INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 14, No. 1, p. 204-212, 2019
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Morphological, agronomic and oil quality Studies of two new olive (*Olea europaea*. L) progenies in Tunisia

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Article published on January 31, 2019

Key words: Olive- Characterization- hybrids.

Abstract

The most cultivated olive oil variety in Tunisia "Chemlali Sfax" has low oleic acid level and high palmitic acid level. In order to resolve this problem, a genetic improvement program throught controlled crosses between Chemlali Sfax and other Tunisian and foreign varieties was started in 1993 and had generated a collection of 1200 hybrids. The aim of this study is to characterize two selected hybrids on the morphological, agronomic and oil quality plans (Hd1= Meski/Chemlali Sfax and Hd2 = selfpollinated Chemlali Sfax). Morphological characters showed a wide variability. Also, the results of tolerance to Verticillium showed that the average infection rate of Hd2 hybrid (48.46%) is significantly lower than that of the hybrid Hd1 (76.08%). Moreover, the acid composition of both hybrids was more interesting. In November, palmitic and linoleic acids concentrations for Hd2 were low (11.5 and 14.1%), while the oleic acid concentration was high (70.5%) as compared to the hybrid Hd1 (65.3%). Thus, the database analysis revealed that the plant material was clustered into two main groups according to the year. Consequently, the principal component analysis generated two principal compounds which accumulated 91.1% of the total variance.

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Introduction

The world olive growing is facing many challenges these years due to the intensification of olive cultivation and the diversification of consumer needs. These challenges are particularly in relation to oil quality and tolerance to pests and fungi. Thus, to improve these characters for olive tree, the hybridization technique has been widely adopted around the world. Olive hybridization was recently adopted in the second half of the 20th century.

In Tunisia, the crossing program was undertaken from 1993 to 1996 and interested the most cultivated varieties, Chemlali Sfax, Chetoui and Meski. In fact, the oil variety Chemlali Sfax is characterized by its adaptation to different environments and its productivity (Trigui, 1996). However, it has problems with the acidic composition of its oil: low oleic acid and the high palmitic acid (Grati-Kamoun and khlif, 2001). Therefore, the hybridization of this variety tends to select new genotypes with better acid composition while keeping the good qualities of this variety. The obtained hybrids are planted in collection in the region of Sfax and a preliminary selection of hybrids on the basis of their oleic acid composition was undertaken (IO, 2015).

Verticillium has been among the most frequently encountered fungal diseases coming from the attack by a soil fungus "Verticilluim dahliae". This disease has been reported in many countries of the Mediterranean basin (Italy, Greece, France, Turkey, Spain, Syria, Australia and USA). In North Africa, it has been reported in Algeria (Matallah *et al.*, 1996, Matallah *et al.*, 1997, Bellhacene *et al.*, 1997), Morocco (Serrhini and Zeroual, 1995) and more recently in Tunisia (Triki and *et al.,* 2006).

The best way to control Verticillium wilt in olive trees is based on an integrated approach involving the application of control measures before and after the establishment of the olive grove (Tjamos and *et al.,* 1993, Lopez and Jimenez, 1995). The fight against the verticilliose has to be made at first as a precautionary measure by the application of the agronomic best practices and also by the use of healthy and tolerant plant material. The tolerant varieties are Frantoio, Coratina and Cipressino from Italy, the American variety Oblanga and the Spanish variety Empeltre (Gratrand and Pinatel, 2011). In this paper, the main objective is to characterize two new olive plant varieties in Sfax on morphological, agronomic, pathological and oil composition plans in intensive and irrigated cultivation mode.

Materials and methods

Plant material

Hybrids of our study were planted since 2005 in an irrigated and intensive orchard at the experimental station "Ettaous" of the Olive Tree Institute (Longitude = 10°37' Est, Latitude = 34°55' North). The follow-up was done on two years 2013 (1) and 2014 (2). The used plant material consists of two hybrids and each one is represented by three trees. The crosses are Meski/Chemlali Sfax for the hybrid Hd1 and self pollinated Chemlali Sfax for the hybrid Hd2.

