

# 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.

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**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 et al. 2004 ; Pannelli et al. 2006 ; Bartolini et al. 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. For that, the cultivar ‘Chemlali Sfax’ have been crossed with both autochthonous 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; Manai 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 31 qualitative 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<sup>-1</sup> (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.

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

<b>FS</b>	Fruit symmetry (positionA)	(1) Symmetrical (2) Lightly asymmetrical (3) Asymmetrical	COI, 1997
<b>FD</b>	Fruit position of maximum diameter (B position)	(1) To bottom (2) Medium (3) To top	COI, 1997
<b>FASH</b>	Fruit apex shape(positionA)	(1) Sharp (2) Rounded	COI, 1997
<b>FBSH</b>	Fruit base shape(positionA)	(1) Cut (2) Rounded	COI, 1997
<b>FK</b>	Knoll	(1) Absent (2) Outlined (3) Evident	COI, 1997
<b>FPL</b>	Presence of lenticels	(1) Little numerous (2) Numerous	COI, 1997
<b>FDL</b>	Dimension of lenticels	(1) Small (2) Big	COI, 1997
<b>SW</b>	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
<b>SSH</b>	Stone shape (positionA)	(1) Spherical (2) Oval (3) Elliptic (4) Longer	COI, 1997
<b>SNG</b>	Stone number of grooves	(1) Reduced (<7) (2) Medium (7–10) (3) High (>10)	COI, 1997
<b>SSA</b>	Stone symmetry (positionA)	(1) Symmetrical (2) Lightly asymmetrical (3) Asymmetrical	COI, 1997
<b>SSB</b>	Stone symmetry (positionB)	(1) Symmetrical (2) Lightly asymmetrical	COI, 1997
<b>SD</b>	Stone position of maximum diameter (B position)	(1) To bottom (2) Medium (3) To top	COI, 1997
<b>SM</b>	Stone mucron	(1) Absent (2) Present	COI, 1997
<b>SASH</b>	Stone apex shape(A)	(1) Sharp (2) Rounded	COI, 1997
<b>SBSH</b>	Stone base shape(positionA)	(1) Cut (2) Sharp (3) Rounded	COI, 1997
<b>SS</b>	Stone surface	(1) Smooth (2) Rough (3) Knotty	COI, 1997
<b>SDG</b>	Stone distribution of grooves	(1) Uniform (2) Grouped in proximities of suture	COI, 1997
<b>SCG</b>	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<sup>2</sup>) whereas the largest one was noted on ‘Chemlali’ × ‘Coratina’ (8.14cm<sup>2</sup>). 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	
<b>Ch *Cor</b>	Min	0,35	7,30	2,90	3,96	0,91	2,28	4,37	0,81	14,10	9,56	1,26	<u>0,16</u>	10,88	<u>5,34</u>	1,91	<u>6,40</u>	<u>2,91</u>
	Max	0,60	<u>14,80</u>	<u>4,55</u>	<u>8,39</u>	<u>1,65</u>	<u>8,14</u>	<u>7,09</u>	2,35	20,41	14,16	<u>1,82</u>	0,42	16,44	7,39	<u>2,49</u>	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	<b>14,64</b>	<b>20,92</b>	<b>10,98</b>	<b>16,54</b>	<b>13,75</b>	<b>26,14</b>	<b>12,94</b>	<b>32,93</b>	<b>12,02</b>	<b>11,82</b>	<b>8,36</b>	<b>30,91</b>	<b>11,81</b>	<b>10,64</b>	<b>9,00</b>	<b>17,62</b>	<b>21,48</b>
	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	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	***
<b>Ch F</b>	Min	0,32	6,30	3,00	4,08	0,79	2,15	3,88	<u>0,77</u>	<u>13,21</u>	<u>9,38</u>	<u>1,12</u>	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	<u>8,05</u>	<u>28,94</u>	<u>23,52</u>	1,49	0,97	<u>18,91</u>	10,21	2,25	<u>11,48</u>	<u>8,80</u>
	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	<b>14,89</b>	<b>20,02</b>	<b>11,15</b>	<b>13,10</b>	<b>13,96</b>	<b>23,79</b>	<b>13,88</b>	<b>80,91</b>	<b>23,02</b>	<b>25,79</b>	<b>7,93</b>	<b>58,22</b>	<b>17,42</b>	<b>18,34</b>	<b>9,91</b>	<b>16,18</b>	<b>30,26</b>
	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	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	***
<b>Ch S</b>	Min	<u>0,22</u>	<u>5,70</u>	<u>2,50</u>	<u>3,59</u>	<u>0,76</u>	<u>1,72</u>	<u>3,61</u>	1,09	13,53	10,78	1,12	0,17	<u>9,15</u>	5,78	<u>1,43</u>	6,63	3,60
	Max	<u>0,65</u>	10,70	4,20	5,87	1,15	4,49	6,91	7,94	28,58	22,74	1,57	<u>1,02</u>	18,44	<u>11,30</u>	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	<b>28,29</b>	<b>20,55</b>	<b>13,81</b>	<b>14,78</b>	<b>10,73</b>	<b>22,95</b>	<b>16,44</b>	<b>74,88</b>	<b>22,11</b>	<b>22,89</b>	<b>10,38</b>	<b>61,48</b>	<b>19,03</b>	<b>20,31</b>	<b>14,19</b>	<b>13,88</b>	<b>23,81</b>
	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<sup>2</sup>), 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-spread 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.

