PHENOTYPIC DIVERSITY OF SOME OLIVE TREE PROGENIES ISSUED FROM A TUNISIAN BREEDING PROGRAM

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Abstract

This work was done to quantify and to evaluate the distribution of the genetic diversity observed within and between olive tree seedlings issued from a Tunisian breeding program. Forty-eight 'Chemlali' olive tree seedlings which were issued from free-, self-, and cross pollination of cultivar 'Chemlali' with 'Coratina' were characterized by combining 17 quantitative and 32 qualitative traits. Principal component analysis was used for the identification of the pattern of morphological variation. Variance analysis revealed significant differences between progenies. Variation coefficients ranged from 8.36 to 32.93% for 'Chemlali' ×'Coratina' descendants, from 7.93 to 80.91% for 'Chemlali' free pollination descendants and from 10.38 to 74.88% for 'Chemlali' self pollination descendants. Shannon and Nei indices indicated also the variation between descendants within crossings. However, some seedlings showed tree, leaf, fruit and endocarp shapes and sizes which differs from the typical of 'Chemlali' cultivar. An increase of the fruit size and an improvement of the flesh to stone ratio were noted; thus the first three principal components explained 72% of the total observed variability. PC1 was mainly correlated to fruit and endocarp sizes and flesh to stone ratio; and PC2 was mainly correlated to fruit and endocarp shapes. Descendants'clustering was done according to the main discriminant parameter which is the fruit size. Most 'Chemlali' × 'Coratina' descendants were closely clustered. 'Chemlali' free and self pollination descendants were not closely grouped, but shows clear overlapping data, which suggests that these two types of pollination can induce a comparative morphological variability.

Keywords: 'Chemlali' seedlings, cluster analysis, morphological descriptors, principal component analysis, Shannon and Nei indices

Introduction

The olive tree (*Olea europaea* L.) has a great economic importance in the Mediterranean basin. A wide variability in the olive germplasm has been generated, which accounted for more than 2000 cultivars. During the last period, the olive oil has shown rapid changes, due to both technological advancement with new machinery available for harvesting the olive, and the changes in agricultural policies and market liberalization. These changes are occurring both in traditional olive-producing countries and in new countries where the growth of olive is rapidly expanding. Thus, the modern olive oil industry requires new and more competitive cultivars which can adapt better to the new trends in the growth of olive. Hence, these varieties should results to oils and olives with high and stable quality **(**Bellini et al. 2008).

In order to select new interesting genotypes, cross breeding can be used to increase the genetic variability. For this reason, breeding programs are currently being carried out in most olive-producing countries: Israel (Lavee 1990), Italy (Bellini 1993 ; Bellini etal. 2004 ; Pannelli et al. 2006 ; Bartolini etal. 2006 ; Padula et al. 2008), Spain (Rallo 1995 ; León et al. 2004 ; León et al. 2006 ; Díaz et al. 2007), Turkey (Arsel and Cirik 1994), Morocco (Charafi et al. 2007), Iran (Zeinanloo et al. 2009) and Egypt (Laz et al. 2006). However, olive breeding is known to be particularly difficult due to flower morphology, high degree of self-incompatibility and low fruit set of most cultivars, long juvenility and a high level of heterozygosis which hinders the expression of recessive genes and reduces the heritability of the desired characters. However, these reasons make the cross breeding technique long and its results poor (Bellini et al. 2003). In this context, any genetic improvement program by cross breeding will require strong efforts and a long time to obtain the next generation and its agronomical evaluation in the field. Moreover, the knowledge derived from current cross-breeding programs, in terms of parental value and heritability is still limited and not always coherent (Bellini et al. 2008).

In Tunisia, a breeding program by controlled crosses has been carried out since 1994 among the most outstanding cutivars 'Chemlali Sfax', an olive variety of high oil content which is well adapted to arid conditions. Its intrinsic qualities of vigor, productivity and oil content have contributed to its wide distribution (Trigui et al. 2006). Although its oil is appreciated for organoleptic characteristics, its low content of oleic acid is considered as a deficiency that needs to be resolved (IOOC 1997). This breeding program aimed to improve the qualities of this variety and to obtain new cultivars for a sustainable modern olive industry. Foe that, the cultivar 'Chemlali Sfax' have been crossed with both autochtonous and foreign pollinators, yielding 500000 fruits, of which only 1685 have produced viable seedlings, and among them, only 1200 have started producing. Most studies are interested in screening progenies for a high oil content and for a chemical composition more interesting than that of the cultivar 'Chemlali Sfax' , which allows the selection of some descendants which are currently under evaluation (Fourati et al. 2002; Manaï et al. 2007, 2008; Rjiba et al. 2009, 2010, 2011; Dabbou et al. 2010, 2011). However, to our knowledge few works were dedicated to the understanding of the phenotypic diversity observed within and between crossings in these progenies and how variability depends on the type of pollination (free, self or cross pollination).

Therefore, the aims of this study are (1) to study the phenotypic variability observed among the 'Chemlali' seedlings obtained from free, self and cross pollination with 'Coratina' by using 17 quantitative and 31qualitative descriptors that are related to different parts of the tree and, (2) to study the distribution of these seedlings using a principal component and a hierarchical cluster analysis realized.

Materials and methods

Plant material

The study was carried out on olive trees from 48 seedlings of 'Chemlali'. In details, 16 descendants were obtained from 'Chemlali' with free pollination, 16 from self pollinated 'Chemlali' and 16 from cross-pollination 'Chemlali' ×'Coratina' . Crosses were performed by pollination of flowers on bagged branches, and forced growth of seedlings was carried out in a greenhouse, to shorten the juvenile period. Seedlings were planted in open fields during 1997-1998 with a density of 1250 trees ha^{-1} (4m x 2m): seedlings from cross 'Chemlali' ×'Coratina' were installed in the experimental station of the Olive Institute at Sfax (Central Tunisia 34° 44′ Nord, 10° 46′ Est), and those from *'Chemlali'* in free and self pollination were installed in the Research Station of Taoues, which is about 40 km far from Sfax (34° 56′ Nord, 10° 36′ Est). The olive trees were grown in similar pedoclimatic conditions and have received the same crop management practices.

Characters evaluated

The olive seedlings were characterized using biometric and morphological parameters related to the tree, the leaf, the fruit and the stone during the year 2010. Every descendant was represented by one olive tree sample. For each tree, morphological observations were made on 40 leaves and on 40 fruits. After fruit characterization, the stone was removed and subjected to characterization; hence the morphological study integrated both quantitative and qualitative variables. For tree; the height and the circumference of both canopy and the trunk were determined. For leaf; the length, width, area and length/width ratio were determined. For fruit; the polar length, cross-sectional width, weight and length/width ratio were determined. For stone; the polar length, cross-sectional width, weight, length/width ratio, numbers of grooves and flesh to stone ratio were determined. Furthermore, other qualitative variables were also recorded according to the methodology for primary characterization of olive varieties cited by the International Olive Oil Council (IOOC, 1997) and by other morphological studies on olive cultivars (UPOV, 1985; Mehri, 1995) (tab.1).

Table 1 List of morphological descriptors and their codes and meanings.

