check for updates

RELIABILITY OF MORPHOLOGICAL CHARACTERS IN IDENTIFICATION OF OLIVE (*Olea europaea L.*) VARIETIES IN *Ex-Situ* CONDITIONS

Fathi Ben Amar[1+]
Hana Souabni[2]
Olfa Saddoud-Debbabi[3]
Mohamed Ali Triki[4]

[1,4]*University of Sfax, Olive Institute, Sfax, Tunisia.*
[1]*Email:* fathibenamar@yahoo.fr
[4]*Email:* trikimali@yahoo.fr

[2]*University of Tunis-El Manar, Faculty of Mathematics, Physics and Natural Sciences, Campus Universitaire, El Manar II, Tunis, Tunisia.*
[2]*Email:* Souabni.hana@gmail.com

[3]*Banque Nationale de Genes, Boulevard du Leader Yesser Arafet, Tunisia.*
[2]*Email:* Olfa.lf@gmail.com

(+ Corresponding author)

ABSTRACT

The study of the morphological characters of olive fruit and endocarp was carried out for 48 varieties planted in the collection of Boughrara (Sfax, Tunisia) and was compared to their characterization in the Tunisian catalog in their centers of origin. The study interested 8 characters for the fruit and 7 characters for the endocarp. The similarity rate was 73.3% for fruit characters and 64.6% for endocarp characters and eight characters of the two organs had similarity rates greater than 70%, particularly the weight, the apex shape and the presence of the nipple. Most varieties had similar identification for a total number of characters between 8 to 14 characters. The data revealed that the fruit contributed with 5 to 6 characters in the similarity of the varieties in the two databases against 4 to 5 characters for the endocarp. The results obtained showed a relatively strong discriminating power of the morphological characters in the identification of the different olive varieties in-situ and ex-situ conditions with an advantage for the fruit characters.

Contribution/Originality: In the present work, we used morphological characterization in situ conditions compared to in-situ conditions for 48 olive varieties. The comparison of endocarp and fruit characters allowed to see how much is the importance of these characters in the characterization of varieties in different environments.

1. INTRODUCTION

Around 2,629 cultivars are attributed to olive, according to FAO [1]. The problem of olive germplasm classification is not only complicated by the richness of its genetic patrimony, but also by the absence of reference standards and by the confusion regarding the cultivar names, with numerous cases of homonymy (one denomination for several genotypes) and synonymy (one genotype with several denominations) [2]. Thus, the identification of olive cultivars is hindered by the high number of synonyms and homonyms commonly used in the areas of traditional cultivation [3].

Since the 80s, much work has been done to characterize olive germplasm mainly by morphological and molecular tools [3]. The first adopted aspect of characterization was the morphology of the tree organs. The work of FAO [4] proposed a collaborative program on the genetic resources of the olive tree and then the olive varieties of Andalusia had been characterized [5]. Later, the primary characterization guide for the olive tree was published by the International Olive Council and introduced a more exhaustive number of characters relating to the tree, the leaf, the inflorescence, the fruit and the endocarp (30 characters in total) [6]. The morphological characterization of

56

olive varieties on a world scale based on the IOC protocol has permitted to edit the world catalog of the olive tree [7]. This catalog brought together 139 varieties of olive trees from 23 countries. The same IOC guide was used to edit a number of national catalogs, such as the Spanish catalog [8] the Tunisian catalogs [9, 10] and the Italian catalog [2].

The systematic utilization of descriptive morphological characters of the tree and various tree organs has enabled the characterization and the discriminatory identification of varieties [2]. On the other hand, the morphological characterization has permitted to elucidate more than 3000 synonyms in Italian olive germplasm [11-13] which include about 800 cultivars [2].

In Tunisia, morphological characterization has been able to reveal homonymy cases mainly regarding to the most cultivated variety 'Chemlali' which has been split into several varieties [9]. This result was confirmed by molecular analyzes [14].

