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Morphometric variability induced by cross breeding of 'chemlali sfax' under different pollination treatments: self-, free-, and cross-pollination

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ABSTRACT

A breeding program has been developed since 1989 (done by Dr. A. Trigui) for improving the oil content and the quality of the most important olive variety cultivated in Tunisia 'Chemlali'. Studies realized on these new obtained descendants, showed that some progenies presented higher oil percentage and a better chemical composition, comparing with the original cultivar 'Chemlali'. However, there is a little understanding about phenotypic diversity distribution according to their genetic origins. The present work was carried out in order to study the morphological variability noted on thirty 'Chemlali Sfax' seedlings issued from self, free and cross pollination with 'Coratina'. Seventeen morphometric parameters were used according to the different parts of the olive tree (tree, leaf, fruit and endocarp). For identification the patterns of morphological variation within the progenies, Principal Component Analysis (PCA) was used and cluster analysis was performed to decide the ultimate numbers of clusters by which the accessions could be assessed. Quantitative characters revealed significant differences between seedlings within all crossbreeding (P≤0.001-test Duncan). Wide ranges of variation were noted. The highest CV was recorded for fruit weight, which was equal to 89.4%, 72.3% and 39.2%, respectively for free, self and cross pollination. Results revealed that the first three Principal Components (PCs) explained 75.5% of the total variation observed in-situ. Fruit, endocarp and leaf size were strongly associated with the first principal component. Fruit and endocarp shape showed the highest contribution to inertia on the second one. Trunk circumference and leaf shape were strongly related to the last one. Projection of seedlings in the plane determined by the first two principal components, showed that seedling issued from 'Chemlali' x 'Coratina' were grouped and showed the smallest fruits and endocarps, the biggest leaves and vigorous trees. While 'Chemlali' free and self pollination seedlings were characterized by medium fruit and endocarp weight, medium leaf size and medium tree vigor. However, cluster analysis revealed four groups for the studied descendants related to the great discriminated effect which was fruit size. The results of morphological evaluation confirmed the usefulness of phenotypic markers for olive genetic resources but the number of descriptors used in this study could be reduced.

Key words: *olive tree seedlings, phenotypic variability*, descriptors, principal component analysis, cluster analysis.

INTRODUCTION

Today the need for new olive cultivars is an on going process due to the continuous developments of new cultivation techniques [1] and to the changes in agricultural policies and market liberalization [2]. For developing new olive cultivars, cross breeding technique have been carried out in some olive-producing countries [3, 4, 5, 6, 7, 8, 9]. These programs focused on cross breeding of the most outstanding cultivars in their respective countries. However, the heterozygosis level of recessive alleles, the scarce knowledge about characters hereditability and the long

unproductive period of seedlings, make the cross breeding technique long and poor of results [10]. In this context, any genetic improvement program by cross breeding will need strong efforts and long time to obtain next generation and its agronomical evaluations in the field [11].

In Tunisia, the variety-population 'Chemlali Sfax' has been crossed with both autochtonous and foreign pollinators. One thousand six hundred and eighty five seedlings have been produced and one thousand and two hundred have started producing. Most studies concerning these descendants were interested in screening some progenies with a higher percentage of oil and a chemical composition more interesting than the variety 'Chemlali Sfax'. This allowed the preselection of forty descendants wich are currently under evaluation [12, 13]. However, fewer of these studies have provided information about the phenotypic diversity distribution. There is little understand about 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).

The morphological traits have been used in different olive collections over the world for the identification, characterization, and evaluation of cultivars [14, 15, 16, 17, 18, 19, 20, 21]. Moreover, these descriptors have been also used for the characterisation and selection of new olive genotypes derived from breeding programs [4, 11, 22, 23, 24, 25].

This study consisted to evaluate and to compare the impact of three different pollination treatments (self-, free-, cross-) on the morphologic variability observed among chemlali Sfax seedlings. The main objectives of this study were (1) to study the variability of different morphological characteristics observed on 'Chemlali Sfax' seedlings according to tree, leaves, fruit and endocarp, (2) to identify the most relevant descriptors and to study distribution of these seedlings by using a principal components and, (3) to classify these seedlings by hierarchical cluster analysis.