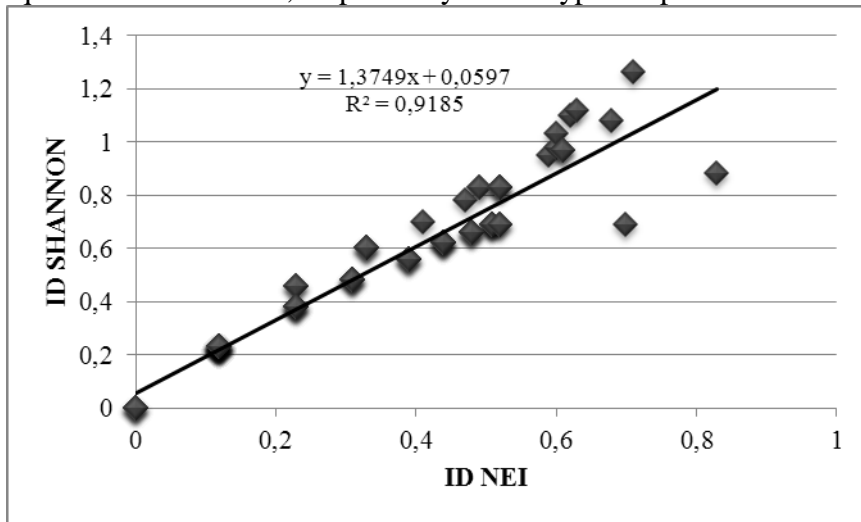
Leaves were mostly with elliptic-lanceolate shape (LSH), flat longitudinal curvature (LLC) and acute apical angle (LAA). Leaves of 'Chemlali' × 'Coratina' descendants were characterized by long length (50 %), medium width (88 %), medium size (63 %) and acute basal angle (88 %). However, 'Chemlali' free 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 R<sup>2</sup> 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.

Trait		Traits states	<i>Ch×Cor</i>			<i>Ch L</i>			<i>Ch A</i>		
			Percent	I Nei	I Shan	Percent	I Nei	I Shan	Percent	I Nei	I Shan
Tree	<b>TV</b>	Weak	0			6			25		
		Medium	56			81			75		
		Strong	44	0,51	0,69	13	0,33	0,60	0	0,39	0,56
	<b>TH</b>	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
	<u>LS</u>	Small	6			88			94		
		Medium	63			13			6		
		Large	31	0,52	0,83	0	0,23	0,38	0	0,12	0,23
	<u>LSH</u>	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
	<u>LAA</u>	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
<u>LBA</u>	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	<u>FW</u>	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
	<u>FSH</u>	Spherical	0			19			50		
		Oval	38			75			38		
		Longer	63	0,48	0,66	6	0,41	0,70	13	0,61	0,97
	<u>FS</u>	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
	<u>FD</u>	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
	<u>FASH</u>	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	<u>SW</u>	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

<b>SSH</b>	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
<b>SNG</b>	Reduced	6			0			0		
	Medium	81			88			94		
	High	13	0,33	0,60	13	0,23	0,38	6	0,12	0,23
<b>SSA</b>	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
<b>SSB</b>	Lightly asymmetrical	81			94			100		
	Symmetrical	19	0,31	0,48	6	0,12	0,23	0	0,00	0,00
<b>SD</b>	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
<b>SM</b>	Absent	13			31			25		
	Present	88	0,23	0,38	69	0,44	0,62	75	0,39	0,56
<b>SASH</b>	Sharp	38			69			69		
	Rounded	63	0,48	0,66	31	0,44	0,62	31	0,44	0,62
<b>SBSH</b>	Cut	25			0			0		
	Sharp	38			63			50		
	Rounded	38	0,68	1,08	38	0,48	0,66	50	0,52	0,69
<b>SS</b>	Smooth	63			0			0		
	Rough	38			81			94		
	Knotty	0	0,48	0,66	19	0,31	0,48	6	0,12	0,23
<b>SDG</b>	Uniform	44			94			69		
	Grouped in proximities of suture	56	0,51	0,69	6	0,12	0,23	31	0,44	0,62
<b>SCG</b>	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.