Regarding the importance of olive morphological characters, it had been concluded that the characters of the leaf, the inflorescence, the fruit and the endocarp were the most discriminators between olive varieties [7]. Nevertheless, several authors estimated that the endocarp characters were the most reliable [9]. On the other hand, the fruit and endocarp weights seemed to be the most discriminating traits [15]. More importantly, the endocarp traits have been described as strong discriminative characteristics and a powerful complementary tool to molecular techniques [16, 17].

The effect of environmental conditions and agronomical factors on the morphological traits was reported by many authors [18-22]. The morphological characters were particularly stables and consequently not affected by the environment such as the tree habit and the shape of fruit, endocarp and leaf [18]. Nevertheless, the environmental effect was not important in the morphological characterization of 48 olive tree seedlings [15]. These authors concluded that the genotype seemed to influence the morphological characters.

While no study has been undertaken to elucidate both the limit of morphological characterization in varietal identification and the most discriminating morphological markers, it is not always possible to identify olive varieties on the basis of morphological characters [18]. Thus, other markers have been used in olive identification, such as enzyme markers [23] agronomic markers [6] and DNA markers [16, 24, 25].

The objective of this work is to compare the results of morphological characterization of 48 Tunisian olive varieties ex-situ conditions of the Boughrara collection (Sfax, Tunisia) with those obtained in their sites of origin (in-situ) in order to test the reliability of the olive morphological characters.

2. MATERIAL AND METHODS

The study was carried out on 48 Tunisian olive varieties Table 1 grown at the national collection of Boughrara- Sfax (south of Tunisia: long. 34°59'05" N, lat. 10°32'56", elevation 125 m.a.s.l). The region of Sfax received an average rainfall of 200 mm per year. Olive trees were planted since 1992 in rainfed and arid conditions with a density of 104 trees per hectare (8m/12m).

The choice of only 48 varieties from the Boughrara collection was dictated by their morphological description in-situ conditions and in the centers of origin [9]. These centers were located in different parts of Tunisia and the in-situ varietal description was done in rainfed conditions and with local farmer's practices.

In Boughrara conditions, it was practically impossible to had olive production for all these varieties in several years and in the same trees. So, we concentrated our effort to had olive production for all varieties in one year.

For each variety in Boughrara collection in November 2018/2019 season, one adult and productive olive tree sample was chosen. For each tree, morphological observations were made on 40 randomly chosen fruits. After fruit characterization, the endocarp was removed and subjected to characterization.

The same characters used in the centers of origin [9] were evaluated in this study. Specifically, eight fruit characters were noted: weight (1), shape (2), symmetry (3), position of maximum transverse diameter (PDM) (4),

apex shape (5), base shape (6), presence of lenticels (7) and presence of nipple (8). Seven characters were selected for the endocarp: weight (9), shape (10), symmetry (11), position of maximum transverse diameter (12), apex shape (13), base shape (14) and roughness of surface (15). For each character, the attribute which had the highest number from the 40 fruits and endocarps was given following the protocol described by IOC [6]. For all characters, attributes were given according to the classification for primary characterization of olive varieties reported by the IOC [6].

The obtained *ex-situ* database for all varieties and characters was compared to that recorded in the *in-situ* areas of the same 48 varieties [9]. According to this comparison, the following parameters were recorded:

- The number of similar attributes in the two databases for each character among all the studied varieties and a percentage was calculated.
- The total number of characters with similar attributes for each variety which consequently give us the distribution of varieties with the same number. In this case, we presented the trend curve of this distribution with the highest coefficient of determination (R^2).
- The number of fruit characters and endocarp characters within the total number with similar attributes for each variety. Thus, data were subjected to analysis of variance with variety as replicate and separation of the means of the fruit and the endocarp characters was obtained using the least significant difference at $P<0.05$. Descriptive statistic parameters (minimum, maximum and mean values) and coefficient of variation were performed.

The statistical analysis was performed using the SPSS 13.0 for windows.