MATERIALS AND METHODS

1.1. Plant material and cultivation

Thirty 'Chemlali Sfax' seedlings were studied which were issued from controlled pollination treatments: 10 seedlings from 'Chemlali' free pollination (ChF), 10 from 'Chemlali' self pollination (ChS) and 10 from cross pollination 'Chemlali' x 'Coratina' (ChC). The seedlings obtained by cross pollination with 'Coratina' were planted in 1997 at the experimental station of the Olive Tree Institute in Sfax central Tunisia (34°N, 21°E). Those obtained by free and self pollination were planted in 1997/1998 at the Research Station of Taoues, about 40 km far from Sfax. All evaluated seedlings were grown in similar pedoclimatic conditions and cultivated with the same agrotechnical treatments. They were conducted under intensive and irrigated conditions with a density of 1250 trees ha^{-1}(4m x 2m).

1.2. Morphological Characteristics

The olive descriptors used in our study were according to those of the Conseil Olive International [26] and UPOV [27]. Seventeen biometric and morphological traits were observed on each olive seedling. All the measurements were evaluated for 40 samples of leaves and fruits per tree. After fruits characterization, endocarps were removed and subject of characterization.

Tree parameters: TC: Trunk circumference (m); CC: Canopy circumference (m); TH: Tree height (m).

Leaf parameters: LL: Leaf: length (cm); LWI: Leaf width (cm); LS: Leaf surface (cm2); LR: Leaf shape (length/width ratio).

Fruit parameters: FW: Fruit weight (g); FL: Fruit polar length (mm); FWI: Fruit cross-sectional width (mm); FR: Fruit shape (length/width ratio).

Endocarp parameters: EW: Endocarp weight (g); EL: Endocarp polar length (mm); EWI: Endocarp cross-sectional width (mm); EFG: Endocarp fibro-vasc.grooves; ER: Endocarp shape (length/width ratio); FSR: Flesh/Stone ratio.

1.3. Data analysis

Descriptive statistics analysis (minimum, maximum and average values) and coefficient of variation were done using SPSS 13.0 and Microsoft Excel 2007 for windows. Means were compared by Duncan's multiple range test at $P<0.05$. Principal component analysis (PCA) and a hierarchic classification (UPGMA) using SPSS 13.0 were done for comparison and clustering analyses of the morphological characters.

RESULTS

1.4. Descriptive Analysis

The mean range, the maximum and minimum values, the coefficient of variation (CV) and the degree of significance for morphological traits are given in table 1. All quantitative parameters showed significant differences (*P≤0.001*) for all 'Chemlali Sfax' seedlings.

The variation coefficient varied from 7.80% to 39.19% for cross pollination, from 10.02% to 89.36% for free pollination and from 10.11% to 72.34% for self pollination. The highest CV was recorded for fruit weight whereas the lowest one was noted for fruit ratio for all crossbreeding. CV was higher than >20% for trunk and canopy circumference (TC,CC), for leaf surface (LS), for fruit parameters like weight (FW), polar length (FL), cross-sectional width (FWI) and for endocarp parameters like weight (EW), polar length (EL), cross-sectional width (EWI) and flesh/stone ratio (FSR) for descendants obtained by self and free pollination. Moreover, tree height (TH) showed a high CV (21.51%) for 'Chemlali' self pollination descendants. For seedlings issued from coss pllination 'Chemlali' x 'Coratina', just five traits: Canopy circumference (CC), leaf surface (LS), fruit weight (FW), endocarp weight (EW) and endocarp fibro-vasc.grooves (EFG) presented high CV which varied from 21.10% to 39.19%. However, neither low CV (<10%) has been noted for all crossbreeding except the CV of the characters fruit shape (FR) and endocarp shape (ER) for *ChC* descendants. They were equal to 7.80% and 9.02% respectively.

Concerning the tree characters, the most extreme of trunk circumference (TC) and tree height (TH) characters were noted on self and free pollination descendants respectively. In fact, tree circumference (TC) varied from 0.21 to 0.86 m.Tree height (TH) varied from 2.50 to 5.20 m.

The smallest leaf was noted on 'Chemlali' self pollination seedlings (LL=3.59 cm, LWI=0.76cm, LS=1.72 cm^2) while the largest one was noted on crossbreeding *ChC* seedlings (LL=8.39 cm, LWI=1.65 cm, LS=8.14 cm^2). The lowest and the greatest fruit size were recorded on free pollination seedlings. Indeed, fruit weight (FW) ranged from 0.77 to 8.05 g. Length (FL) and width fruit (FWI) ranged from 13.21 to 28.94 mm and from 9.38 to 23.52 mm respectively. For endocarp characters, the highest and lowest weights (EW) were found among self pollination seedlings. It ranged between 0.15 and 1.02 g. The extremities values of endocarp ratio (ER) and flesh to stone ratio (FSR) were noted on free pollination progenies. They varied from 1.39 to 2.56 and from 3.11 to 8.8 respectively. The upper and lowest extrimities of the number of endocarp fibro-vasc grooves (EFG) were noted among these issued from crossbreeding *ChC*. It varied from 6 to 11.