Code	Variable	Intensity	Described in
TV	Tree vigour	(1) Weak (2) Medium (3) Strong	COI, 1997
TH	Tree habit	(1) Erected (2) Spread out (3) Falling down (4) semi dwarf	Aîachi, 2009
TCD	Canopy density	(1) Loose (2) Medium (3) Compact	COI, 1997
LBL	Leaf blade length	(1) Short (2) Medium (3) Long	COI, 1997
LBW	Leaf blade width	(1) Narrow (2) Medium (3) Wide	COI, 1997
LS	Leaf size	(1) Small (2) Medium (3) Large	COI, 1997
LSH	Leaf shape (length/width)	(1) Elliptic (2) Elliptic-lanceolate (3) Lanceolate	COI, 1997
LAA	Leaf apical angle	(1) Very acute(<45°) (2) Acute(45-60°) (3) Obtuse(>60°)	Mehri and Helali, 1995
LBA	Leaf basal angle	(1) Very acute(<45°) (2) Acute(45-60°) (3) Obtuse(>60°)	Mehri and Helali, 1995
LLC	Longitudinal curvature of the blade	(1) Hyponastic (2) Flat (3) Epinastic (4) Helicoid	COI, 1997
FW	Fruit weight	(1)Low (<2 g) (2) Medium (2–4 g) (3) High (4–6 g) (4) Very high (>6 g)	COI, 1997
FSH	Fruit shape	(1) Spherical (2) Oval (3) Longer	COI, 1997

FS	Fruit symmetry (positionA)	(1) Symmetrical (2) Lightly asymmetrical (3) Asymmetrical	COI, 1997
FD	Fruit position of maximum diameter (B position)	(1) To bottom (2) Medium (3) To top	COI, 1997
FASH	Fruit apex shape(positionA)	(1) Sharp (2) Rounded	COI, 1997
FBSH	Fruit base shape(positionA)	(1) Cut (2) Rounded	COI, 1997
FK	Knoll	(1) Absent (2) Outlined (3) Evident	COI, 1997
FPL	Presence of lenticels	(1) Little numerous (2) Numerous	COI, 1997
FDL	Dimension of lenticels	(1) Small (2) Big	COI, 1997
SW	Stone weight	(1) Low (<0.3 g) (2) Medium (0.3–0.45 g) (3) High (0.45–0.7 g) (4) Very high (>0.7 g)	COI, 1997
SSH	Stone shape (positionA)	(1) Spherical (2) Oval (3) Elliptic (4) Longer	COI, 1997
SNG	Stone number of grooves	(1) Reduced (<7) (2) Medium (7–10) (3) High (>10)	COI, 1997
SSA	Stone symmetry (positionA)	(1) Symmetrical (2) Lightly asymmetrical (3) Asymmetrical	COI, 1997
SSB	Stone symmetry (positionB)	(1) Symmetrical (2) Lightly asymmetrical	COI, 1997
SD	Stone position of maximum diameter (B position)	(1) To bottom (2) Medium (3) To top	COI, 1997
SM	Stone mucron	(1) Absent (2) Present	COI, 1997
SASH	Stone apex shape(A)	(1) Sharp (2) Rounded	COI, 1997
SBSH	Stone base shape(positionA)	(1) Cut (2) Sharp (3) Rounded	COI, 1997
SS	Stone surface	(1) Smooth (2) Rough (3) Knotty	COI, 1997
SDG	Stone distribution of grooves	(1)Uniform (2) Grouped in proximities of suture	COI, 1997
SCG	Stone Continuance of grooves	(1) Including apex (2) Excluding apex	COI, 1997

Data analysis

Descriptive statistics analysis (minimum, maximum and average values) and coefficient of variation were performed for all quantitative parameters. In addition, the analysis of variance (ANOVA), which applies a Duncan's test at a significant level of ($p<0.05$), was performed for all measured parameters in order to test the significance of variance among descendants within the same crossing. The statistical analysis was performed using the SPSS 13.0 for windows.

For qualitative parameters, the Shannon-Weaver and Nei index were computed using the phenotypic frequencies to assess the phenotypic diversity for each character on each crossing. The Shannon-Weaver diversity index is given by: $H = -\sum_{i=1}^{n} P_i \, Ln\,(P_i)$
where P_i is the proportion of descendants in the i^{th} class of an n-class character and n is the number of phenotypic classes for a character.

The diversity was also estimated by Nei a diversity index which is defined as: $H' = \frac{2n}{2n-1}(1-\sum P_i^2)$; where P_i refers to the frequency of descendants in each class for each character and n is the number of studied descendants. Mean diversity was estimated for each descendants within crossings by pooling the values of H of all the traits and dividing the sum by the total number of traits.

Moreover, the traits mean values were used to perform principal component (PCA) and cluster analyses using the SPSS 13.0 for windows and Microsoft Excel 2007. Finally, in order to group olive descendants based on morphological similarity, cluster analysis was conducted on the Squared Euclidean Distance matrix with the Unweighted Pair Group Method based on Arithmetic Averages (UPGMA).

Results

Quantitative traits analysis (Descriptive statistics and variance analysis)

All quantitative analysis studied for the 48 accessions (3 combinations), were reported in table 2. Thus, the descendants within crossings were significantly different ($p<0,001$) for all evaluated quantitative parameters (Table 2).

The variation coefficients ranged from 8.36 to 32.93% for 'Chemlali' ×'Coratina' descendants, from 7.93 to 80.91% for 'Chemlali' free pollination descendants and from 10.38 to 74.88% for 'Chemlali' free pollination descendants. The highest variation coefficient was noted for fruit weight (FW) whereas the lowest values were recorded as fruit ratio (FR) for the three types of pollination. Canopy circumference (CC), leaf area (LA), fruit weight (FW), stone weight (SW) and flesh to stone ratio (FSR) showed also important variability (CV>20%) among descendants for all crossings. Moreover, fruit length and width (FL, FWI) noted high variation coefficient between descendants obtained through self and free pollination. Also, trunk circumference (CT) and stone width (SWI) showed high variability among descendants issued from ‘Chemlali’ self pollination.

The weakest tree was noted for ‘Chemlali’ self pollination descendants which showed a height of 2.5m and a canopy circumference of 5.7m, while the most vigorous tree was observed for ‘Chemlali’ ×’Coratina’ which gave a tree height equals to 4.55m and a canopy circumference equal to 14.8 m. According to the leaf, the smallest one was noted among the ‘Chemlali’ self pollination progenies (1.72cm^2) whereas the largest one was noted on ‘Chemlali’ ×’Coratina’ (8.14cm^2). The smallest and the greatest olive fruit were recorded among free pollination seedlings; hence fruit weight (FW) ranges from 0.77 to 8.05 g. For the fruit length and width, they ranged from 13.21 to 28.94 mm and from 9.38 to 23.52 mm, respectively. The smallest stone was recorded among ‘Chemlali’ ×’Coratina’ descendants (SW=0.16g) and the greatest one was recorded on self pollination (SW=1.02g). The ratio between fruit flesh and stone varied from 2.91 (‘Chemlali’ ×Coratine descendants) to 8.80 (‘Chemlali’ self pollination descendants).

Table 2 Descriptive statistical analysis of 17 quantitative morphometric traits evaluated for 48 olive tree seedlings of ‘Chemlali’ (Values underlined are the upper and lower extremes for each trait).