Table-1. Number, name and origin of local varieties used in the study.

Variety	Name	Origin	Variety	Name	Origin
1	Chemlali Tataouine	Tataouine	25	Chetoui	Ariana
2	Chemlali Ontha	Tataouine	26	Meski	Ariana
3	Fakhari Douirat	Tataouine	27	Besbessi	Ariana
4	Zarrazi Injassi Douirat	Tataouine	28	R'khami	Ariana
5	Toffahi	Tataouine	29	Barouni du nord	Ariana
6	Dhokar ben Gardene	Medenine	30	Marsaline	Ariana
7	Chemlali Zarzis	Medenine	31	Sayali du Nord	Nabeul
8	Zarrazi Zarzis	Medenine	32	Zarrazi du Nord	Nabeul
9	Chemlali meliane	Medenine	33	Jemri ben Gardene	Medenine
10	Chemlali Balhi	Medenine	34	Dhokar Nafti	Medenine
11	Indouri Jerba	Medenine	35	Injassi Hchichina	Sfax
12	Chemlali Chouamekh	Medenine	36	Kbiret Louzir	Sfax
13	Jemri Bouchouka	Medenine	37	Zarbout Louzir	Sfax
14	Chemlali Sfax	Sfax	38	Semni Jebeniana	Sfax
15	Chemlali Ghraiba	Sfax	39	Khechinet sig	Sfax
16	Chemlali O Msallem	Sfax	40	Zalmati Zarzis	Medenine
17	Ech-Chahla	Sfax	41	Chemlali Bent Louzir	Sfax
18	Chemchali Gafsa	Gafsa	42	Sahli Gafsa	Gafsa
19	Fougi Asli	Gafsa	43	Chemlali du Nord	Ariana
20	Baldi Gtar	Gafsa	44	Gerboui du Nord	Beja
21	Tounsi Gafsa	Gafsa	45	Balhi Sig	Sfax
22	Injassi Gafsa	Gafsa	46	Mlouki Bletteche	Sfax
23	Mengar Erragma	Kasserine	47	Bidh Hmam	Kasserine

3. RESULTS AND DISCUSSION

3.1. General Similarity Matrix

The comparison of our data with those *in-situ* conditions allowed us to obtain Table 2. For all characters and varieties combined, the number of characters similar to the two databases was 493, which represented 69.2% of the total.

Table-2. Matrix of similarity of fruit and endocarp characters in the two databases (White: similar, Gray: different, Black: not noted by Trigui and Msallem [9]).

Variety	Fruit characters								Endocarp characters						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1			G	G											
2			G	G				G							G
3	G		G	G			G	B					G		
4			G	G					G		G				
5							G	G							
6		G								G	G			G	G
7												G			
8														G	G
9		G								G	G				G
10			G	G		G	G	G			G		G		
11		G									G				
12		G	G			G							G		
13					G		G								G
14										G					
15			G				G								
16			G				G			G	G	G			
17			G												
18			G				G				G				
19	G						G		G					G	G
20	G		G				G		G						
21		G			G			G						G	
22			G	G					G					G	
23			G								G				
24		G								G	G		G	G	G
25			G					G		G		G	G	G	
26		G		G					G			G	G		
27		G									G				
28					G	G		G			G				
29		G							G						
30			G			G						G	G		
31		G			G			G	G					G	
32							G		G					G	
33			G							G		G			G
34							G				G		G		G
35		G					G								
36								G							
37			G		G		G	B			G	G	G	G	
38				G			G								
39			G			G	G				G				
40				G		G				G					
41		G						B							
42			G				G				G				
43		G						B		G	G			G	
44					G					G				G	
45				G		G			B		G			G	G
46									B					G	G
47			G					B		G	G			G	
48	G			G	G			B		G	G			G	G

For the fruit, the number of similar cases was 277 out of a total of 378, giving a rate of 73.3%, while this number was 216 out of a total of 334 for the endocarp, which gave a rate of 64.6. %.