1.5. Principal Component Analysis

The PCA is generally used before the cluster analysis in order to determine the relative importance of morphological characters (descriptors) and to study the inter-relationships between all the studied Chemlali seedlings. The principal components analysis was performed on average data by descendant. The first three principal components (PC1, PC2 and PC3) accounted for 47%, 18% and 10% of the total variance respectively. The three components accumulated 75% of total variability (Table 2).

The first principal component (PC1) showed that width and weight of fruit and stone (FWI, EWI, FW and EW), fruit length (FL) and flesh to stone ratio (FSR) were important attributes for the classification of seedlings. The PC1 was also correlated negatively with leaf size (LS, LL and LWI) and tree parameters (CC, TH). The inertia accounted for the second principal component (PC2) was due to the contribution of fruit and stone shape (FR, ER) and to endocarp length (EL). The third principal component (PC3) was associated positively with trunk circumference (TC) while it was negatively related with leaf shape (LR).

Figure 1 shows a projection of seedlings in the reduced space determined by the first two principal components. The 'Chemlali' x 'Coratina' descendants were associated together on the right presenting a considerable percentage of similarity and appears as a homogeneous group. These descendants showed the smallest fruits and endocarps, the greatest leaves and vigorous trees. 'Chemlali' free and self pollination seedlings were distributed and overlapped randomly on the center cloud. They presented medium fruit and endocarp weight, medium leaf size and medium tree vigor. *ChF7*, *ChF8* and *ChS4*, *ChS7* were set on the left apart from all rest of seedlings. They had high contribution on the plan and presented big fruits and endocarps, small leaves and low tree vigor.

1.6. Hierarchical Cluster Analysis

To assess the genotyps variability, the olive progenies were storted into clustering UPGMA method analysis based on the morphological characters (Figure 2). The generated dendrogram revealed five major groups of descendants, mainly according to fruit size. The first group included olive seedlings with high fruit weight, whereas the last group enclosed progenies with a small fruit size.

The first group consisted of four progenies which have very high fruit and endocarp size, important flesh to stone ratio, small leaf and weak tree vigour, obtained from 'Chemlali' self pollination crossing (*ChS7*, *ChS4*) and 'Chemlali' free pollination crossing (*ChF8*, *ChF7*).

The second group included just one olive seedling obtained from 'Chemlali' free pollination crossing that had high fruit and endocarp size, medium leaf size and vigorous tree.

The third group comprised five olive descendants obtained through 'Chemlali' self pollination (*ChS1*, *ChS2*), 'Chemlali' free pollination (*ChF2*, *ChF4*) and 'Chemlali' x 'Coratina' crossing (*ChC4*) wich have slightly high fruit and endocarp size, large leaf and medium tree vigour.

The fourth group contained twelve olive seedlings wich composed by 'Chemlali' self pollination progenies (*ChS5*, *ChS9*, *ChS6*, *ChS3*, *ChS2*, *ChS10*), also 'Chemlali' free pollination progenies (*ChF1*, *ChF9*, *ChF10*, *ChF3*, *ChF6*) and finally cross 'Chemlali' x 'Coratina' progenies (*ChC10*). They had medium fruit and endocarp size, large leaf and medium tree vigour.

The last group is composed exclusively by 'Chemlali' x 'Coratina' descendants (*ChC6*, *ChC8*, *ChC1*, *ChC2*, *ChC3*, *ChC9*, *ChC7*, *ChC5*) wich were characterized by a very high leaf size, small fruit and endocarp size and vigorous tree and low flesh to stone ratio.

DISCUSSION

Most morphological traits noted on 'Chemlali Sfax' olive seedlings showed a wide range of variation. High coefficients of variation and highly significant differences were noted between progenies within same crossbreeding for most traits evaluated. These differences can be explained mainly by the genetic variation because all seedlings within crossings had the same age and were grown under the same agro-climatic conditions. The effect of environemental conditions, like cited by many authors [28] and [25], was not important in our study. It can be concluded that the genotype seemed to influence the morphological characters of descendants. This result agrees the works of [4], [14], [23] and [24].

High variability noted in our study was also observed in these progenies for other crossings and other characteristics [29, 12, 13, 30]. Our results are likewise in agreement with the degree of variability reported for other olive characteristics in other olive crossbreeding programs [3, 4, 31, 32, 24, 22, 23, 25]. Thus, these results confirm that crossbreeding is an efficient technique to increase the genetic variability in olive for the selection of new interesting genotypes.