Methodology

The morphological characterization was performed on 40 fruits and their stones and 40 mature leaves in November and 40 inflorescences in spring (three replicates for each samples). The qualitative characterization of leaves, fruits and stones was done as described by IOC (1997). For qualitative characters, we calculated the phenotypic frequency of each class of the character as well as the diversity index of Nei by the following formula (Nei, 1978): $(2n / 2n-1) * (1-Pi^2)$. The classes of each character are compared with the phenotype of both original varieties Chemlali Sfax and Meski reported by Trigui and Msallem (2002). For the agronomic characterization, we marked three trees for each hybrid in February and for every tree, 10 one-year-old shoots distributed on the entire tree canopy were chosen.

For quantitative characters, we calculated the average followed by the standard deviation to appreciate the intra-hybrid variability and the coefficient of variation to compare the variability of the various characters. The tables include the minimum and the maximum

values. We calculated also the Pearson correlation coefficients between all quantitative characters and the statistical significance of every coefficient at the levels of 1 and 5%.

To study the behavior against V. dahliae, both hybrids as well as chek variety Chemlali Sfax were inoculated by this fungi. The reaction notation was made by two methods. The first one is based on Campbell's (Campbell and Madden, 1990) study. Sesli (Sesli and al.2010) postulate that the Area Under the Disease Progress Curve "(AUDPC) is estimated for each range according to the following formula:

AUDPC = (t / 2 * (S2 + 2S3 + ...+ 2Si + Si) / 4n * 100.

Where,

t = interval in days between observations.

Si = average severity.

4 = maximum score of disease.

n = number of observations.

The second method is based on the percentage of dead plants (PDP).

Variance analysis with one factor was carried out for each scoring method and the separation of means was performed by the Duncan test at 5% level. For fatty acid composition, three representative samples from each hybrid were handpicked at the same harvest date (November) for two years 2013 and 2014. Olive oil is produced by grinding 2.5-kg stoned olives and extracting the oil by mechanical means. The fatty acid composition of the oils was determined by GC as fatty acid methyl esters (FAMEs).

The FAMEs were prepared as described by the EU official method EEC/1429/92.

Data analysis

For each hybrid the data of the means of three replications for quantitative morphological parameter, agronomic parameters and the fatty acid composition were used to cluster analysis was conducted on the squared Euclidean Distance matrix with the Unweighted Pair Group method based on Arithmetic Averages (UPGMA). The same data were used to perform principal component analysis. These analyses were undertaken by using the XLSTAT 2014.

Results

Qualitative characters

The leaves are usually of the same type of the two hybrids (Table 1). There are no differences in the blade longitudinal curvature which is flat. The fruit morphological characterization shows a wide variability according to the character (table 2). These data have resulted in relatively low Nei indices for the nipple, the base and the apex (less than 0.19) and high for the other characters ranging between 0.24 and 0.65.

Compared with the original varieties, we noticed that the two hybrids have mostly the class of the two varieties for the apex, the base and the nipple. Nevertheless, the classes found in the remaining characters do not primarily reflect those of the two varieties since new classes have emerged such as the fruit asymmetric position which is dominant (87.5%).

Table 1. Qualitative characteristics of the leaf in comparison with the original varieties.

Character	Class	Percentage	Nei index	Chemlali Sfax	Meski
Shape	Elliptic	5	0,16	Elliptic-lanceolate	Elliptic-lanceolate
	Elliptic-lanceolate	93,75			
	Lanceolate	1,25			
Length	Medium	82,5	0,39	Medium	Medium
	Short	17,5			
	Long	0			
Width	Medium	98,75	0,03	Medium	Medium
	Narrow	1,25			
	Broad	0			
longitudinal curvature of blade	Flat	100	0	Flat	Flat
	Epinastic	0			
	Hyponastic	0			
	Helicoid	0			

Table 2. Qualitative characteristics of the fruit compared with the original varieties.