The similarity rates not exceeding 74% indicated mainly the effect of the environment on the expression of morphological characters, in agreement with the conclusions of several studies [14, 20]. In our case, the problem was to compare the olive varieties in the centers of origin (in-situ) and in a varietal collection (ex-situ).

The level of similarity for the fruit was significantly higher than that for the endocarp, which contradicted the ideas pronounced by several authors [9, 16, 17]. This contradiction can be explained by the differences in climatic and cultural conditions between the in-situ and ex-situ conditions but also by the subjectivity of the description.

The distribution of the varieties according to the number of similar characters Figure 1 showed that only six varieties having a number of similar characters less than or equal to 7. Most of the studied varieties had a similarity at the level of 8 to 14 characters with a peak at the class level of 11 characters (11 varieties).
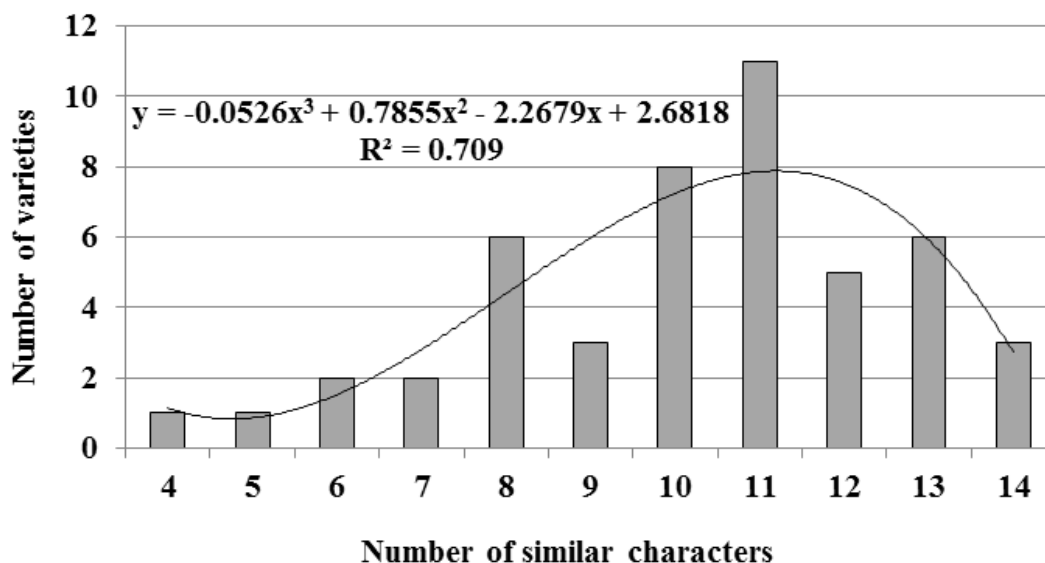

Figure-1. Distribution of olive varieties according to their similar characters number between *in situ* and *ex situ* situations and the distribution curve with equation.

According to Table 2, the number of similar characters varied widely among varieties but it did not depend on original sites. Thus, varieties with similar characters higher than 12 belonged to south (Chemlali Tataouine, Chemlali Zarzis and Indouri Jerba) and central (Chemlali Sfax, Kbiret Louzir and Semni Jebeniana) parts of Tunisia. On the other hand, eight varieties having less than 8 similar characters were located in different regions of Tunisia, Fakhari and Chemlali Balhi from the south west, Fougi Asli, Souabaa Aljia and Oueslati from the Centre west, Zarbout Louzir from the centre east and Meski, Barouni du Nord, Sayali du Nord and Chemlali du Nord from the north east. These results confirmed that the ex-situ morphological characterization might be considered more suitable for olive varieties since it reduced the maximum environment effect in the in-situ situation.