		Tree			Leaf				Fruit				Stone					
		TC	CC	TH	LL	LWI	LA	LR	FW	FL	FWI	FR	SW	SL	SWI	SR	SG	FSR
Ch *Cor	Min	0,35	7,30	2,90	3,96	0,91	2,28	4,37	0,81	14,10	9,56	1,26	0,16	10,88	5,34	1,91	6,40	2,91
	Max	0,60	14,80	4,55	8,39	1,65	8,14	7,09	2,35	20,41	14,16	1,82	0,42	16,44	7,39	2,49	11,40	6,34
	Mean	0,47	10,16	3,99	6,79	1,30	5,71	5,26	1,34	17,27	11,49	1,51	0,26	13,26	6,06	2,19	8,35	4,22
	SD	0,07	2,13	0,44	1,12	0,18	1,49	0,68	0,44	2,08	1,36	0,13	0,08	1,57	0,65	0,20	1,47	0,91
	CV	**14,64**	**20,92**	**10,98**	**16,54**	**13,75**	**26,14**	**12,94**	**32,93**	**12,02**	**11,82**	**8,36**	**30,91**	**11,81**	**10,64**	**9,00**	**17,62**	**21,48**
	F value	-	-	-	133,78	73,28	100,94	65,02	176,02	173,63	160,49	120,57	186,09	182,85	180,13	130,54	94,52	-
	Sig level	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	-
Ch F	Min	0,32	6,30	3,00	4,08	0,79	2,15	3,88	0,77	13,21	9,38	1,12	0,17	10,44	5,59	1,56	6,68	3,11
	Max	0,55	12,60	4,30	6,39	1,24	4,44	6,52	8,05	28,94	23,52	1,49	0,97	18,91	10,21	2,25	11,48	8,80
	Mean	0,43	8,83	3,54	5,06	1,02	3,17	5,07	2,78	19,35	14,59	1,34	0,39	13,96	7,24	1,94	8,54	5,69
	SD	0,06	1,77	0,39	0,66	0,14	0,76	0,70	2,25	4,45	3,76	0,11	0,23	2,43	1,33	0,19	1,38	1,72
	CV	**14,89**	**20,02**	**11,15**	**13,10**	**13,96**	**23,79**	**13,88**	**80,91**	**23,02**	**25,79**	**7,93**	**58,22**	**17,42**	**18,34**	**9,91**	**16,18**	**30,26**
	F value	-	-	-	93,37	73,05	95,78	68,80	378,48	293,27	444,37	46,80	312,66	244,62	304,11	68,19	64,40	-
	Sig level	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	-
Ch S	Min	0,22	5,70	2,50	3,59	0,76	1,72	3,61	1,09	13,53	10,78	1,12	0,17	9,15	5,78	1,43	6,63	3,60
	Max	0,65	10,70	4,20	5,87	1,15	4,49	6,91	7,94	28,58	22,74	1,57	1,02	18,44	11,30	2,37	10,90	8,41
	Mean	0,41	7,54	3,45	4,89	0,97	2,96	5,13	2,48	18,42	14,42	1,29	0,36	12,90	7,28	1,79	8,30	5,64
	SD	0,12	1,55	0,48	0,72	0,10	0,68	0,84	1,86	4,07	3,30	0,13	0,22	2,46	1,48	0,25	1,15	1,34
	CV	**28,29**	**20,55**	**13,81**	**14,78**	**10,73**	**22,95**	**16,44**	**74,88**	**22,11**	**22,89**	**10,38**	**61,48**	**19,03**	**20,31**	**14,19**	**13,88**	**23,81**
	F value	-	-	-	109,39	52,91	82,91	83,98	444,27	409,70	647,66	178,56	263,04	327,50	542,22	239,25	49,46	-
	Sig level	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	-

TC: trunk circumference (m), CC: canopy circumference (m), TH: tree height (m), LL: leaf length (cm), LWI: leaf width (cm), LA: leaf area (cm^2), LR: leaf (length/width) ratio, FW: fruit weight (g), FL: fruit length (mm), FWI: fruit width (mm), FR: fruit (length/width) ratio, SW: stone weight (g), SL: stone length (mm), SWI: stone width (mm), SR: stone (length/width) ratio, SG: number of grooves, FSR: fruit flesh to stone ratio.
SD: standard deviation, CV: variation coefficient (%), Sig Level: significance level, *** significant at 1‰ level.

Qualitative traits analysis (Diversity's index)

Shannon index and Nei index, as a measure of morphological trait diversity across 'Chemlali' seedlings, were calculated for all qualitative parameters and different type pollinations were presented in Table 3.

Trees had mostly medium vigour (TV), erected-spead out habit (TH) and medium canopy density (TCD). Semi dwarf habit was rarely presented on self pollination descendants (13%). Weak vigour was mainly observed on 'Chemlali' self pollination descendants while strong vigour were noted on 'Chemlali' ×'Coratina' descendants by which the percentage of each class is equal to 25% and 44%, respectively.

Leaves were mostly with elliptic-lanceolate shape (LSH), flat longitudinal curvature (LLC) and acute apical angle (LAA). Leaves of 'Chemlali' ×'Coratina' descendants were characterized by long length (50 %), medium width (88 %), medium size (63 %) and acute basal angle (88 %). However, 'Chemlali' free and self pollination descendants presented essentially small, short and narrow leaves with very acute basal angle.

Most Fruits of 'Chemlali' seedlings were slightly asymmetrical (SSA), truncate base's shape (FBSH) with low weight (FW), central maximum diameter (FD), numerous (FPL) and small lenticels (FDL) but without mamelon (FM). Fruits issued from 'Chemlali' × 'Coratina', were long shaped (63%) with sharp apex shape (75%). However, fruits issued from 'Chemlali' free pollination, were oval shaped (75%) with rounded apex shape (75%). Moreover, fruits of 'Chemlali' self pollination, were spherical shaped (50%).

Most stones were slightly asymmetrical (SSA) and sharp base's shape (SBSH). They noted low weight (SW), medium number of grooves (SNG) which were continuous in apex (SCG) and with mucron (SM). 'Chemlali' ×'Coratina' and 'Chemlali' free pollination fruits were elliptic shaped for 56% and 69% of the total, respectively. However, 'Chemlali' self pollination descendants, noted ovoid stone (63%). Also, 'Chemlali' descendants issued from free and self pollination, had stone with sharp apex shape (SASH), a central maximum diameter (SMD), a rough surface (SS) and a uniform grooves (SDG). 'Chemlali' ×'Coratina' descendants had mainly stone with round apex shape (63%), maximum diameter toward apex (63%), smooth surface (63%) and grouped grooves (65%).

Furthermore, both Shannon and Nei indices showed similar trends of phenotypic diversity. High correlation coefficient was noted between these two diversity indices; hence R2 was equal to 0.91 (Fig.1). The highest diversity index values were noted on stone weight (SW) for 'Chemlali' free and self pollination descendants. Average Shannon and Nei diversity index was equal to 0.54 and 0.34, respectively for all types of pollination.

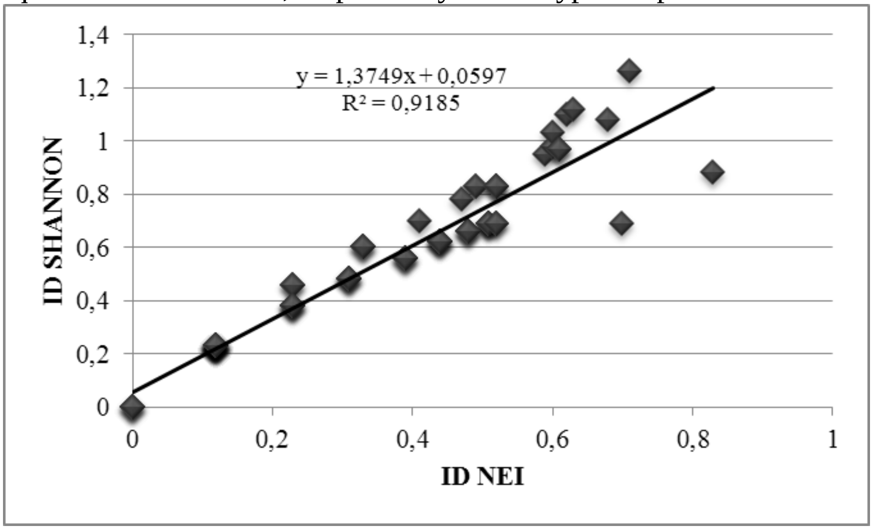

Figure 1 Plot illustrating the correlation between Shannon and Nei indices assessed via correspondence analysis of 48 'Chemlali' olive seedlings on 31 qualitative traits.