The three principal components accounted 75% of the morphological variation. This percentage was consistent with the result of the descriptive analysis and indicated the high morphological variability observed in the 'Chemlali Sfax' descendants. The first PC was mainly correlated to fruit and endocarp size and flesh to stone ratio, whereas the second PC was mainly correlated to fruit and endocarp shape. The same observation has been reported by [33] in the characterization of 61 accessions of the olive germplasm collection (Argentina). They noted that the first PC were correlated to weight, length, fruit and endocarp widths and flesh to stone ratio and the second PC was due to the contribution of endocarp and fruit form.

The cluster analysis classifies the descendants according, mainly, to the fruit size suggesting the great discriminant power of this character. This result corroborates with other studies carried out in other classic cultivars based both on morphometric characters [34, 16] and on molecular markers [35, 36, 37, 19, 38].

Moreover, 'Chemlali' x 'Coratina' progenies were closely clustered in the same homogenous group, except two descendants, suggesting that morphometric characters were able to discriminate between genotypes with different genetic origins. Similar clustering according to the genetic origins has been previously reported in other olive seedlings based on other descriptors [39, 30]. However, it is worth mentioning that descendants derived from free and self pollination in this study, were divided into overlapping clusters. This result confirm the high degree of variability already mentionned by descriptive analysis among these descendants suggesting that free and self pollination can induce a comparative phenotypic variability.It can be explained by the high heterozygosity of olive and the high chromosome number of the species [40]. Moreover, the 'Chemlali' variety was considered previously as a polyclonal variety characterized by a high heterogeneity [41].

Table 1 Mean range, maximum and minimum values, coefficient of variation (CV) and signifacance level for 17 morphological traits noted on 'Chemlali Sfax' seedlings. Values underlined are the upper and lower extremes for each trait.

		Tree			**Leaf**				**Fruit**				**Endocarp**					
		TC	CC	TH	LL	LWI	LS	LR	FW	FL	FWI	FR	EW	EL	EWI	EFG	ER	FSR
Ch *Cor	Min	0,35	7,80	2,90	6,01	1,07	4,40	4,73	0,81	14,10	9,56	1,26	0,16	10,88	5,34	6,40	1,91	3,28
	Max	0,60	14,80	4,55	8,39	1,65	8,14	7,09	2,35	20,41	14,16	1,65	0,42	15,29	7,39	11,40	2,47	5,03
	Mean	0,48	10,67	4,07	7,21	1,33	6,17	5,50	1,35	16,99	11,55	1,48	0,26	13,02	6,16	8,34	2,13	4,13
	CV(%)	16,38	21,10	12,41	11,18	13,57	21,62	12,58	39,19	12,77	14,19	7,80	34,79	11,53	12,23	19,96	9,02	17,99
	F calculated	-	-	-	59,99	62,18	62,50	68,07	225,59	198,25	217,43	100,84	216,727	174,795	239,582	124,106	141,568	-
	Sig Level	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	-
Ch F	Min	0,21	6,7	3	4,2	0,79	2,23	3,88	0,77	13,21	9,38	1,12	0,17	10,49	5,59	6,68	1,39	3,11
	Max	0,86	12,6	4,6	6,38	1,39	4,43	6,64	8,05	28,94	23,52	1,55	0,97	18,91	10,21	9,88	2,56	8,8
	Mean	0,43	8,49	3,70	5,01	1,06	3,32	4,88	3,01	19,32	14,90	1,31	0,42	13,87	7,47	8,02	1,88	5,43
	CV(%)	39,64	21,26	14,41	13,79	14,64	24,08	18,82	89,36	26,70	29,78	10,02	63,38	20,80	20,80	13,26	17,04	28,97
	F calculated	-	-	-	122,71	45,14	65,34	15,61	403,85	368,94	526,35	67,23	326,66	94,43	335,77	32,74	48,39	-
	Sig Level	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	-
Ch S	Min	0,22	5,70	2,50	3,59	0,76	1,72	3,61	1,07	13,53	11,64	1,12	0,15	9,15	5,59	6,63	1,43	3,85
	Max	0,65	10,70	5,20	5,87	1,15	4,49	6,91	7,94	28,58	22,74	1,57	1,02	18,44	11,30	10,90	2,37	8,41
	Mean	0,44	7,86	3,58	4,72	0,97	2,85	4,94	3,02	19,50	15,58	1,25	0,42	13,21	7,71	8,30	1,73	5,96
	CV(%)	29,56	21,16	21,51	17,55	13,52	29,95	18,83	72,34	25,03	23,30	10,11	62,72	23,29	22,27	15,63	14,85	22,27
	F calculated	-	-	-	168,13	80,21	158,79	129,77	442,09	516,16	693,06	211,80	248,35	455,47	579,77	69,82	270,37	-
	Sig Level	-	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	-

