variety (‘Chiquitita’) was selected and released in Spain [10].

Following the study of Fontanazza and Baldoni [11], a controlled breeding genetic improvement project was initiated in 1993 and concerned some Mediterranean countries. In fact, in Tunisia, this program has interested the Chemlali Sfax variety to improve the acidic composition of its oil. Indeed, Chemlali Sfax has low oleic acid (53 to 56%) and high palmitic acid (17 to 21%) according to Grati-Kamoun and Khlif [12] and Zarrouk et al. [13]. Thus, the new cultivars obtained by controlled crosses between the Tunisian oil variety Chemlali Sfax and other Tunisian and foreign varieties [14] were planted in 1997 at the experimental farm of Olive Tree Institute in Sfax region. Since 2000, a preliminary selection based on the acid composition has been carried out [15] and the selected crossbreeds were planted in an orchard of behavior in the Sfax region in 2005.

Many works were undertaken in order to quantify and to evaluate the genetic diversity observed within and between olive seedlings issued from the Tunisian cross breeding program.

The new progenies of ‘Chemlali Sfax’ showed a high morphological variability and highly significant differences are observed in the most descendants of crossbreeding [16]. Previous studies realized in new olive progenies of ‘Chemlali sfax’ showed great variability in morpho-agronomical [17] and architectural characters [18].

Moreover, many other works were undertaken on Chemlali Sfax seedlings regarding the acidic composition of the oil [19-24]. These studies revealed high variability in the main fatty acid concentrations and several seedlings had a more interesting lipid composition than the original variety. All the above studies were carried out on the original seedling collection.

Recently, five new cultivars obtained in the Tunisian crossbreeding program were proposed for release, accepted in 2016, and published in the Official Journal of Republic of Tunisia [25].

The aim of this study was to characterize the main morphological characters and the fatty acid composition in the five released cultivars planted in the comparative field trial established in 2005.

2. MATERIAL AND METHODS

Five new olive trees were obtained through controlled crossings between Chemlali Sfax and other Tunisian and foreign varieties (Table 1). These cultivars were planted since 2005 in an orchard at the experimental station "Ettaous" of the Olive Institute at Sfax (south of Tunisia, Longitude = 10°37' Est, Latitude = 34°55' North), where the olive trees cultivated in the same pedoclimatic conditions at a planting distance of 4m / 6m and drip irrigation.

Table 1. Names, codes and crosses of new released cultivars in Tunisia

Name	Abbreviation	Cross
Janouby Mwarref	JM	Unknown
Chemlali Mhassen	CM	Chemlali Sfax self pollinated
Zeitoun Allyssa	ZA	Chemlali Sfax * Chemchali Gafsa
Zeitoun Ennwader	ZEW	Chemlali Sfax * Lucques
Zeitoun Ennour	ZEN	Chemlali Sfax * Chemchali Gafsa

- The morphological characterization was performed on 40 fruits and their stones and 40 mature leaves in November (three trees for each samples) during three years (2013-2015). The qualitative characterization of olive was done as described by the IOC (1997) [26]. Concerning the distribution of qualitative characteristics we used the average of the three repetitions for each year then the average of the three years for each character to describe the morphology of the different parts for each hybrid.

The morphological follow-up included both quantitative and qualitative variables (table 2). For leaf, the shapes (LS) (length (LL)/width (LWI) ratio) were determined. For fruit, fresh average weight of the fruit (FW), shape (FS) (length (FL)/width (FWI) ratio), symmetry (FSy), position of the maximum transverse diameter (FPDM), Apex (FA), base (FB), lenticels (FLe), nipple(FN), location of start color change and maturity color were determined. Moreover, to stone we have noted the weight (SW), Shape (SS) (length (SL)/width (SWI) ratio), symmetry (SSy), position of the maximum transverse diameter (SPDM), apex (SA), base (SB), surface (SSu), distribution of groves (SG) and termination of the apex. Morphological description was compared to that of Chemlali Sfax reported in literature [27,28].

- For oil production, from each hybrid, three fruit samples were taken at the same harvest date (November) for two years 2013 and 2014 and at three harvest dates (November (N), December (D) and January (J)) for only 2014. After harvesting, about one kilogram of olive fruit of each sample were immediately transported to the laboratory and transformed into oil using an experimental oil mill following 4 steps which are milling, malaxation for 30 min at 25°C, centrifugation at 2,000xg for 3 min. and natural decantation. Oils samples were stored in bottle in the dark at 4°C until use.