The mathematical model for this distribution was a polynomial type with an inflection point at the 11th character class level. The maximum area between the curve and the abscissa axis involved the classes of 8 to 14 characters and a total of 42 varieties. This finding demonstrated the significant discriminatory power of morphological characters in accordance with previous conclusions [2, 9, 15].

3.2. Similarity of Fruit and Endocarp Characters

Figure 2 showed the percentages of similarity of varieties between the site of origin and the Boughrara olive collection for each character of the fruit. It was clear that this percentage varied according to the character with values ranging from 45.8 to 91.6%.
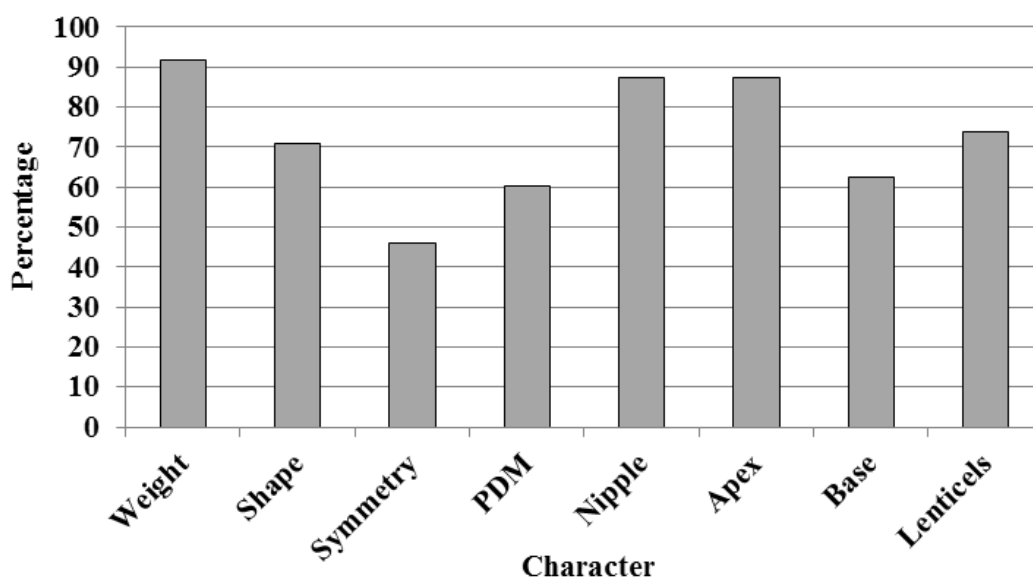
Figure-2. Similarity percentages of the different fruit characters between *in situ* and *ex situ* conditions.

The symmetry of the fruit was the only character to have a percentage below 50% of similarity. The highest values were recorded for weight, presence of the nipple and shape of the apex characters (> 85%). The weight of the fruit showed the maximum similarity of the olive varieties in ex-situ and in-situ situations, with 91.6%.