Character	Class	Percentage	Nei index	Chemlali Sfax	Meski
Shape	Elongated	8,75	0,65	Ovoid	Ovoid
	Ovoid	66,25			
	Spherical	25			
Symmetry	Symmetric	12,5	0,29	Symmetric	Symmetric
	Lightly asymmetric	0			
	Asymmetric	87,5			
Apex	Rounded	95	0,13	Rounded	Rounded
	Pointed	5			
Base	Rounded	0	0	Truncate	Truncate
	Truncate	100			
Position of maximum diameter	Medium	90	0,24	Medium	Towards apex
	Towards base	10			
	Towards apex	0			
Nipple	Absent	92,5	0,19	Absent	Present or absent
	Tenuous	7,5			
	Obvious	0			

Table 3. Qualitative characteristics of the stone compared with the original varieties.

Character	Class	Percentage	Nei index	Chemlali Sfax	Meski
Shape	Elongated	0	0,47	Elliptic	Ovoid
	Ovoid	22,5			
	Elliptic	77,5			
Symmetry	Symmetric	0	0	Symmetric	Slightly asymmetric
	Slightly asymmetric	0			
	Asymmetric	100			
Apex	Rounded	51,25	0,67	Rounded	Pointed
	Pointed	48,75			
Base	Truncate	5	0,25	Pointed	Pointed
	Rounded	90			
	Pointed	5			
Position of maximum diameter	Central	7,5	0,19	Central	Towards apex
	Towards base	0			
	Towards apex	92,5			
Mucro	Present	100	0	Present	Absent or little mucro
	Absent	0			
Surface	Rugose	40	0,64	Smooth	Rugose
	Smooth	0			
	Scabrous	50			

The morphological characterization of the stone (Fig. 1, table3) of the two hybrids shows a wide variability for most characters, compared with the two original varieties, we can notice that the dominant class is that of Chemlali Sfax for mucro and shape and that of Meski for position of maximum diameter. However, the dominant class is not that of the two varieties for symmetry, surface and base and equal to that of the two varieties for apex. The quantitative characteristic's data were presented in Table 4 and show significant variation between the two hybrids. For fruit characters, it can be noticed that the variation is relatively low since the coefficient of variation is less than 11%. However, the variation of the weight is relatively large and the coefficient of variation is around 25.74%. Thus, the average fresh fruit weight varies from 2.27 to 3.28g.

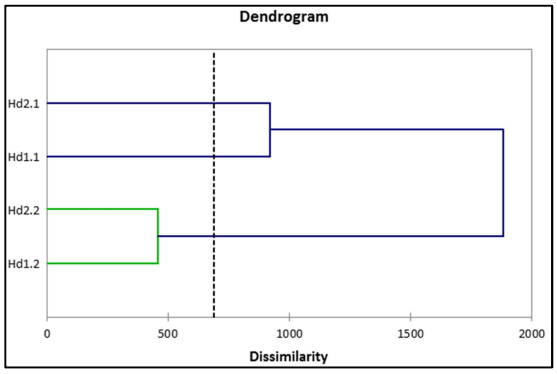

Fig.1. UPGMA dendrogram of the two hybrids for two years.

Quantitative characteristics: descriptive analysis

The stone characters have the same trend as the fruit since the length; the width and their ratio have a coefficient of variation less than 20%.

While, the stone weight vary widely from 0.29 to 0.42g with a coefficient of variation of 25.89%. The leaf characters (length, width, area and their ratio) exhibited a very small variation since the coefficient of variation does not exceed 6.19%. Similarly, the characters of the inflorescence (length and number of flowers) have a coefficient of variation less than 13%. The characters of the shoot show an important variation. Indeed, the flowering rate varies from 18.08 to 30.32% and the fruit set rate varies from 12.55 to 19.85% and the annual vegetative elongation varies from 18.47 to 36.78cm.

Table 4. Descriptive analysis of quantitative traits.