The most discriminative descriptors showed values of diversity higher than 0,85. Leaf blade length (LBL), stone base shape (SBSH), tree habit (TH), leaf size (LS), fruit position of maximum diameter (FD) and stone diameter (SD) were the most discriminative traits on 'Chemlali' ×'Coratina' descendants. Also, Stone weight (SW), fruit weight (FW), stone diameter (SD), canopy density (TCD) and leaf blade length (LBL) were the most determinant descriptors on 'Chemlali' free pollination descendants. Finally, stone weight (SW), tree habit (TH), fruit shape (FSH), fruit weight (FW), stone shape (SSH) and stone base shape (SBSH) have a great importance on 'Chemlali' self pollination descendants.

Table 3 Number and percentage of observed trait states, Shannon and Nei indices of 31 qualitative morphological traits used in the analysis of 48 'Chemlali' olive seedlings.

			Ch×Cor			***Ch L***			***Ch A***		
	Trait	**Traits states**	Percent	I Nei	I Shan	Percent	I Nei	I Shan	Percent	I Nei	I Shan
Tree	**TV**	Weak	0			6			25		
		Medium	56			81			75		
		Strong	44	0,51	0,69	13	0,33	0,60	0	0,39	0,56
	TH	Erected	31			25			50		
		Spread out	56			69			38		
		Falling down	13			6			0		

		Semidwarf	0	0,59	0,95	0	0,47	0,78	13	0,61	0,97
	TCD	Loose	0			19			13		
		Medium	94			56			69		
		Compact	6	0,12	0,23	25	0,60	0,98	19	0,49	0,83
Leaf	LBL	Short	6			50			56		
		Medium	44			50			44		
		Long	50	0,83	0,88	0	0,52	0,69	0	0,51	0,69
	LBW	Narrow	6			44			56		
		Medium	88			56			44		
		Wide	6	0,23	0,46	0	0,51	0,69	0	0,51	0,69
	LS	Small	6			88			94		
		Medium	63			13			6		
		Large	31	0,52	0,83	0	0,23	0,38	0	0,12	0,23
	LSH	Elliptic	0			6			6		
		Elliptic-lanceolate	94			81			81		
		Lanceolate	6	0,12	0,23	13	0,33	0,60	13	0,33	0,60
	LAA	Very acute	0			31			19		
		Acute	94			69			81		
		Obtuse	6	0,12	0,23	0	0,44	0,62	0	0,31	0,48
	LBA	Very acute	13			50			69		
		Acute	88			50			31		
		Obtuse	0	0,23	0,38	0	0,52	0,69	0	0,44	0,62
	LLC	Hyponastic	0			81			0		
		Flat	94			19			56		
		Epinastic	6			0			44		
		Helicoid	0	0,12	0,23	0	0,31	0,48	0	0,70	0,69
Fruit	**FW**	Low	94			56			56		
		Medium	6			25			31		
		High	0			6			6		
		Very high	0	0,12	0,23	13	0,62	1,10	6	0,60	1,03
	FSH	Spherical	0			19			50		
		Oval	38			75			38		
		Longer	63	0,48	0,66	6	0,41	0,70	13	0,61	0,97
	FS	Symmetrical	0			6			0		
		Lightly asymmetrical	88			88			94		
		Asymmetrical	13	0,23	0,38	6	0,23	0,46	6	0,12	0,23
	FD	To bottom	6			6			0		
		Medium	63			94			94		
		To top	31	0,52	0,83		0,12	0,23	6	0,12	0,23
	FASH	Sharp	75			25			31		
		Rounded	25	0,39	0,56	75	0,39	0,56	69	0,44	0,62
	FBSH	Cut	100			94			100		
		Low	0	0,00	0,00	6	0,12	0,23	0	0,00	0,00
	FM	Absent	81			94			94		
		Outlined	19			0			6		
		Evident	0	0,31	0,48	6	0,12	0,23	0	0,12	0,23
	FPL	Little numerous	6			6			13		
		Numerous	94	0,12	0,23	94	0,12	0,23	88	0,23	0,38
	FDL	Small	94			100			94		
		Big	6	0,12	0,23	0	0,00	0,00	6	0,12	0,23
Stone	**SW**	Low	63			38			56		
		Medium	38			38			19		
		High	0			13			19		
		Very high	0	0,48	0,66	13	0,71	1,26	6	0,63	1,12

	SSH	Spherical	0			0			0		
		Oval	0			19			63		
		Elliptic	56			69			31		
		Longer	44	0,51	0,69	13	0,49	0,83	6	0,52	0,83
	SNG	Reduced	6			0			0		
		Medium	81			88			94		
		High	13	0,33	0,60	13	0,23	0,38	6	0,12	0,23
	SSA	Symmetrical	6			0			19		
		Lightly asymmetrical	88			100			81		
		Asymmetrical	6	0,23	0,46	0	0,00	0,00	0	0,31	0,48
	SSB	Lightly asymmetrical	81			94			100		
		Symmetrical	19	0,31	0,48	6	0,12	0,23	0	0,00	0,00
	SD	Excluding apex	6			13			0		
		Medium	31			50			56		
		To top	63	0,52	0,83	38	0,61	0,97	44	0,51	0,69
	SM	Absent	13			31			25		
		Present	88	0,23	0,38	69	0,44	0,62	75	0,39	0,56
	SASH	Sharp	38			69			69		
		Rounded	63	0,48	0,66	31	0,44	0,62	31	0,44	0,62
	SBSH	Cut	25			0			0		
		Sharp	38			63			50		
		Rounded	38	0,68	1,08	38	0,48	0,66	50	0,52	0,69
	SS	Smooth	63			0			0		
		Rough	38			81			94		
		Knotty	0	0,48	0,66	19	0,31	0,48	6	0,12	0,23
	SDG	Uniform	44			94			69		
		Grouped in proximities of suture	56	0,51	0,69	6	0,12	0,23	31	0,44	0,62
	SCG	Including apex	94			75			88		
		To bottom	6	0,12	0,23	25	0,39	0,56	13	0,23	0,38
				0,34	0,52		0,35	0,55		0,35	0,54

TV: Tree vigour; TH: Tree habit; TCD: Canopy density; LBL: Leaf blade length; LBW: Leaf blade width; LS: Leaf size; LSH: Leaf shape (length/width); LAA: Leaf apical angle; LBA: Leaf basal angle; LLC: Longitudinal curvature of the blade; FW: Fruit weight; FSH: Fruit shape; FS: Fruit symmetry (positionA); FD: Fruit position of maximum; Diameter (B position); FASH: Fruit apex shape (positionA); FBSH: Fruit base shape (positionA); FK: Knoll; FPL: Presence of lenticels; FDL: Dimension of lenticels; SW: Stone weight; SSH:Stone shape (positionA); SNG: Stone number of grooves; SSA: Stone symmetry (positionA); SSB: Stone symmetry (positionB); Stone position of maximum; SD: Diameter (B position); SM: Stone mucron; SASH: Stone apex shape (A); SBSH: Stone base shape (positionA); SS: Stone surface; SDG: Stone distribution of grooves; SCG: Stone Continuance of grooves.