The oil fatty acid composition was recorded by gas chromatography (GC) as fatty acid methyl esters (FAMEs). The FAMEs were prepared as described by the EU official method EEC/1429/92. In this study, we have focus for three acid while the palmitic acid (C16:0, a saturated fatty acid), the oleic acid (C18:1, a monounsaturated fatty acid) and the linoleic acid (18:2, a di-unsaturated fatty acid)

- Data analysis: In this work we utilized three method for analysis, the first is linear discriminant analysis (LDA), the second is hierarchical cluster analysis (HCA) and the third is principal component analysis (PCA).

For the first analysis: A table contains in columns8 character of olive and 13 character of stone and in rows 40 fruits of 5 hybrids in 3 years a total is 600 samples (40 samples per hybrid (average of three replicate) per year).This analysis was undertaken by using the SPSS 23.0.

For the other both analysis (HCA and PCA): A same data set was used, a table contains in columns morphological parameter (the means of three years) and the fatty acid composition (the mean values of three replications in each analysis) in November 2013 and three months for 2014, and in rows was the hybrids. These analyses were undertaken by using the XLSTAT 2014.5.03.

Cluster analysis was done on the squared Euclidean Distance matrix with the Unweighted Pair Group method based on Arithmetic Averages (UPGMA).

3. RESULTS

3.1. Morphological description

The qualitative parameters were described in table 2; we only mentioned the variables that have differences between hybrids. Accordingly, from the analysis of the most discriminating category variables (table5) we can extract some identification for each hybrid:

- Janouby Mwarref had lanceolate leaf shape and scabrous surface stone.
- Cultivar Chemlali Mhassen had both position of maximum transverse diameter towards apex and rugose surface of the stone.
- Cultivar Zeitoun Allyssa had elongated stone shape.

- Cultivar Zeitoun Ennwader was mainly characterized by smooth surface.
- Cultivar Zeitoun Ennour had apex termination without mucro and uniform color change.

Table 2. Most discriminanting category variables

Variable	JM	CM	ZA	ZEW	ZEN
Shape leaf	Lanceolate	Elliptic-Lanceolate	Elliptic-Lanceolate	Elliptic-Lanceolate	Elliptic-Lanceolate
Shape fruit	Ovoid	ovoid	elongated	elongated	ovoid
Apex fruit	pointed	rounded	rounded	pointed	rounded
Color change of fruit	From the apex	From the apex	From the apex	From the apex	uniform
Shape Stone	Elliptic	Elliptic	elongated	Elliptic	Elliptic
PDM stone	central	Towards apex	central	Towards apex	central
Base stone	pointed	rounded	rounded	rounded	rounded
Surface stone	scabrous	rugose	rugose	smooth	rugose

PDM: Position of Maximum Transverse Diameter

Linear discriminant analysis

The purpose of this work was to estimate if the data sets of olives and / or stones have enough information to allow the development of rules of linear discrimination among five hybrids. Consequently, the LDA classification results shown in Table 6 show the percentage of membership expected in the group of original samples, according to the olive fruit, variable data set the correct classification achieves 83.3% for the JM, 79.2% for ZEW, 73.3% for CM, 59.2% for ZA and 55.8% for ZEN. While, according to the stone variable data set the correct classification achieves 85% for the JM, 90% for ZEW, 95% for CM, 77.5% for ZA and 65.8% for ZEN. From these results, it appears that the stones are better classifiers of all hybrids.

Nonetheless, the figure 1(a) of LDA plot of olive shows that there is certain overlapping of the samples, whereas according to the figure 1(b) of the distribution of the hybrids according to the stone parameters shows that the each hybrid has a well-defined area.

Moreover, we have done the Wilks' Lambda and F-tests (table7) to evaluate the discriminating capacity of the variables. According to Paula and al. [29], the lower the Wilks'

Lambda value and the higher the F-tests, the higher the discriminant capacity of the variable. Therefore, the result of Wilks' Lambda and F-tests (table7) shows that the leaf can contribute only with the shape parameter, which shows that leaf parameters do not vary between hybrids. For the olive, four most discriminate variables are length, width, ratio length/width and the apex. For the stone five most discriminating variables are width, the ratio length/width, Shape, PDM and surface.

