Addressing the Challenge of Cultivars Identification and Authentication in Mediterranean Olive Collections: A Case Study in Morocco

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Abstract

Conservation and use of well-characterized olive (Olea europaea L.) genetic resources are the key to future olive improvement and sustainable production. Yet, authentication of plant materials in ex-situ olive collections throughout the world has received little attention. Here we characterized 95 accessions, from a collection maintained in the experimental station of INRA-Meknes, Morocco, by comparing their SSR (14 markers) and morphological (11 endocarp traits) profiles to an international reference dataset with 672 distinct genotypes corresponding to 535 well-described olive cultivars from the two Worldwide Olive Germplasm Banks of Marrakech, Morocco, and Cordoba, Spain (WOGB-M/C). Results revealed 122 alleles in the Meknes collection versus 265 in the reference database, but the difference was not significant. Additionally, forty cultivars were identified in Meknes collection, among which 33 were present in the reference database. Principal Coordinates Analysis revealed that these varieties span the range of all of the 535 varieties in the international database, indicating important genetic diversity within the investigated plant materials. Finally, cases of mislabeling errors, synonyms, and redundant genotypes pertaining mainly to "Picholine marocaine" and

"Frantoio" varieties have been encountered in Meknes collection. Overall, our work highlights the power of coupling modern genetic and morphological tools along with exploring reference databases for authenticating genetic cultivars in olive tree collections.

Keywords: Olea europaea L., Simple sequence repeats (SSR), Meknes olive collection, Worldwide Olive Germplasm Banks (WOGB), Olive database

Introduction

Olive tree (*Olea europaea* L.) is a major agricultural crop in the Mediterranean basin with approximately 95% of the world's olive production (IOOC, 2016), used mostly for oil extraction and canning. Domestication of olive tree in the Mediterranean has taken place over more than 6,000 years through mass selection and clonal propagation (Kaniewski et al., 2012). This has led to the selection of a wide range of cultivars. Specifically, more than 1,200 varieties have been reported in 54 countries and are preserved for conservation and research purposes in nearly 100 distinct collections throughout the globe (Bartolini, 2008). Because of clonal propagation, olive tree has been disseminated greatly across the Mediterranean basin, which resulted in many cases of synonymy (different names for the same cultivar; Barranco et al., 2000), homonymy (same name for different cultivars; Barranco et al., 2005) and molecular variants (intra-varietal variation; Cipriani et al., 2002; Khadari et al., 2008; El Bakkali et al., 2013a). Additionally, mislabeling errors that occurred in the process of establishing germplasm collections have added to the complexity of plant material management (Trujillo et al., 2014; El Bakkali et al., 2019). These issues emphasize the significance of cultivar identification to enhancing our ability for precise classification and authentication of cultivars. Indeed, several studies highlighted the importance of using Simple Sequence Repeats molecular markers (SSR) in characterizing germplasm collections through the exploration of genetic diversity (Sarri et al., 2006; Khadari et al., 2008; Baldoni et al., 2009; Haouane et al., 2011; Belaj et al., 2012; Diez et al., 2012; El Bakkali et al., 2013b; Trujillo et al., 2014). Perhaps more importantly, endocarp traits, being strong discriminative morphological characteristics, have also been described as a powerful complementary tool to molecular techniques that allow increasing identification resolution at the intra-varietal variation level (Belaj et al., 2012; Trujillo et al., 2014; El Bakkali et al., 2019). SSR markers and endocarp traits were routinely used to scrutinize olive germplasm preserved in the two largest worldwide olive germplasm banks (WOGB) in Marrakech-Morocco (Haouane et al., 2011; El Bakkali et al., 2013b) and Cordoba-Spain (Belaj et al., 2012; Diez et al., 2012, Trujillo et al., 2014). Recently, an attempt was conducted to establish one single database by

characterizing and comparing the 1,091 olive accessions from 22 countries in the two WOGB-M/C collections using 20 SSR markers and 11 endocarps traits (El Bakkali et al., 2019). This collaborative effort identified a total of 672 distinct genotypes, corresponding to 535 well-characterized cultivars, in which 211 cultivars were authenticated. The available database of 672 distinct genotypes provides comparable information about Mediterranean olive germplasm and can serve as a repository data for research on the identification of cultivars and management of olive accessions in local and regional collections throughout the Mediterranean basin. Nonetheless, this database needs enrichment over time for a more extensive referential on olive genetic resources.