Principal components Analysis (PCA)

PCA was performed to compare morphological characters (descriptors) and to study the inter-relationships between all the studied 'Chemlali' seedlings. The first three principal compounds (PC1, PC2 and PC3) accounted for 44, 20 and 9% of the total variance respectively, accumulating 72% of variability (Table 4).

The first PC showed that width (FWI), weight (FW) of fruit and stone (SWI, SW) and flesh to stone ratio (FSR) had a more important contribution.

The PC1 was also correlated negatively with leaf size (LA, LL, and LWI) and tree parameters (TH, CC, TC). The inertia which accounted for the second PC was due to the contribution of fruit and stone shape (FR, SF) to stone length (SL). Leaf ratio (LR) and stone grooves (SG) were not used to distinguish descendants due to their low contribution to the total inertia (Table 4).

Table 4 Estimation of variance, accumulated variances and weighting coefficients (autovectors) of the first three principal components for 17 quantitative characters evaluated on 48 'Chemlali' olive descendants

	PC1	PC2	PC3
% Variance	43,80	19,74	8,54
% Accumulation variation	43,80	63,54	72,08
FWI	0,93	0,29	0,21
SWI	0,89	0,33	0,11
FW	0,88	0,41	0,16
SW	0,82	0,53	0,02
FL	0,76	0,63	-0,05
FSR	0,73	-0,12	0,36
LA	-0,71	0,47	0,38
LL	-0,69	0,45	0,40
LWI	-0,64	0,46	0,34
TH	-0,63	0,32	0,10
CC	-0,58	0,37	0,23
SR	-0,56	0,53	-0,52
TC	-0,43	0,34	0,28
SG	0,17	0,17	0,04
LR	-0,16	0,10	0,15
SL	0,49	0,79	-0,32
FR	-0,57	0,60	-0,53

FWI: fruit width (mm),SWI: stone width (mm),FW: fruit weight (g),SW: stone weight (g),FL: fruit length (mm),FSR: fruit flesh to stone ratio,LA: leaf area (cm^2),LL: leaf length (cm),LWI: leaf width (cm),TH: tree height (m),CC: canopy circumference (m),SR: stone (length/width) ratio,TC: trunk circumference (m),SG: number of grooves,LR: leaf (length/width) ratio,SL: stone length (mm),FR: fruit (length/width) ratio.

Figure 2 shows a projection of the different seedlings on the plan determined by the first two principal components. No clear group was found according to the genetic origin (type of pollination). However, the 'Chemlali' ×'Coratina' descendants were represented on the left part which presents a considerable percentage of similarity and which appears as a homogeneous group. 'Chemlali' free (CF) and self pollination seedlings (CS) were distributed and overlapped randomly on the center of the plan. CF2, CF6, CF9 and CS6, CS10, CS11 were located on the left part of the plane which was distinguished from all the rest.

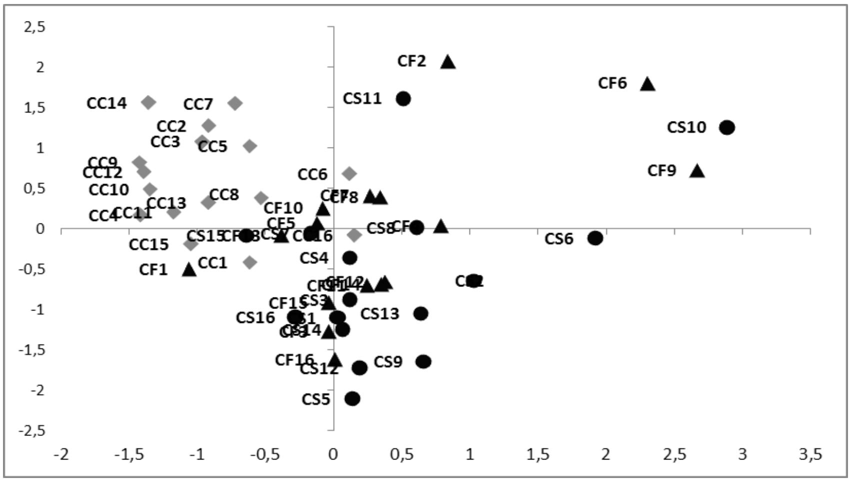

Figure 2 Plot illustrating the relationships among 48 'Chemlali' olive seedlings assessed by 17 quantitative morphological traits

Hierarchical cluster analysis

The dendrogram obtained from the UPGMA cluster (Unweighted Pair Group Method Arithmetic Average) carried out on the 17 quantitative traits and 32 qualitative traits, is shown in Fig. 3. The 48 'Chemlali' olive descendants were clustered into six main groups mainly according to their fruit size.

The first group consist exclusively of CF9, a 'Chemlali' free pollination seedling, which was characterized with the highest fruit weight (8.05g), flesh to stone ratio (8.8) and fruit width (23.2mm). It presented a symmetric spherical fruit with around base, a short and narrow leaf with very acute apical and basal angles. However, this tree had weak vigour.

The second group includes CS6, CS10 (self pollination seedlings) and CF6 (free pollination seedling) which shows very high fruit weight (>6g), weak tree vigour and short leaf. CF6 had the longest fruit (28.94mm) and stone (18.91mm). It had also an elliptical leaf. CS6 presented the lowest canopy circumference (5.7m). CS10 was characterized by a semidwarf tree habit with the lowest tree height (2.5m). It had also the highest weight and width stone (1.02g and 11.30mm respectively).

The third group exclusively contains CF2, a 'Chemlali' free pollination descendant, which presented a vigorous and erected tree, medium leaf size, and a high fruit size with an evident mamelon. It had also the highest numbers of grooves (11).

The fourth group is composed only of CS11, obtained through 'Chemlali' self pollination crossing, which featured medium vigorous and erected tree, medium leaf size, long fruit with high size, sharp apex shape and little numerous and big lenticels. Hence, its stone was long.

The fifth group grouped 21 seedlings (1 ‘Chemlali’ ×’Coratina’, 11 ‘Chemlali’ self pollination, 9 ‘Chemlali’ free pollination) presenting medium vigour tree, small and elliptic-lanceolate leaf and oval-spherical fruit with low weight.

The last group contained 21 seedlings of which 15 were obtained from ‘Chemlali’ ×’Coratina’ crossbreeding, 2 from ‘Chemlali’ self crossing and 4 from ‘Chemlali’ free crossing. They were characterized with vigorous tree, large and long leaf, small and long fruit with sharp apex shape and small stone with smooth surface.