Table 6. Classification results of the test set (percentages of correct classifications)

Data set used in classification	Hybrids	Hybrids				
		JM	CM	ZA	ZEW	ZEN
Olive	JM	**83,3**	6,7	0	6,7	3,3
	CM	0,8	**73,3**	0	2,5	23,3
	ZA	0	0	**59,2**	12,5	28,3
	ZEW	5	8	6,7	**79,2**	8,3
	ZEN	0,8	22,5	14,2	6,7	**55,8**
Endocarp	JM	**85**	0	0	0	15
	CM	1,7	**95**	0	0	3.3
	ZA	0	0	**77.5**	0	22,5
	ZEW	0	0	9.2	**90**	0.8
	ZEN	0,8	24.2	9.2	0	**65,8**

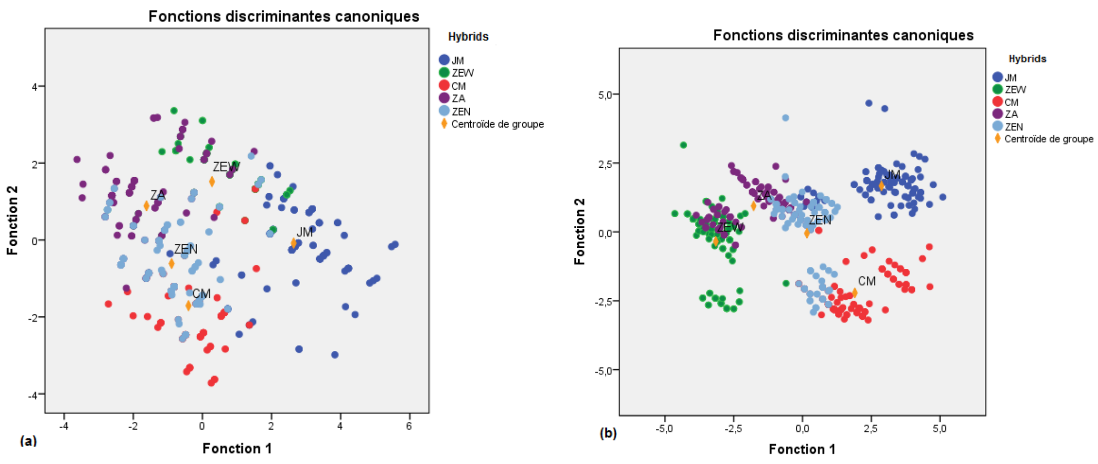

Fig.1. Linear discriminant plots of the olive (a) and stone (b) data s

Table 7. Wilks' Lambda and F-tests of group means

	Variable	Lambda de Wilks	Test-F		Variable	Lambda de Wilks	Test-F
Leaf	LL	0.65	74.763	stone	SL	0.842	27.973
	LWI	0.661	71.246		SWI	**0.652**	**79.451**
	LL/LWI	**0.475**	**153.661**		SL/SWI	**0.614**	**93.451**
	shape	**0.65**	**74.627**		**shape**	**0.697**	**64.650**
Fruit	FL	**0.559**	**117.50**		Symmetry	0.914	13.93
	FWI	**0.387**	**235.395**		Apex	0.962	5.831
	FL/FWI	**0.583**	**106.282**		Base	0.792	39.112
	Shape	0.653	78.938		**PDM**	**0.414**	**210.82**
	Symmetry	0.846	27.126		**surface**	**0.237**	**478.964**
	Apex	**0.441**	**188.765**		Apex termination	0,855	22,345
	Base	0.973	4.175				
	PDM	0.981	2.873				

3.2. Fatty acid composition analysis

In November, the acidic composition of the oils varied according to the hybrids (table 8). The main monounsaturated fatty acid, Oleic acid (18:1), has great importance because of its nutritional incidence on the oxidative stability of oils. Oleic acid is present in a wide range of concentrations from 70% (Janouby Mwarref) to 76.7% (Zeitoun Ennour). Therefore, the rate of palmitic acid (C16:0), the major saturated fatty acid in olive oil, ranged from 10.7 for Zeitoun Ennour to 14% for Zeitoun Ennwader. While, with respect to the linoleic acid (C18:2), the highest percentage was observed in Janouby Mwarref (14.5%), whereas the lowest percentage was found in Zeitoun Ennour (8.5%).