*TC: Trunk circumference (cm); CC: Canopy circumference (m); TH: Tree height (m); LL: Leaf: length (cm); LWI: Leaf width (cm); LS: Leaf surface (cm2); LR: Leaf shape (length/width ratio); FW: Fruit weight (g); FL: Fruit polar length (mm); FWI: Fruit cross-sectional width (mm); FR: Fruit shape (length/width ratio);EW: Endocarp weight (g); EL: Endocarp polar length (mm); EWI: Endocarp cross-sectional width (mm); EFG: Endocarp fibro-vasc.grooves; ER: Endocarp shape (length/width ratio); FSR: Flesh/Stone ratio; CV: variation coeficient (%), Sig Level: significance level, *** significant at 1‰ level.*

Table 2 Estimates of variance, accumulated variances and weighting coefficients of the first three principal components for 17 quantitative characters evaluated on 'Chemlali Sfax' olive descendants.

	PC1	PC2	PC3
% Variance	47,04	18,21	10,23
% Accumulation variation	47,04	65,25	75,49
FWI	0,940	0,216	0,181
EWI	0,927	0,255	0,077
FW	0,900	0,326	0,175
EW	0,859	0,460	0,102
FL	0,803	0,572	0,101
FSR	0,784	-0,155	0,314
LS	-0,723	0,467	0,085
LL	-0,692	0,523	-0,077
LWI	-0,684	0,392	0,400
CC	-0,669	0,056	0,265
TH	-0,645	0,044	0,434
EL	0,541	0,782	-0,057
FR	-0,547	0,718	-0,222
ER	-0,543	0,632	-0,186
TC	-0,429	0,112	0,752
LR	-0,161	0,303	-0,602
EFG	0,127	-0,182	-0,322

FWI: Fruit cross-sectional width (mm); EWI: Endocarp cross-sectional width (mm); FW: Fruit weight (g); EW: Endocarp weight (g); FL: Fruit polar length (mm); FSR: Flesh/Stone ratio; LS: Leaf surface (cm^2); LL: Leaf: length (cm); LWI: Leaf width (cm); CC: Canopy circumference (m); TH: Tree height (m); EL: Endocarp polar length (mm); FR: Fruit shape (length/width ratio); ER: Endocarp shape (length/width ratio); TC: Trunk circumference (cm); LR: Leaf shape (length/width ratio); EFG: Endocarp fibro-vasc.grooves.