Organe	Variable	Minimum	Maximum	Moyenne	Ecart-type	CV
Fruit	Length (LF)	1,78	2,05	1,92	0,19	9,93
	Width (WF)	1,34	1,62	1,48	0,2	13,59
	Ratio length/width (LF/WF)	1,27	1,34	1,3	0,04	3,41
	fruit weight (FW)	2,27	3,28	2,78	0,71	25,74
	ratio pulp/stone (P/N)	6,73	6,74	6,74	0,01	0,1
	maturity index (IM)	2,83	2,95	2,89	0,08	2,94
Stone	Length (LS)	1,19	1,26	1,23	0,05	3,91
	Width (WS)	0,6	0,7	0,65	0,07	10,82
	Ratio length/width (LS/WS)	1,8	1,98	1,89	0,13	6,65
	weight (PMN)	0,29	0,42	0,36	0,09	25,89
Leaf	Length (LL)	5,07	5,45	5,26	0,27	5,13
	Width (WL)	1,11	1,13	1,12	0,01	1,07
	Ratio length/width (LL/WL)	4,53	4,95	4,74	0,29	6,19
	leaf size (Sfe)	4	4,24	4,12	0,17	4,12
Inflorescence	Length (LInf)	2,57	3,09	2,83	0,37	12,99
	Number of flower (NbrF/Inf)	12,39	13,68	13,03	0,92	7,03
	Length (LP)	20,92	22,77	21,84	1,31	5,99
Shoot	Flowering rate (TF)	18,08	30,32	24,2	8,65	35,75
	fruit set rate (TN)	12,55	19,85	16,2	5,16	31,86
	Abortion rate (TA)	1,4	12,99	7,19	8,2	113,98
	Rate of fruit fall (T Ch)	33,85	37,72	35,79	2,74	7,65
	Annual vegetative elongation (AVA)	18,47	36,78	27,62	12,95	46,88
Tree	Production (Pr)	16,77	21,84	19,3	3,59	18,58

The coefficient of variation for these characters is from 31 to 46%. For two other characters (rate of full down the fruits and the length of the shoot), we record a slight variation and the coefficient of variation does not exceed 8%. The production by tree of both hybrids have a small variation because it varies from 16 to 21kg with a coefficient of variation of 18.58%.

Tolerance to verticillium

The tolerance results to verticillium by both PDP and AUDPC methods are shown in Table 5. By AUDPC method, these results show that the hybrid Hd1 statistically has the same performance as the check with 76.08 and 75.23% respectively.

The hybrid Hd2 shows a significantly lower performance than Hd1 and the check with only 48.46%. With the PDP method, the same trend is recorded with high performance for the hybrid Hd1 and the check (100 and 91.67%). However, Hd2 has the lowest performance with 33.33%. Chemlali Sfax and hybrid Hd1 are ranked highly susceptible to verticillium, while the hybrid Hd2 is considered moderately sensitive.

Table 5. Average infection rate of the studied hybrids and Chemlali Sfax by both methods PDP and AUDPC.

Cultivar	AUDPC	PDP	Notation
Hd1	76.08 a	100 a	HS
Hd2	48.46 b	33.33 b	MS
Chemlali Sfax	75.23 a	91.67 a	HS

Fatty acid composition

As can be seen in Table 6, the content of the different fatty acids in November of the studied oils for the both hybrids for two years 2013 and 2014. The main monounsaturated fatty acid, Oleic acid (18:1), has great importance because of its nutritional incidence on the oxidative stability of oils.

In fact, oleic acid is present in a wide range of concentrations from 65.3% (Hd1) to 70.56% (Hd2). Whereas, the level of palmitic acid (C16:0), the major saturated fatty acid in olive oil, ranged from 11.55% for Hd2 to 17.75% for Hd1. With respect to the linoleic acid (C18:2), the highest percentage was observed in Hd2 (14.1%), whereas the lowest percentage was found in Hd1 (10.6%).

Data Analysis

Cluster analysis

The hierarchical analysis in Fig.1 highlights mainly two groups composed by year, the first group was composed by Hd1 and Hd2 for 2013 and the second contains the two hybrids of 2014. This composition shows the alternate bearing phenomenon in these hybrids.

Table 6. Values of fatty acids (%) in olive oils from the new cultivars in November (Average of two years).