However, olive oil of the Chemlali Sfax variety characterized by a high rate of palmitic and linoleic acids and a low rate of oleic acid according to the values of Zarrouk et al [13] and the classification of IOC (1997) [26]. Whereas, olive oil is with better nutritional value when it has low palmitic acid level, moderate linoleic acid level and high oleic acid level. In our study, in comparison to the oil of the Chemlali Sfax cultivar, the new cultivars produced oils with higher rate of oleic acid and lower rate of palmitic and linoleic acids. According to IOC

(1997) norms [26], the analyzed oils were with medium to high levels for palmitic acid, high to very high levels for oleic acid and medium to high levels for linoleic acid. Therefore, a substantial oil quality improvement was done with the crossbreeding program of this variety. The important improvement in oil quality was achieved in oleic acid content, which increased up to 77% (Zeitoun Ennour), with a maximum increase of 40% in comparison with Chemlali Sfax.

Nevertheless, regarding interannual variations (Table 8), the standard deviations for oleic acid measurements were fairly small and did not exceed 2.6, giving rise to coefficients of variation smaller than 3.6%. While the standard deviations for the palmitic and linoleic acids were as high as 2.7, the corresponding coefficients of variation reached their highest levels (up to 20.4% for palmitic acid and 30.6% for linoleic acid).

Table 8. Values of fatty acids (%) in olive oils from the new cultivars in November, as compared to that of the original cultivar Chemlali Sfax

Hybrid	Palmitic acid (C16:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
Zeitoun Ennour	10.7 ± 2,2 (medium)	76.7 ± 0.4 (very high)	8.5 ± 2.6 (medium)
Janouby Mwarref	11.3 ± 1 (medium)	70 ± 1.6 (high)	14.5 ± 2.7 (high)
Zeitoun Allyssa	12.1 ± 0.2 (medium)	72.1 ± 2.6 (high)	11.2 ± 2.7 (high)
Chemlali Mhassen	11.3 ± 0.2 (medium)	71.5 ± 2.1 (high)	13.7 ± 1.4 (high)
Zeitoun Ennwader	14 ± 1.8 (high)	70.7 ± 0.4 (high)	11.2 ± 1.9 (high)
Chemlali Sfax*	19.6 (very high)	55 (low)	18.1 (very high)

*: Values reported by Zarrouk et al. [13]

Else, the fatty acid composition for all the new cultivars varied among harvesting dates (Table 9). Thus, palmitic acid had the minimum levels between 11.2% in November and 9.1%in January, while maximum levels were recorded between 15.3% in November and 13.2% in January. For oleic acid, minimum and maximum levels showed a narrower range of variation, respectively between 70.3 and 70.4% and between 76.9 and 79.2%. Minimum linoleic acid levels ranged from 6.6% (December) to 7.5% (January), while maximum values ranged from 12.7% (November) to 14.4% (January).

Table 9. Minimum and maximum fatty acid values (%) in olive oils of new cultivars for three months, as compared to that of the IOC norm

Month	Palmitic acid (C16:0)		Oleic acid (C18:1)		Linoleic acid (C18:2)	
	min	max	min	max	min	max
November	11.2	15.3	70.3	76.9	6.7	12.7
December	9.7	14.3	70.4	77.8	6.6	13.2
January	9.1	13.2	70.4	79.2	7.5	14.4
IOC (1997)	7.5	20	55	83	2.5	21

3.3. Analysis statistics

Principal Components Analysis (PCA)

The principal component analysis (PCA) revealed two major components totaling 81.51 % (figure 2) of the total variance (42.39 % and 39.12 % respectively) (figure 2).