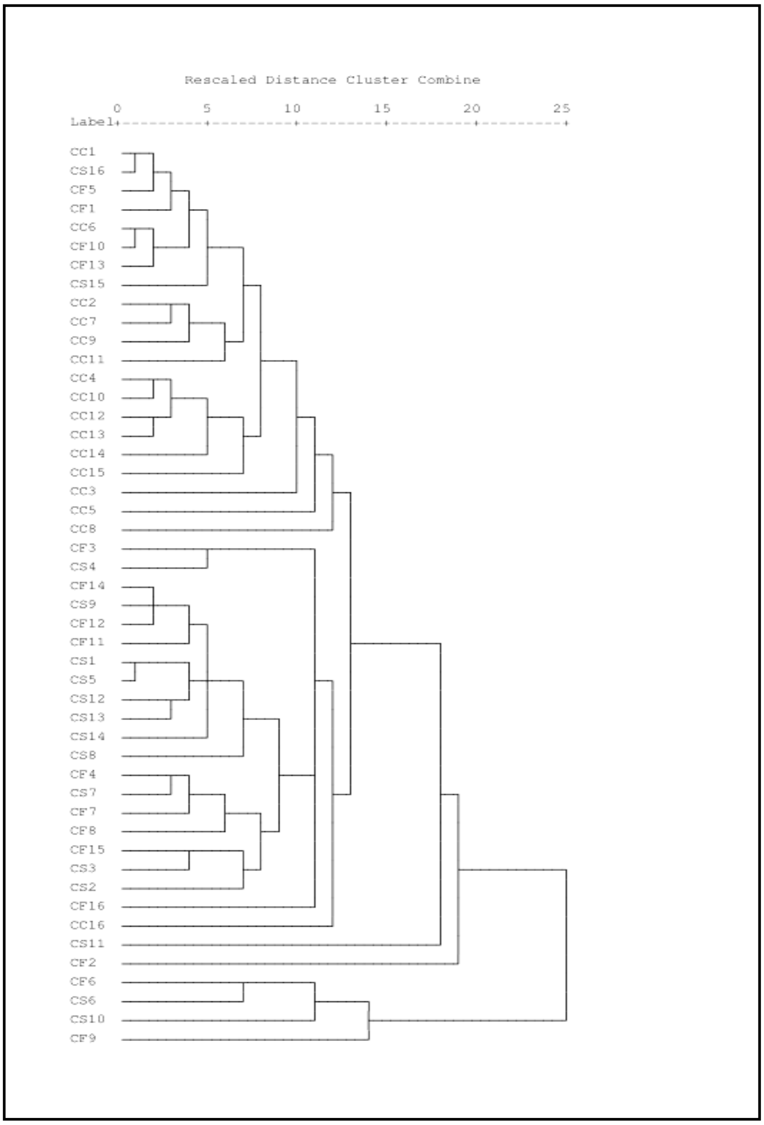

Figure 3 UPGMA dendrogram based on quantitative and qualitative morphological data of 48 ‘Chemlali’ olive seedlings

Discussion

Morphological variability in the 'Chemlali' olive tree seedlings

Morphological characteristics of the 'Chemlali' olive tree seedlings showed a high genetic variability. Most descendants within crossings noted highly significant differences. These differences in morphological characters were due mainly to genetic variation, as all seedlings within crossings had the same agro-climatic conditions (Rjiba et al. 2010). The effect of environmental conditions and agronomical factors on the morphological traits, as cited by many authors Besnard et al. (2001), Hannachi et al. (2007) and Padula et al. (2008), was not important in our study. It can be concluded that the genotype seemed to influence the morphological characters of descendants which is in agreement with the works of Bellini (1993), Cantini et al. (1999), Bartolini et al. (2006) and León et al. (2006).

High variability noted for the morphological characters revealed in the current study, is in accordance with the previous studies carried out in 'Chemlali' olive tree seedlings using morpho-agronomical (Trigui et al. 2006), architectural (Aïachi and Trigui 2001) and chemical characteristics (Fourati et al. 2002; Manaï et al. 2007, 2008; Rjiba et al. 2009, 2010, 2011; Dabbou et al. 2010, 2011). Similar variability was also observed in other olive cross breeding programs (Lavee 1990; Bellini 1993; Fontanazza et al. 1999; León et al. 2004; León et al.2006; Pannelli et al. 2006; Bartolini etal. 2006; Padula et al. 2008). As expected, crossbreeding is an efficient technique to increase the genetic variability in olive for the selection of new interesting genotypes (Ripa et al. 2006; Ripa et al. 2008; Lavee 2010).

Biometric indices should always be accompanied by a detailed morphological description of the different part of olive tree following the UPOV and COI method, like those noted by Bartolini et al. (1998), Barranco et al. (2000) and Rotendi et al. (2003). In this study, both quantitative and qualitative traits of tree, leaf, fruit and endocarp were analyzed. Concerning quantitative traits, the highest variation coefficients were noted for fruit size (FW) on all crossings. Endocarp size (EW), flesh to stone ratio (FSR), leaf size (LA) and canopy circumference (CC) noted also important variation coefficients. Variation coefficients recorded on the studied 'Chemlali' seedlings are similar or even slightly higher than that which was previously reported in olive cultivars collection (Trentacoste and Puertas 2011) and wild olives (Belaj et al. 2011) using morphometic traits; however, the highest one was noted on fruits by the same authors. Concerning qualitative traits, Shannon-Weaver and Nei indexes indicated variation between descendants within crossings. A similar study based on 23 qualitative traits in 48 wild olives had noted comparable diversity index (Belaj et al. 2011).

In our study, some seedlings showed tree, leaf, fruit and endocarp shapes and sizes which differed from the typical of 'Chemlali' cultivar, described by Barranco et al. (2000) and Trigui and Msallem (2002). Bartoloni et al. (2006) noted that five hybrids issued from the same crossing clearly differed from the original parents.

An increase of the fruit size and the improvement of the flesh to stone ratio, were noted in comparison to the small size of ‘Chemlali’ fruit. In fact, more than half of the studied seedlings, especially those issued from self and free pollination, presented a medium with a high and very high size. These can be considered as two important criteria of the improvement of olive oil content.

Discrimination and identification of ‘Chemlali’ olive tree seedlings

The first three principal components accounted for 72% of the total variance which was consistent with the high morphological variability observed in the studied descendants. This percentage was relatively higher compared to those reported by Cantini et al. (1999) and Trentacoste et al. (2011). The principal component analysis performed on morphological traits, was useful for identifying the most important traits associated with variations among the olive tree seedlings. The most important discriminating traits were fruit and stone widths, fruit and stone weights, fruit length, flesh to stone ratio, leaf area, leaf length, leaf width, tree height, canopy circumference, stone ratio, trunk circumference, number of grooves, leaf ratio, stone length and fruit ratio. Fruit and endocarp sizes seemed to be the most discriminating traits. These results are in agreement with those reported previously by several researchers on olive tree (Bellini 1993; Idrissi and Ouazzani 2004; Pinheiro and Esteves de Sliva 2005; Bartollini et al. 2006; Hannachi et al. 2007; Poljuha et al. 2008).

The first principal component was mainly correlated to fruit and endocarp size and flesh to stone ratio, whereas the second principal component was mainly correlated to fruit and endocarp shape. The same results were reported by Trendacoste and Puertas (2011) who studied 61 accessions of the olive germplasm collection in Argentina using 21 morpho-phenological and agronomic characteristics.

Correlations between quantitative traits showed a strong association among the fruit and stone dimensions, as previously reported in the studies of wild (Hannachi et al. 2008) and cultivated olive trees (Cantini et al. 1999; Belaj et al. 2011). Furthermore, negative correlations were noted between the tree parameters (trunk and canopy circumference, tree height) and fruit parameters (fruit weight, fruit width, and fruit length). These results indicated the possibility to select descendants by presenting a tree with medium and compact vigor and a tree which had big fruit. Therefore, these selections can be interesting for expansion of intensification and mechanization of olive.