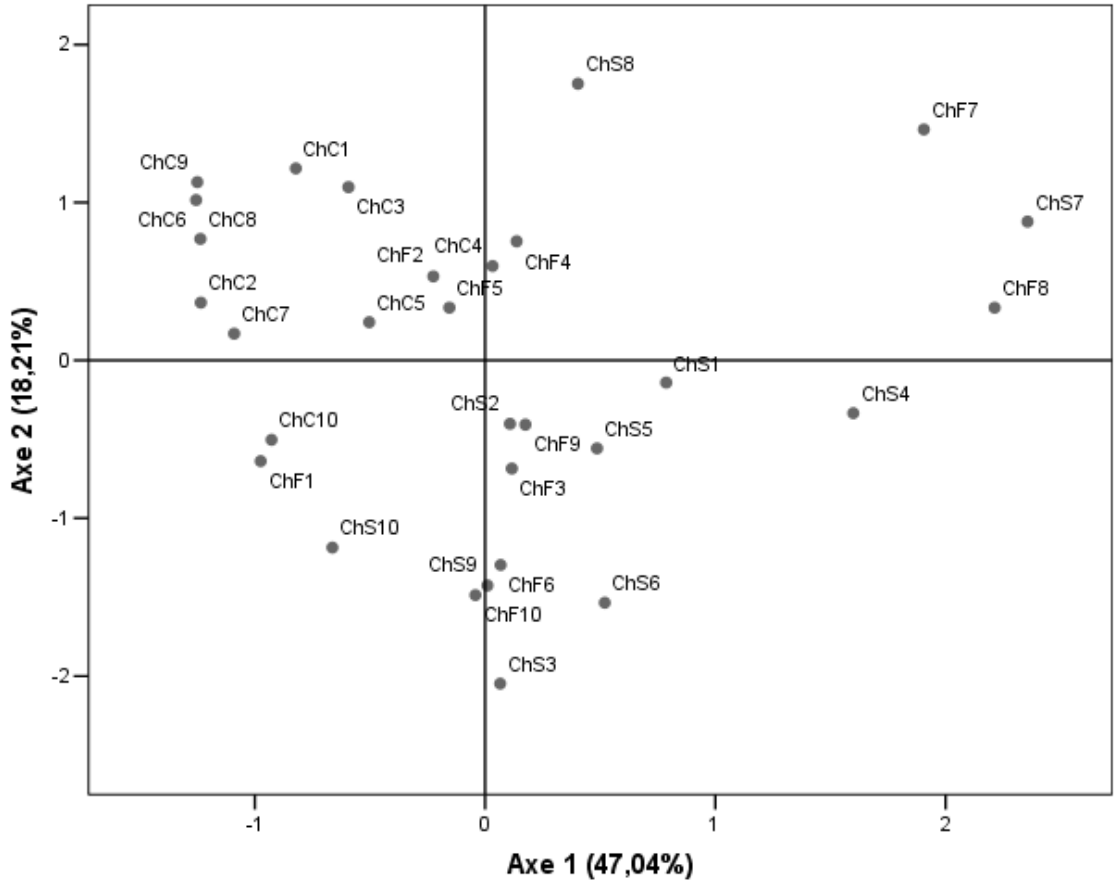

Fig. 1. Plot illustrating the relationships among 30 'Chemlali Sfax' olive seedlings assessed via 17 quantitative morphological traits.

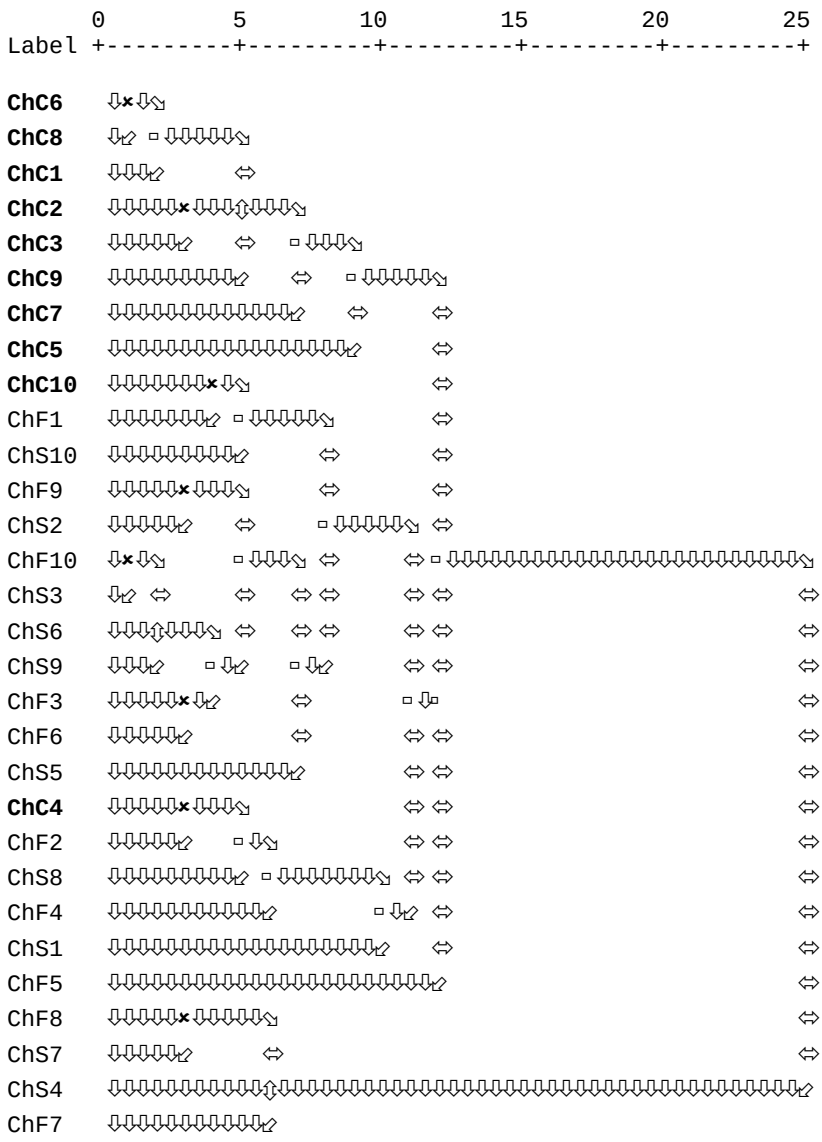

Fig. 2. Dendrogram of the clustering of the morphological characters from olive seedlings obtained from controlled pollination of 'Chemlali Sfax'.