The percentages of similarity of the endocarp characters presented in Figure 3 showed a wide variability ranging from 43.75% for symmetry to 79.16% for the endocarp apex shape. The weight and the endocarp surface also showed high percentages (> 70%).
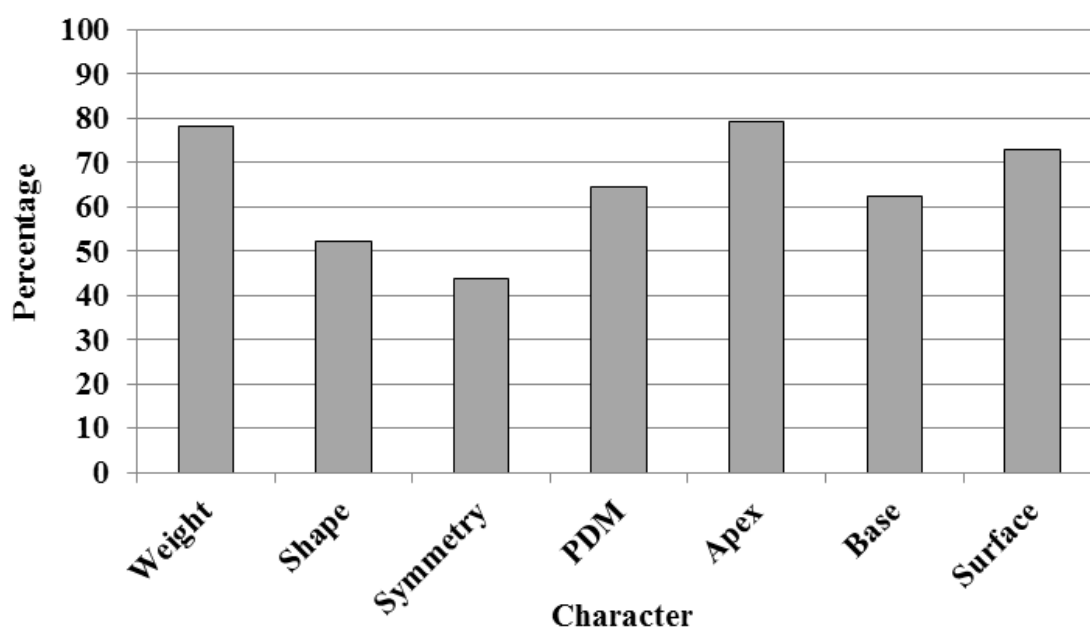

Figure-3. Similarity percentages of the different endocarp characters between *in situ* and *ex situ* situations.

The comparison of the similarity percentages of the fruit characters and the endocarp characters allowed us to present the following observations.

First, the symmetry of the two organs recorded the lowest percentages (43 and 46% respectively). The weakness of this character could be explained by the following reasons:

- This qualitative character was appreciated visually and thus led to an experimental error, as for all qualitative characters.

61

- The symmetry of the fruit and the endocarp was strongly affected by the endogenous factors of the tree (olive production) and the exogenous factors (crop management, climate).

The strong similarity of the weight of the two organs might be due to the quantitative nature of this character. The good reliability of fruit and endocarp weight had been confirmed on olive progenies [15]. On the other hand, previous studies had demonstrated the importance of the quantitative characters of the fruit and the endocarp in the analysis of the olive biodiversity [9, 14, 26].

This study revealed that eight morphological characters of the two organs had a similarity rates higher than 70%. Consequently, at least 7 varieties out of 10 could be identified based on these characters, 35 varieties in our case. Since the similarities found for the different varieties were not necessarily related to the same characters as presented in Table 1, other varieties could be identified by other fruit and endocarp characters having a similarity rates less than 70% such as the shape, the position of maximum transverse diameter, the symmetry and the base shape. As significative example, the variety Fougi Asli was easily identified by only four similar characters, fruit apex and base shapes, nipple and endocarp shape. In fact, none of the other studied varieties had rounded fruit apex and base with tenuous nipple and rounded endocarp apex with mucro, as reported by in-situ study [9]. Therefore, it could be noted that most of the studied olive varieties might be correctly characterized by the morphology of its organs, which was in harmony with the mathematical curve presented in Figure 1. This conclusion did not confirm the suggestions of several authors [14, 18, 23] but confirmed on the other hand other studies [15]. It could be concluded that the genotype strongly affected the expression of the most morphological characters and the effect of the environment, although existing, was not decisive in the identification of olive varieties.

The examination of the similarity in terms of number of characters of the fruit and the endocarp revealed that the average number of similar characters of the fruit (between 5 and 6) was significantly higher than that of the endocarp (between 4 and 5), according to Table 3. These data indicated that the average total number of similar characters varied from 9 to 11, which confirmed the results presented in Figure 1.

The number of similar characters of the fruit varied from 2 to 8 depending on the variety and from 1 to 7 for the endocarp. However, the variation of this number for the fruit was lower than that for the endocarp with coefficients of variation of 23.8 and 36.3% respectively.

Table-3. Statistical parameters of similar characters number related to fruit and endocarp of studied olive varieties.