Hybrids	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Hd1	17,7±1,5	2,5±0,4	2,7±0,28	65,3±1,2	10,6±0,6	0,5±0,03	0,4±0,1
Hd2	11,5±0,13	0,5±0,001	2,3±0,07	70,5±0,5	14,1±0,7	0,56±0,04	0,26±0,02

Principal components Analysis (PCA)

All collected data were submitted to the principal component analysis (Fig. 2). Two principal components were found to be significant and explained 91.1% of total variance (52.74% and 38.37% respectively). The annual vegetative elongation was positively correlated with CP2 while production was correlated negatively. At the level of fatty acid composition, the palmitic acid (satured acid) was correlated positively with CP1 and CP2 controversy with oleic and linoleic acid were correlated negatively with CP1 and CP2.

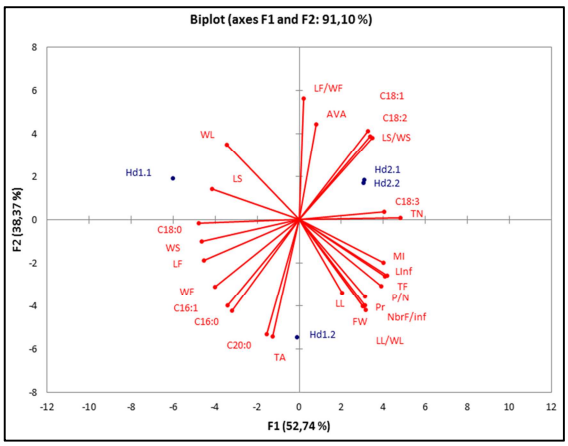

Fig. 2. Principal Components Analysis of the quantitative characters related to morphology, agronomy and fatty acid composition.

In fact, CP1 clearly separates the hybrids by the fruit set rate from the lowest rate to the highest rate, in addition the three parameters such as oleic acid, length fruit and width stone were correlated to CP1 and separate the hybrids from the highest value to the lowest value. In accordance with CP1 the hybrids are classed in 3 groups the first one contains Hd1.1, the second contains Hd1.2, finally the third contains the Hd2 for 2013 and 2014.

While CP2 separates the hybrids according to annual vegetative elongation, the abortion rate and the ratio length/width of fruit. In accordance with CP2 these hybrids were classed in two groups, the first one contains only Hd1.2 characterized by the highest value of the abortion rate and the lowest value of the both other parameters; and the second group contains Hd1.1, Hd2.1 and Hd2.2 characterized by the value controversy to the first group.

Discussion

According to this work, we found a relatively important phenotype variation for the various organs of both studied hybrids. These differences in morphological characters were mainly due to the genetic variation since these hybrids were planted in the same agro-climatic conditions. Rjiba (rjiba and al. 2010) and laaribi (Laaribi and *et al.,* 2014) confirmed that weather conditions have no influence on the morphological characters, while the studies of Besnard (Besnard and *et al.,* 2001), Hannachi (Hannachi and *et al.,* 2007) and Padula (Padula and al. 2008) estimated that the environmental and cultural conditions affected the morphological characterization. The qualitative characters of the fruit, the stone and the leaf of hybrids were closer in

several cases to the variety Chemlali Sfax and in others of the variety Meski. On the other hand, the dominant class of several characters is that of one of both varieties. However, other characters were dominant for a class which is not the one of both varieties such as the fruit shape and the stone symmetry. These results indicate that the genetic control of different characters is not the same and studies should be undertaken in this way. On the other hand, the studied hybrids were different morphological from the two original varieties which were described by Barranco (Barranco and *et al.,* 2000) and Trigui (Trigui and Msallem, 2002), similar results were reported by Laaribi (Laaribi and *et al.,* 2014).