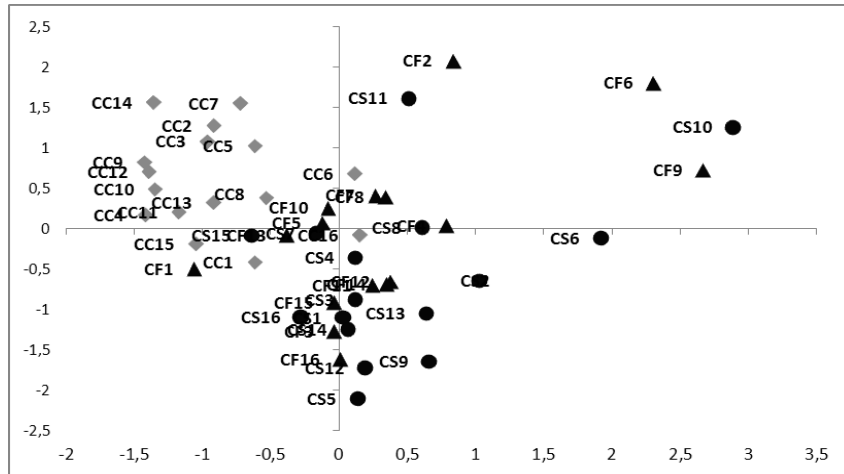
The PC1 was also correlated negatively with leaf size (LA, LL, and LWI) and tree parameters (TH, CC, TC). The inertia which accounted for the second PC was due to the contribution of fruit and stone shape (FR, SF) to stone length (SL). Leaf ratio (LR) and stone grooves (SG) were not used to distinguish 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<sup>2</sup>),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.

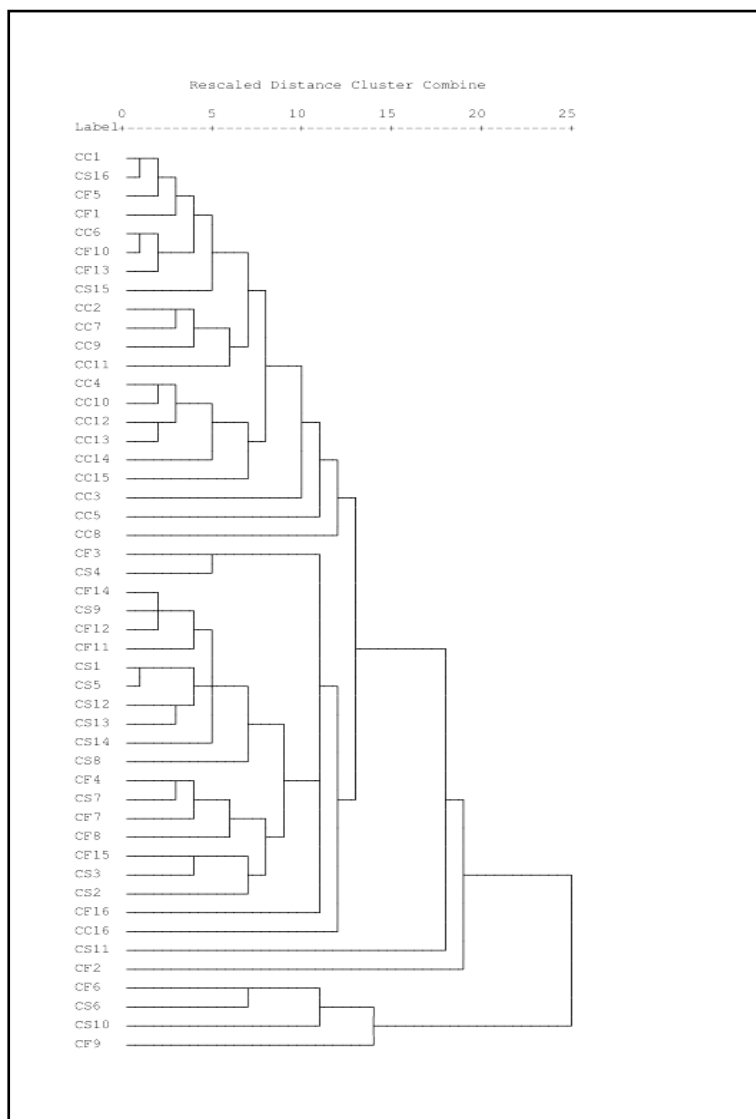
The first group consist exclusively of CF9, a ‘Chemlali’ free pollination seedling, which was characterized with the highest fruit weight (8.05g), flesh to stone ratio (8.8) and fruit width (23.2mm). It presented a symmetric spherical fruit with around base, a short and narrow leaf with very acute apical 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 et al. 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 morphometric 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.

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