Statistical parameter	Fruit	Endocarp
Minimum	2	1
Maximum	8	7
Mean	5.68 a	4.54 b
SD	1.35	1.65
CV (%)	23.82	36.32

Note: Means with the same letter were not statistically different

The data presented in table 3 showed that the characters of the fruit allowed better identification of olive varieties by the high average number of similar characters and the small variation in this number between varieties. It seemed that the fruit was the organ of the olive tree which allowed better discrimination of olive varieties. This result did not confirm the idea of several authors [9, 16, 17] for whom the endocarp had better power of discrimination than the fruit.

4. CONCLUSION

The morphological comparison study carried out between in-situ and ex-situ conditions of the olive tree showed that the characters of the fruit were more discriminating than those of the endocarp. Eleven characters from the two organs were able to identify more than 70% of olive varieties, particularly the weight, the apex shape and the presence of the nipple. Thus, morphological characterization of olive varieties could be a useful tool for olive

characterization in plant propagation program and plant certification procedure. However, molecular tools will be necessary for varieties showing high level of synonymy. This work on similarity of olive morphological characters might be extended to the olive varieties planted at the same time in national collections and international collections (Cordoba and Marrakech) in order to confirm the reliability of these characters.

REFERENCES

[1]   FAO, "The second report on the state of the world's plant genetic resources for food and agriculture, FAO commission on genetic resources," ed Italia: Ed FAO, 2010, p. 402.

[2]   I. Muzzalupo, "Olive Germplasm – Italian Catalogue of olive varieties," ed United Kingdom: INTECH Open Science (Ed), 2012, p. 420.

[3]   E. Bellini, E. Giordani, and A. Rosati, "Genetic improvement of olive from clonal selection to cross-breeding programs," *Advances in Horticultural Science*, vol. 22, pp. 73-86, 2008.

[4]   FAO, "Proposition d'un programme coopératif sur les ressources génétiques de l'olivierc," presented at the Report of the FAO Olive Production Committee. 4th session. Madrid June 9-12, 1981.

[5]   D. Barranco and L. L. Rallo, *The olive varieties grown in Andalusia*. Spain: Instituto de Estudios Agrarios, Pesqueros y Alimentarios (Ed), 1984.

[6]   IOC, "Methodology of primary and secondary characterization of olive varieties," ed: European Union/International Olive Council (Ed), 1997, p. 10.

[7]   D. Barranco, A. Cimato, P. Fiorino, L. Rallo, A. Touzani, C. Castaneda, F. Serafini, and I. Trijillo, *World catalogue of olive varieties*. Madrid , Spain: International Olive Oil Council, 2000.

[8]   D. Barranco and L. Rallo, "Olive cultivars in Spain," *Hort Technology*, vol. 10, pp. 107-110, 2000.

[9]   A. Trigui and M. Msallem, *Olive trees from Tunisia: Ministry of agriculture, IRESA (Ed)* vol. 1. Tunisia: Institut de l'Olivier, 2002.

[10]   F. Ben Amar, S. Ben Maachia, and A. Yengui, *Catalog of genetic resources of the olive tree of the oasis of Degache*. Rome, Italy: FAO, 2017.

[11]   G. Bartolini, C. Messeri, and G. Prevost, "Olive tree germplasm: Descriptor lists of cultivated varieties in the world," *Acta Horticulturae*, vol. 356, pp. 116-118, 1994.

[12]   G. Bartolini, G. Prevost, C. Messeri, and G. Carignani, *Olive germplasm: Cultivars and world-wide collections*. Rome, Italy: FAO Library, 1998.

[13]   G. Bartolini, G. Prevost, C. Messeri, and G. Carignani, "Olive cultivar names and synonyms and collections detected in a literature review," *Acta Horticulturae*, vol. 474, pp. 159-162, 1999.