The less important variability of the qualitative characters give indications of low Nei diversity index (<0.38). The leaf longitudinal curvature and the stone mucro characters are not practically variable between hybrids (Nei index lower than 0.03). Only the characters of fruit shape, stone shape, stone surface and apex stone shape are variable with Nei index from 0.47 to 0.67. The wide diversity in the qualitative characters was also reported by Laaribi (Laaribi and al. 2014) within the hybrids of Chemlali Sfax variety and by Belaj (Belaj and *et al.,* 2011) in the wild olive tree.

The variation of the quantitative characters has the same trend with the qualitative characters. Indeed, the characters of the fruit, the stone, the leaf and the inflorescence are weakly variable between hybrids since the coefficient of variation does not exceed 14% except for the weight of the fruit and the stone have the coefficients about 25%. So, characters related to the shape (length, width and their ratio) are less variable. On the other hand, the characters related to productivity (fruit weight, stone weight, flowering rate, fruit set rate and annual vegetative elongation) are widely variable between hybrids with a coefficient of variation higher than 20%. Therefore, we notice especially a clear improvement of the fruit weight of these two hybrids (minimum 2.27g) in comparison with Chemlali Sfax for which fruit weight is around 1g (Fourati and al. 2003, Manai and *et al.,* 2006).

A similar result was reported by Laaribi (Laaribi and *et al.,* 2014) for hybrids obtained by auto pollination, free pollination and cross pollination. The ratio P/N of Chemlali Sfax is around 5 according to Fourati (Fourati and al. 2003) and around 4.3 according to Trigui (Trigui and Msallem, 2002) while both studied hybrids have a ratio higher than the reference variable (P/N = 6.73). We can conclude that the hybridization will contribute to a significant improvement of the characters related to the oil content of the fruit (fruit weight and ratio P/N).

The production variability between both hybrids is small (coefficient of variation is equal to 18.58%). This result can be explained by the harvest variation recorded in 2013 (the production is equal to 0.2kg for Hd1 and 13.5kg for Hd2) and by the similarity in 2014 where the two hybrids have the same production with an average of 30kg per tree. The alternate bearing phenomenon is present in these two hybrids as well as in the original variety Chemlali Sfax, according to Trigui (Trigui and Msallem, 2002). Therefore, any selection in Chemlali Sfax hybrids must be oriented to lowest index of alternation bearing. The hierarchical classification of these hybrids are grouped by year rather than by hybrid. This grouping may be the result of the important variation of agronomic characters across years and the presence of an alternate bearing phenomenon.

It appears from the pathological results that the hybrid Meski/Chemlali Sfax is highly sensitive against verticillium. This behavior makes reference to that of the check Chemlali Sfax. The autopollinated hybrid is moderately susceptible. The difference of result between the check and the hybrid Hd2 seems to indicate several essential conclusions:

- The transmission of susceptibility to verticillium for Chemlali Sfax is not related to the role of this variety in the crossing (male or female).
- The character of susceptibility in these two crosses comes mainly from the check variety Chemlali Sfax.
- The character of susceptibility to the verticillium appears to be dominant in these crosses.

The sensibility observed in the autopollinated hybrid can prove that this character is obtained by a genotype in which alleles are homozygous. Indeed, autopollination of Chemlali Sfax can generate homozygous genotype for all alleles.

The oil quality was interested for study, the hybrid Hd1 obtained from the crossing between Meski and Chemlali contains the highest palmitic acid levels and the lowest oleic acid levels this may be due to the variety Meski, its oil content to 16% palmitic acid and 55% oleic acid (IO, 2008). But the hybrid Hd2 obtained by Chemlali selfed had a low palmitic acid content (11.82% in November) although the Chemlali variety is characterized by a high palmitic acid content (Zarrouk *et al*., 2009).

Although the difference of acidic composition between hybrids is of genetic order, where the cultivar remain the major variable which make diversify the characteristics of olive oil, but also the evolution of the rate of each of the different fatty acids during the maturation process olives for assuming a certain partition balance between molecular species. Indeed, there or the oil has high levels of oleic acid, it is certainly less rich in linoleic and palmitic acid and vice versa. The controlled crosses contribute to the improvement of the acidic composition of the oil; they allow us to obtain hybrids characterized by oils rich in oleic acid.

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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