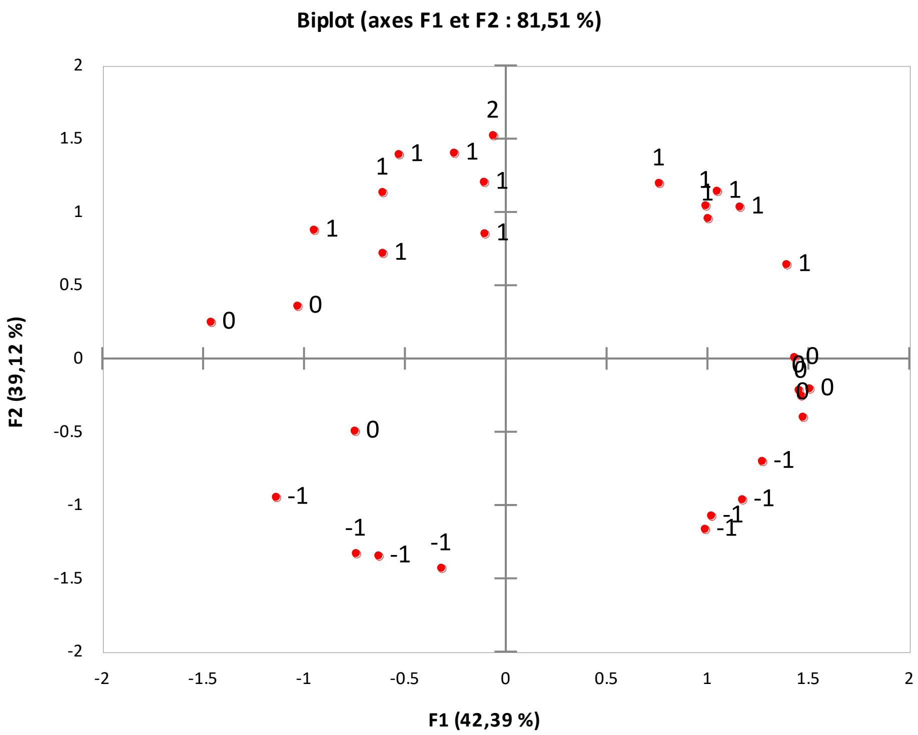

Fig.2. Principal components analysis based on the morphologic characteristics and fatty acid composition

The CP1 is correlated with morphological parameters and the second component is correlated with the oil parameters. These oil parameters are fairly stable characteristics of the different hybrids since the averages of each parameter for November, December and January are grouped together.

In fact, CP1 clearly separated the hybrids by the average fresh weight from the lowest olive weight to the highest, also by the endocarp weight from the lowest to the highest and by leaf parameters. In accordance with CP1 the hybrids are classed in two groups, the first one contains ZEW, ZA, ZEN and CM; the second contains only with JM. Moreover, CP2 separated the hybrids according to their fatty acid composition. In accordance with CP2 the hybrids are classed in three groups, the first one contains CM and ZEW characterized by the lowest palmitic acid; the second contains ZEN and ZA characterized by the lowest oleic acid; finally, the third contains only by the JM characterized by the lowest linoleic acid.

Hierarchical Cluster Analysis (HCA)

The dendrogramme resulting from the analysis of the morphological parameters and fatty acid composition is shown in figure 3. The cluster of dendrogramme composed the hybrids in the same way as the PC2 of the principal component analysis.

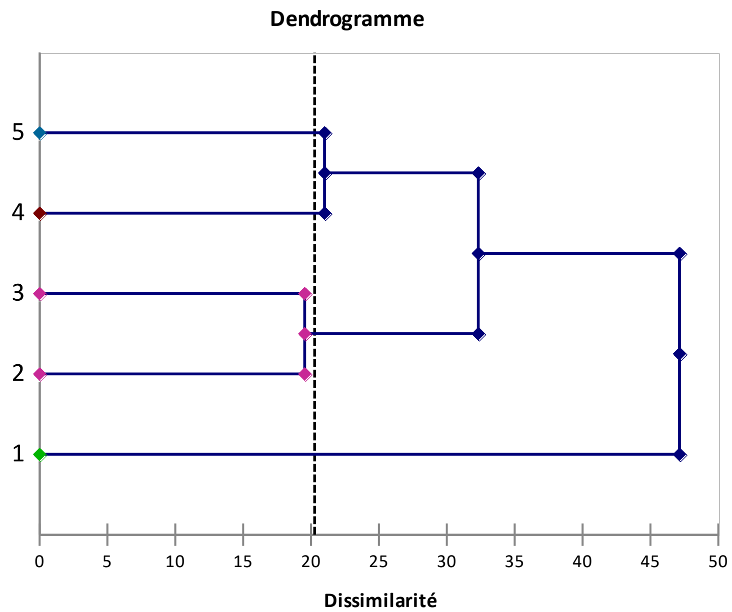

Fig.3. UPGMA dendrogram of the two hybrids based on the morphologic characteristics and fatty acid composition

The first group is composed of two progenies obtained from crossbreeding of Chemlali × Chemlali (CM) and the progenies from Chemlali × Lucques (ZEW), these hybrids are characterized by a very high level palmitic acid rate, the lowest value of leaf parameters and estone weight. The second group contains the Chemlali × Chemchali progenies (ZA and ZEN), which are characterized by a very high level oleic acid rate. The last group is constituted from JM progenies which has characterized by the high linoleic acid rate and the highest for stone end fruit weight, it is also the only hybrid with lanceolate leaf.

4. DISCUSSION

The selected cultivars were similar to Chemlali Sfax for five variables, the low number of characters (5) similar to the original variety showed a high genetic variability in these seedlings, in accordance with the results of Laaribi and al. [14] and Guellaoui and al. [30].