However, it can be explained probably by a foreign pollen contamination, especially in the case of selfing descendants which present characteristics widely different from 'Chemlali'. Although, descendant noted fruit weight equal to 7.94 g while fruit weight of 'Chemlali' do not exceed 2 g [15, 42], as mentionned on selfings of 'Picholine marocaine' [7] and 'Picual', 'Arbequina' and 'Frantioi' [39]. This can be conclued following a verifiying reliability of seedlings by molecular markers.

In conclusion, the results prove the interested heterogeneity of the studied seedlings. In the future, it is important to extent the research with more descriptors for higher number of descendants to facilitate future selections. This study can be completed by the use of molecular methods which are very suitable to reach a better understanding of the material's genetic diversity.

REFERENCES

[1] S. Lavee, Proceeding Symposia ICH Lisboa, **2010**, 371.
[2] V. Ripa, F. Rose, M. A. De Caravita, M. R.Parise, E. Perri, A. Rosati, S. Pandolfi, A. Paoletti, G. Pannelli, G. Padula, E. Giordani, E. Bellini, A. Buccoliero, Mennone C., *Advances in horticultural science*, **2008**, 22 (2), 95-103.
[3] S. Lavee, *Acta Horticulturae*, **1990**, 286, 23-36.
[4] E. Bellini, *Olivae*, **1993**, 49, 21-34.
[5] L. Rallo, Olivae, **1995**, 59, 46-53.