Genetic relationships

Descendants' clustering has been done accordingly, mainly to the fruit size suggesting the great discriminating power of this character, which classified seedlings in six groups. This result corroborates with other studies carried out in other classic cultivars based on both morphometric characters (Lansari and Tahri Hassani 1996; Idrissi and Ouazzani 2004) and on molecular markers (Hagidimitriou et al. 2005; Marra et al. 2006; Grati Kamoun et al. 2006; Taamalli et al. 2006; Gregoriou 2006) in which clustering cultivars was principally according to fruit size.

Principal component analysis as well as cluster revealed that morphological characteristics were able to discriminate between descendants with different genetic origins (genetic combinations). Indeed, most olive seedlings obtained through 'Chemlali' × 'Coratina' pollination were closely clustered. Thus, a clustering of olive seedlings with similar genetic combination has also been observed in other studies performed on morphological, chemical and molecular descriptors (Díaz et al. 2007; Rjiba et al. 2010).

However, descendants obtained through free and self pollination of 'Chemlali' were not closely grouped, showing clear overlapping data. These results confirms a high variability already mentioned by descriptive analysis among these descendants suggesting that free and self pollination can induce comparative morphological variability. It can be explained both by the high heterozygosity of olive and the high chromosome number of the species (Bellini et al. 2008) or by the high heterogeneity of the polyclonal cultivar 'Chemlali' (Fendri et al. 2010). It can also be explained by the possibility of foreign pollen pollution, especially in the case of selfings descendants which presents characteristics widely different from 'Chemlali'. The same aspect was already mentioned for selfings of 'Picholine marocaine' (Charafi et al. 2007) and 'Picual', 'Arbequina' and 'Frantoio' (Díaz et al. 2007). Hence, this can be tested by molecular markers. Furthermore, descendants presenting big size of fruit unlike the typical small size of 'Chemlali' can confirm the low fruit size which is heritably reported by Zeinanloo et al. (2009).

Conclusion

In conclusion, this present study proves the interesting genetic diversity of the studied progenies and underlines the necessity to extend this research with more descriptors for higher number of descendants in order to confirm these data and facilitate future selections. This study can be completed by the use of molecular markers such as microsatellites that are very suitable to reach a better understanding of the material's genetic diversity.

References:

Arsel H and Cirik N, 1994. Aperçu sur les activités d'amélioration de l'olivier en Turquie. Olivae 52 : 25-27

Barranco D, Cimato A, Fiorino P, Rallo L, Touzani A, Caatanedo C, Serafini F and Trijillo I , 2000.World catalogue of olive varieties. International Olive Council, Madrid

Bartolini G, Prevost G, Messeri C, Carignani G, Menini UG, 1998.Olive germplasm. Cultivars and World-Wide collections, FAO, Rome

Bartolini S, Andreini L, Guerriero R and Gentili M, 2006. Improvement of the quality of table olives in Tuscany through cross-breeding and selection: preliminary results of Leccino x Konservolia hybrids. In: Proceedings Second International Seminar Olivebioteq 2006- November 5th-10th-Mazara del Vallo, Marsala (Italy). Volume 1: 143-146

Belaj A, León L, Satovic Z, De La Rosa R, 2011.Variabilty of wild olives (*Olea europeae* subsp. Europaea var. sylvestris) analyzed by agro-morphological traits and SSR markers. Scientia Horticulturae.doi: 10.1016/j.scienta.2011.04.025

Bellini E,1993. Variabilité génétique et héritabilité de certains caractères chez des plants de semis d'olivier issus de croisement. Olivae 49: 21-34

Bellini E, Giordani E and Nin S, 2003.Genetica e Miglioramento.In: Olea. Trattato di olivicoltura (Fiorino P) Edagricole, Bologna 116-129

Bellini E, Giordani E, Parlatti M V, 2004. Arno, Tevere et Basento : nouveaux cultivars d'olivier obtenus par croisements. Olivae 102:42-46

Bellini E, Giordani E and Rosti A, 2008. Genetic improvement of olive from clonal selection to cross-breeding programs.Adv.Hor.Sci. 22(2): 73-86

Besnard G, Baradat P and Berville A, 2001.Genitic relationships in the olive (*Olea europaea* L.) reflect multilocal selection of cultivars. Theoretical and Applied Genetics 102:251-258

Cantini C, Cimato A and Sani G (1999) Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109 (3): 173-181

Charafi J, Rahioui B, El Meziane A, Moukhli A, Boulouha B, Modafar C E and Khadari B , 2007.Verifying the reliability of hybrid issued from the cross "Picholine marocaine clones X Picholine du Languedoc".African Journal of Biotechnology 6 (24): 2776-2779

C.O.I., 1997. Méthodologie pour la caractérisation primaire des variétés d'olivier. Projet RESGEN-CT (67-97), Union Européenne/Conseil Oléicole International.

Dabbou S, Rjiba I, Echbili A, Gazzah N, Mechri B and Hammami M, 2010.Effect of controlled crossing on the triglyceride and fatty acid composition of virgin olive oils. Chemistry & Biodiversity.doi: 10.1002/cbdv.200900385

Dabbou S, Chaieb I, Rjiba I, Issaoui M, Echbili A, Nakbi A, Gazzah N and Hammami M , 2011. Multivariate data analysis of fatty acid content in the classification of olive oils developed through controlled crossbreeding. Journal of the American Oil Chemists'Society 89 (4): 667-674

Diaz A, De La Rosa R, Rallo P, Munoz-Diez, C, Trujillo I, Barranco D, Martin A and Belaj A, 2007. Selections of an olive breeding program identified by microsatellite markers. Crop Science 47: 2317-2322

Fendri M, Trujillo I, Trigui A, Rodríguez-García M I and AlchéRamírez J D, 2010. Simple sequence repeat identification and endocarp characterization of olive tree accessions in a Tunisian germplasm collection. HortScience 45:1429-1436

Fontanazza G, Vergari G, Patumi M and Giorio G, 1999. Preliminary results of the evaluation of yield components in an F1 segregant population of olive seedlings from the cross (Leccino x Kalamata). Acta Hort. (ISHS) 474: 97-102

Fourati H, Cossentini M, Karray B and Khlif M, 2002. Classification of olive trees according to fruit and oil characterisation. Acta Hort. (ISHS) 586:141-145

GratiKamoun N, Lamy Mahmoud F, Rebai A, Gargouri A, Panaud O and Saar A, 2006. Genetic diversity (inter and intra-varietal) of some tunisian olive tree cultivars detected by AFLP markers. In: Proceedings Second International Seminar Olivebioteq 2006 - November 5th-10th-Mazara delVallo, Marsala (Italy) Volume 1: 45-52

Gregoriou C, 2006. Genetic diversity and evaluation of thirty-one clones of the Local or Ladoelia olive variety in Cyprus In: Proceedings Second International Seminar Olivebioteq 2006 - November 5th-10th-Mazara del Vallo, Marsala (Italy) Volume 1:117-121

Hagidimitriou M, Katsiotis A, Menexes G, Pontikis C and Loukas M , 2005.Genetic diversity of major Greek olive cultivars using molecular (AFLPs and RAPDs) markers and morphological traits.Journal of the American Society for Horticultural Science 130: 211-217

Hannachi H, Msallem M, Ben Elhadj S and El Gazzah M, 2007. Influence du site géographique sur les potentialités agronomiques et technologiques de l'olivier (*Olea europaea* L.) en Tunisie. Comptes Rendus Biologies 330: 135-142