In fact, all the selected cultivars showed new morphological states. We revealed the appearance in seedlings of lanceolate leaf shape, medium weight, elongate, asymmetric and pointed apex for the fruit. For the stone, most of the characters of the cultivars showed new states (medium weight, elongate shape, asymmetric, maximum diameter towards apex, pointed apex, rounded base, rugose and scabrous surface and apex without mucro). These differences in morphological characters of the new cultivars were mainly due to the genetic variation since these hybrids were planted in the same agro-climatic conditions, as cited by Manai et al. [20]. Even, morphological differences were noted for two hybrids derived from the same cross (Chemlali Sfax x Chemchali Gafsa). Thus, Zeitoun Allyssa and Zeitoun Ennour were different for five morphological characters in accordance with Bartoloni and al. [31] whonoted in his work that five hybrids issued from the same crossing were clearly different from the original parents.

In comparison to the ‘Chemlali Sfax’, which had low fruit weight, 1 g as reported by Grati-Kamoun and Khlif [12], the new cultivars have olives with a higher average fresh fruit weight.In fact, most of the studied seedlings presented medium fruit weight (2 to 4 g according to IOC 1997 [26]). The increase in olive weight improves the olive oil content as reported by Bellini [3]. Also, according to Zarrouk and al. [13] it is important to study the fresh average weight of the fruit for the new cultivars if it is olive oil or table or even both uses.

According to study of Maestro-Duran and Borja-Padilla [32] in olive oil, when the ratio oleic/linoleic is greater than 7 the oil produced is a good quality this is assured when the rate of oleic acid is greater than 73 % and the rate of linoleic acid less than 10%. Chemlali Sfax showed an oleic/linoleic ratio lower than three, according to the values reported by Zarrouk et al. [13] while this ratio for the new cultivars ranged from 4.8 to 9. As revealed by many authors [33-35], hybridization is an important method to increase the genetic variability in olive fallowing the selection of new interesting genotypes. In our study, an improvement of the oil quality was noted in comparison to the original Chemlali Sfax oil quality. The significant improvement of oil quality for the selected progenies Zeitoun Allyssa and Zeitoun Ennour can be explained by the high oil quality of Chemchali Gafsa reported by Grati-Kamoun and Khlif [12].

Also, it is clear that differences were found between years for all the fatty acid concentrations of the new cultivars. Hence, it is well-known in literature that the fatty acid composition of olive oil is strongly influenced by environmental factor such as year of harvest, location, season or climatic conditions [36-39]. Nevertheless, these variations did not influence the high oil quality of the different cultivars and their superiority on the standard cultivar Chemlali Sfax.

According to these results, the variation of the different fatty acids concentrations among harvest dates was always in concordance with the International Olive Council norm [26]. Thus, the selected oils could be commercialized without any problems and will improve the Tunisian olive oil exportation. From November to January, the oleic and linoleic acid levels increased when harvest time progressed while the palmitic acid level decreased over time. These findings were not always in agreement with those reported in literature. Thus, according to previous studies on the fatty acid composition of olive oils, the rate of oleic acid increases during ripening [40,41] while other studies report decreases [42] in the rate of oleic acid with ripeness.

These tendencies facilitate the determination of an optimum harvest date of the new cultivars. In the other hand, the variation of fatty acid concentrations during the maturation of olive fruit in our study was similar to that of Chemlali Sfax reported by Grati-Kamoun and Khlif [12].

5. CONCLUSION

In this study, we have proved the interesting genetic diversity of the new progenies of olive cultivation in the morphological and oil chemical characters. It allowed us to depict five released olive cultivars that showed better fatty acid composition and specific fruit and endocarp characters when compared to Chemlali Sfax. Our study can be completed by the use of molecular markers such as microsatellites SSR which are very effective to understand the material’s genetic diversity.

6. ACKNOWLEDGEMENTS

This work has been done as a part of research project in our laboratory. We thank the Tunisian Ministry of Agriculture, Hydraulic Resources and Fisheries (especially IRESA) for financially supporting this program. The Tunisian universities of Sfax and Sousse approved our work.

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How to cite this article:
Guellaoui I, Ben Amar F, Ayadi M, Boubaker M. Characterization of five new olive (*olea europaea* l.) cultivars obtained in a crossbreeding program in Tunisia. J. Fundam. Appl. Sci., 2019, *11(3), 1318-1333.*