[14]   N. Grati Kamoun, F. Lamy Mahmoud, A. Rebai, A. Gargouri, O. Panaud, and A. Saar, "Genetic diversity of Tunisian olive tree (Olea europaea L.) cultivars assessed by AFLP markers," *Genetic Resources and Crop Evolution*, vol. 53, pp. 265–275, 2006.

[15]   I. Laaribi, M. Mezghani-Aiachi, and M. Mars, "Phenotypic diversity of some olive tree progenies issued from a Tunisian breeding program," *European Scientific Journal*, vol. 10, pp. 292-313, 2014.

[16]   M. Fendri, I. Trujillo, A. Trigui, M. I. Rodriguez-Garcia, and J. D. Alché Ramirez, "Simple sequence repeat identification and endocarp characterization of Olive tree accessions in a Tunisian germplasm collection," *HortScience*, vol. 45, pp. 1429-1436, 2010.

[17]   A. El Bakkali, A. Mekkaoui, S. El Iraqui El Houssaini, A. Essarioui, and B. Khadari, "Addressing the challenge of cultivars identification and authentication in mediterranean olive collections: A case study in Morocco," *European Scientific Journal*, vol. 16, pp. 339-35, 2020.

[18]   G. Fontanazza, *Genetic and technical aspects of propagation for intensive plantation," In World Olive Encyclopedia. IOC (Eds.).* Madrid, Spain: International Olive Oil Council, 1997.

[19]   G. Besnard, P. Baradat, and A. Berville, "Genetic relationships in the olive (Olea europaea L.) reflect multilocal selection of cultivars," *Theoretical and Applied Genetics*, vol. 102, pp. 251-258, 2001.

[20]   A. Belaj, G. Cipriani, R. Testolin, L. Rallo, and I. Trujillo, "Characterization and identification of the main Spanish and Italian olive cultivars by simple-sequence-repeat-markers," *HortScience*, vol. 39, pp. 1557–1561, 2004.

[21]   H. Hannachi, M. Msallem, S. Ben Elhadj, and M. E. Gazzah, "Influence of the geographical site on the agronomic and technological potentialities of the olive tree (Olea europaea L.) in Tunisia," *Comptes Rendus Biologies*, vol. 330, pp. 135-142, 2007.

[22]   G. Padula, E. Giordani, E. Bellini, A. Rosati, S. Pandolfi, A. Paoletti, G. Pannelli, V. Ripa, F. De Rose, E. Perri, A. Buccoliero, and C. Mennone, "Field evaluation of new olive (Olea europaea L.) selections and effects of genotype and environment on productivity and fruit characteristics," *Advanced Certificate in Horticulture*, vol. 22, pp. 87-94, 2008.

[23]   I. Trujillo, L. Rallo, and P. Arus, "Identifying olive cultivars by isozyme analysis," *Journal of the American Society for Horticultural Science*, vol. 120, pp. 318–324, 1995.

[24]   A. Belaj, I. Trujillo, R. De la Rosa, L. Rallo, and J. MGime´Nez, "Polymorphism and discriminating capacity of randomly amplified polymorphic markers in an olive germplasm bank," *Journal of the American Society for Horticultural Science*, vol. 126, pp. 64–71, 2001.

[25]   O. Saddoud Debbabi, M. Marilena Miazzi, O. Elloumi, M. Fendri, F. Ben Amar, M. Savoia, S. Sion, H. Souabni, S. Rahmani Mnasri, S. Ben Abdelaali, F. Jendoubi, G. Mangini, F. Famiani, F. Taranto, C. Montemurro, and M. Msallem, "Recovery, assessment, and molecular characterization of minor olive genotypes in Tunisia," *Plants*, vol. 9, p. 382, 2020.

[26]   R. S. Mnasri, D. O. Saddoud, and A. Ferchichi, "The study of olive oil quality and morphological biodiversity of Olea europaea L. in the region of "Hbebsa," *Journal of Biodiversity and Environmental Sciences,*, vol. 4, pp. 59-66, 2013.