[6] H. Arsel, Cirik, N., Olivae, **1994**, 52, 25-27.
[7] J. Charafi, B. Rahioui, A. El Meziane, A. Moukhli, B. Boulouha, C. E. Modafar, Khadari, B., *African Journal of Biotechnology*, **2007**, 6 (24), 2776-2779.
[8] A. Zeinanloo, A. Shahsavari, A. Mohammadi, R.Naghavi M., *Scientia Horticulture*, **2009**, 123, 68-72.
[9] S.I. Laz, The olive industry in Tunisia, Proceedings Second International Seminar Olivebioteq 2006, Special Seminars and Invited Lecture, Mazara Del Vallo (TP), 5-10 November, **2006**, 51-64.
[10] E. Bellini, E. Giordani, Nin S, Genetica e Miglioramento, In *Olea* Trattato di olivicoltura, (Fiorino, P.), Edagricole, Bologna, **2003**, pp. 116-129.
[11] V. Ripa, F. De Rose, A. Tucci, S. Scalercio, P. Tucci, Pellegrino M., Preliminary observations on the agronomical behaviour of olive cross breedings cultivated in Rossano Calabro. Proceedings Second International Seminar Olivebioteq **2006** - November 5th-10th-Mazara del Vallo, Marsala (Italy), **2006**, Volume 1. 139-142.
[12] H. Manaï, F. Mahjoub Haddada, O. Imen, A. Trigui, D. Daoud, Zarrouk M., Olivae, **2006**, 106, 17-23.
[13] A. Trigui, A. Yengui, Belguith H., Olea, *FAO OLIVE NETWORK*, **2006**, 25, 19-23.
[14] C. Cantini, A. Cimato, Sani G., *Euphytica*, **1999**, 109 (3), 173-181.
[15] D. Barranco, A. Cimato, P. Fiorino, L. Rallo, A. Touzani, C. Caatanedo, F. Serafini, Trijillo I., **2000**, World catalogue of olive varieties, International olive council, Madrid.
[16] A. Idrissi, Ouazzani, N., *Plant Genetic Resources Newsletter*, **2004**, 136, 1–10.
[17] A. Rotondi, M. Magli, C. Ricciolini, Baldoni, L., *Euphytica*, **2003**, 132 (2), 129-137.
[18] M. T. Ozkaya, E. Cakir, Z. Gokbayrak, H. Ercan, Taskin N., *Scientia Horticulture*, **2006**, 108, 205-209.
[19] W. Taamalli, F. Geuna, R. Banfi, D. Bassi, D. Daoud, Zarrouk M., *Electronic Journal of Biotechnologiy*, **2006**, 9(5), 467-481.
[20] H. Hannachi, C. Breton, M. Msallem, S. B. El Hadj, M. El Gazzah, Berville A., Sci Hort., **2008**, 116, 280–290.
[21] D. Poljuha, B. Sladonja, K. Brkić Bubola, M. Radulović, K. Brščić, E. Šetić, M. Krapac, Milotić A., Food Technol, *Boitechnol*., **2008**, 46(4), 347-354.
[22] G. Pannelli, A. Rosati, S. Pandolfi, G. Padula, C. Mennone, E Giordani, Bellini E., Field evaluation of olive selections derived from a breeding program. Proceedings Second International Seminar Olivebioteq 2006- November 5th-10th-Mazara del Vallo, Marsala (Italy), Volume 1, **2006**, 95-102.
[23] S. Bartolini, L. Andreini, R. Guerriero, Gentili M., Improvement of the quality of table olives in Tuscany through cross-breeding and selection: preliminary results of Leccino x Konservolia hybrids, Proceedings Second International Seminar Olivebioteq 2006- November 5th-10th-Mazara del Vallo, Marsala (Italy), Volume 1, **2006**, 143-146.
[24] L. León, R. De La Rosa, D. Barranco, Rallo L., Agronomic characterization of 15 selections of then olive crossbreeding program of Cordoba, Spain, Proceedings Second International Seminar Olivebioteq **2006** - November 5th-10th-Mazara del Vallo, Marsala (Italy), Volume 1, **2006**, 87-93.
[25] G. Padula, E. Giordani, E. Bellini, A. Rosati, S. Pandolfi, A. Paoletti, G. Pannelli, V. Ripa, F De Rose, E. Perri, A. Buccoliero, Mennone C., *Adv. Hort. Sci*., **2008**, 22(2), 87-94.
[26] C.O.I. Méthodologie pour la caractérisation primaire des variétés d'olivier, **1997**, Projet RESGEN-CT (67-97), Union Européenne/Conseil Oléicole International.
[27] U.P.O.V. Principes directeurs pour la conduite de l'examen des caractères distinctifs, de l'homogénéité et de la stabilité de l'olivier *Olea europaea* L., **1985**, Union internationale pour la Protection des Obtentions Végétales (UPOV), pp 21.
[28] H. Hannachi, M. Msallem, S. Ben Elhadj, El Gazzah M., *Comptes Rendus Biologies*, **2007**, 330, 135-142.
[29] M. Mezghani Aïachi, Trigui A., Olivae, **2001**, 87, 45-49.
[30] I. Rjiba, S. Dabbou, N. Gazzah, Hammami M., *Chemistry & biodiversity*, **2010**, 7, 649-655.
[31] G. Fontanazza, G. Vergari, M. Patumi, Giorio G., *Acta Hort.* (ISHS), **1999**, 474, 97-102.
[32] L. León, M. Uceda, A. Jiménez, L. M Martin, Rallo L., *Spanish Journal of Aricultural Research*, **2004**, 2 (3), 353-359.
[33] E. R. Trentacoste, Puertas C.M., *Euphytica*, **2011**, 177, 99-109.
[34] A. Lansari, Tahri Hassani J. B., Olivae, **1996**, 60, 42-47.
[35] M. Hagidimitriou, A. Katsiotis, G. Menexes, C. Pontikis, Loukas M., Jo*urnal of the American Society for Horticultural Science*, **2005**, 130, 211-217.
[36] F. P. Marra, R. Buffa, G. Campisi, C. F. Osta, C. Di Vaio, M. La Farina, M. La Mantia, R. Mafrica, A. Motisi, R. Zappia, Caruso T., Morphological and SSR molecular markers based genetic variability in 39 olive cultivars (*Olea europaea* L.) originated in Southern Italy. Proceedings Second International Seminar - November 5th-10th-Mazara del Vallo, Marsala (Italy), **2006**, Volume 1, 213-216.
[37] N. Grati Kamoun, F. Lamy Mahmoud, A. Rebai, A. Gargouri, O. Panaud, Saar A., Genetic diversity (inter and intra-varietal) of some tunisian olive tree cultivars detected by AFLP markers. Proceedings Second International Seminar Olivebioteq **2006** - November 5th-10th-Mazara del Vallo, Marsala (Italy), **2006**, Volume 1, 45-52.

[38] C. Gregoriou, Genetic diversity and evaluation of thirty-one clones of the Local or Ladoelia olive variety in Cyprus. Proceedings Second International Seminar Olivebioteq **2006** - November 5th-10th-Mazara del Vallo, Marsala (Italy), **2006**, Volume 1, 117-121.
[39] A. Diaz, R. De La Rosa, P. Rallo, C. Munoz-Diez, I. Trujillo, D. Barranco, A. Martin, A. Belaj, *Crop Science*, **2007**, 47, 2317-2322.
[40] E. Bellini, E. Giordani, Rosti A., *Adv.Hor.Sci*., **2008**, 22(2), 73-86.
[41] M. Fendri, I. Trujillo, A. Trigui, M. I. Rodríguez-García, Alché Ramírez J. D., *Hort Science*, **2010**, 45, 1429-1436.
[42] A. Trigui, Msallem M., 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, **2002**, Volume I, 159 pp.