Hannachi H, Breton C, Msallem M, El Hadj S B, El Gazzah M and Berville A, 2008. Differences between native and introduced olive cultivars as revealed by morphology of drupes, oil composition and SSR polymorphisms: a case study in Tunisia. Sci Hort 116 : 280–290

Idrissi A and Ouazzani N, 2004. Apport des descripteurs morphologiques à l'inventaire et à l'identification des variétés d'olivier (*Olea europaea* L.). Plant Genetic Resources Newsletter 136 : 1–10

Lansari A and Tahri Hassani J B, 1996. Contribution à l'étude de la variabilité morphologique au sein de la population de "Picholine Marocaine" dans la région de Zerhoun au Maroc. Olivae 60**:** 42-47

Lavee S, 1990. Aims, methods, and advances in breeding of new olive (*Olea europaea* L.) cultivars. Acta Horticulturae 286:23-36

Lavee S, 2010. Genetic resources, from conservation to new cultivars. In: Proceeding SymposiaICH Lisboap 371

LAZ S I, 2006. The olive industry in Tunisia. In: Proceedings Second International Seminar Olivebioteq 2006, Special Seminars and Invited Lectures. Mazara Del Vallo (TP) 5-10 November 51-64

León L, Uceda M, Jiménez A, Martin L M and Rallo L, 2004. Variability of fatty acid composition in olive (*Oleaeuropaea* L.) progenies. Spanish Journal of Aricultural Research 2 (3): 353-359

León L, De La Rosa R, Barranco D and Rallo L, 2006. Agronomic characterization of 15 selections of then olive crossbreeding program of Cordoba, Spain. In: Proceedings Second International Seminar Olivebioteq 2006 - November 5th-10th-Mazara delVallo, Marsala (Italy) Volume 1: 87-93

Manaï H, MahjoubHaddada F, Trigui A, Daoud D and Zarrouk M, 2007. Compositional quality of virgin olive oil from two new Tunisian cultivars obtained through controlled crossings. Journal of the science of food and agriculture 87 (4): 600-606

Manaï H, MahjoubHaddada F, Oueslati I, Daoud D and Zarrouk M, 2008.Characterization of monovarietal virgin olive oils from six crossing varieties.Scientia Horticulturae 115: 252–260

Marra F P, Buffa R, Campisi G, Costa F, Di Vaio C, La Farina M, La Mantia M, Mafrica R, Motisi A, Zappia R and Caruso T, 2006. Morphological and SSR molecular markers based genetic variability in 39 olive cultivars (*Olea europaea* L.) originated in Southern Italy. In: Proceedings Second International Seminar – November 5th-10th-Mazara del Vallo, Marsala (Italy), Volume 1: 213-216

Mehri H and Hellali R, 1995. Etude pomologique des principales variétés d'oliviers cultivées en Tunisie. Ezzaitouna Numéro Spécial. Éd. IRESA, Institut de l’Olivier, Tunisie

Mezghani Aïachi M and Trigui A (2001) Contribution à l'analyse de l'architecture de l'olivier: étude du comportement de la descendance des croisements dirigés de la 'Chemlali de Sfax'. Olivae 87 : 45-49

Padula G, Giordani E, Bellini E, Rosati A, Pandolfi S, Paoletti A, Pannelli G, Ripa V, De Rose F, Perri E, Buccoliero A and Mennone C, 2008.Field evaluation of new olive (*Oleaeuropaea* L.) selections and effects of genotype and environment on productivity and fruit characteristics. Adv. Hort. Sci. 22(2): 87-94

Pannelli G, Rosati A, Pandolfi S, Padula G, Mennone C, Giordani E and Bellini E, 2006. Field evaluation of olive selections derived from a breeding program. In: Proceedings Second International Seminar Olivebioteq 2006- November 5th-10th-Mazara delVallo, Marsala (Italy) Volume 1: 95-102

Pinheiro P B M and Esteves da Silva J C G, 2005. Chemometric classification of olives from there Portuguese cultivars of *Oleaeuropaea* L. AnalyticaChimicaActa 544:229-235

Poljuha D, Sladonja B, BrkićBubola K, Radulović M, Bršćić K, Šetić E, Krapac M and Milotić A, 2008. A multidisciplinary approach to the characterisation of autochthonous Istrian Olive (*Olaeeuropaea* L.) varieties.Food Technol, Boitechnol. 46(4) : 347-354

Rallo L, 1995. Sélection et amélioration génétique de l'olivier en Espagne. Olivae 59: 46-53

Ripa V, De Rose F, Tucci A, Scalercio S, Tucci P and Pellegrino M, 2006.Preliminary observations on the agronomical behaviour of olive cross breedings cultivated in Rossano Calabro. In; Proceedings Second International Seminar Olivebioteq 2006 - November 5th-10th-Mazara del Vallo, Marsala (Italy), Volume 1: 139-142

Ripa V, Rose F, De Caravita M A, Parise M R, Perri E, Rosati A, Pandolfi S, Paoletti A, Pannelli G, Padula G, Giordani E, Bellini E, Buccoliero A and Mennone C, 2008.Qualitative evaluation of olive oils from new olive selections and effects of genotype and environment on oil quality. Advances in horticultural science 22 (2):95-103

Rjiba I., S. Debbou, N. Gazzah, I. Chreif, M. Hammami, 2009.Profiles of volatile compounds from nine new hybrids obtained by controlled crossings on olive ‘Chemlali’ cultivar and mediterranean varieties. Natural Product Research, 23 (7): 622-632.

Rjiba I, Dabbou S, Gazzah N andHammami M, 2010. Effect of crossbreeding on the chemical composition and biological characteristics of tunisiens new olive progenies. Chemistry & biodiversity 7: 649-655

Rjiba I,Gazzah N, Dabbou S, Hammami M, 2011. Evaluation of virgin olive oil minor compounds in progenies of controlled crosses. Journal of Food Biochemistry 35 (5): 1413–1423. doi: 10.1111/j.1745-4514.2010.00462.x

Rotondi A, Magli M, Ricciolini C and Baldoni L, 2003. Morphological and molecular analyses for the characterization of a group of Italian olive cultivars. Euphytica 132 (2):129-137

Taamalli W, Geuna F, Banfi R, Bassi D, Daoud D and Zarrouk M, 2006.Agronomic and molecular analyses for the characterisation of accessions in Tunisian olive germplasm collections. Electronic Journal of Biotechnologiy 9(5): 467-481

Trentacoste E R and Puertas C M , 2011.Preliminary characterization and morpho-agronomic evaluation of the olive germplasm collection of the Mendiza province (Argentina).Euphytica 177: 99-109

Trigui A and Msallem M, 2002. Oliviers de Tunisie : Catalogue des Variétés Autochtones & Types Locaux : Identification variétale & Caractérisation morpho-pomologique des Ressources Génétiques Oléicoles de Tunisie. IRESA (Ministère de l’Agriculture), Institut de l’Olivier, Tunisia Volume I pp 159

Trigui A, Yengui A and Belguith H, 2006. Olive germplasm in Tunisia.Olea, FAO OLIVE NETWORK 25: 19-23

U.P.O.V., 1985. Principes directeurs pour la conduite de l'examen des caractères distinctifs, de l'homogénéité et de la stabilité de l’olivier *Olea europaea* L. Union internationale pour la Protection des Obtentions Végétales (UPOV), 21 pp.

Zeinanloo A, ShahsavariA, Mohammadi A and Naghavi M R, 2009. Variance component and heritability of some fruit characters in olive (*Oleaeuropaea* L.). Scientia Horticulture 123: 68-72.