In addition to the genetic material preserved in the WOGB of Marrakech, Morocco has another *ex-situ* large collection of olive germplasm maintained in the experimental station of the National Institute for Agricultural Research (INRA) in the Meknes region (long. 33.931031; lat. -5.274508). This valuable collection has remained poorly explored since its establishment in the fifties (CND, 1955), and recently underwent a rejuvenation process through cutting transplantation with a high risk of mislabeling errors. Such constraints impede the potential of its exploitation as an-yet untapped resource for advancing breeding programs. In this investigation, we build up on previous results from the work carried out on the two WOGBs of Marrakech and Cordoba to characterize the Meknes collection using SSR markers and endocarp traits. Specifically, the objective of this study is to perform accurate identification of accessions and unravel mislabeling errors in the collection to construct a more comprehensive understanding of olive genetic diversity in Morocco and the Mediterranean. This study emphasizes the importance of the use of a comprehensive database to identify and authenticate olive varieties towards use in breeding programs.

Materials & Methods

Plant material and SSR genotyping

Ninety five olive trees from the Meknes collection corresponding to 83 accessions and 79 denominations from 9 countries were used in this study (Table 1). For each individual tree, total DNA was extracted from 1g of young leave tissue as described by Khadari et al. (2008). DNA was quality-checked using 0.8% agarose gel electrophoresis and quantified by spectrofluorometry (GENios Plus, TECAN, Grödig, Austria). Fourteen SSR loci (Table 2) were PCR-amplified in the same conditions as described by El Bakkali et al. (2019). These markers were selected based on their clear amplification, high polymorphism and reproducibility as observed previously by many authors (Baldoni et al., 2009; Haouane et al., 2011; El Bakkali et al., 2013b & 2019; Trujillo et al., 2014). PCR products were separated using an automatic

capillary sequencer (ABI prism 3130XL Genetic Analyzer Applied Biosystems, Foster City, CA, USA), using GeneScan 400 HD-Rox as internal standard, and chromatograms were then visualized and analyzed with GeneMapper 3.7 software (Applied Biosystems). The generated dataset was compared to a reference database of 672 genotypes identified from the two WOGB-M/C collections (El Bakkali et al., 2019).

Table 1. List of the accessions in Meknes collection with their codes, origins, corresponding cultivar names and main cultivation areas

	Code in collection	Accession name	Origin	Cultivar name	Origin	Comment
1	MEK025	Alfafara	Spain	Picholine marocaine	Morocco	Mislabelling
2	MEK015	Amellau	France	Amellau	France	
3	MEK028	Americana	Italy	Frantoio	Italy	Mislabelling
4	MEK070	Arbequina	Spain	Arbequina-70	Spain	Mislabelling
5	MEK0V3	Arbequina	Spain	Arbequina	Spain	
6	MEK0V7	Ayvalik	Turkey	Leccino	Italy	Mislabelling
7	MEK005(1)	Azeradji	Algeria	Azeradji-005	Algeria	Mislabelling
8	MEK005(2)	Azeradji	Algeria	Morisca	Italy	Mislabelling
9	MEK026	Barouni du Nord	Tunisia	Lechin de Sevilla	Spain	Mislabelling
10	MEK073(1)	Blanqueta	Spain	Blanqueta	Spain	
11	MEK073(2)	Blanqueta	Spain	Blanqueta	Spain	
12	MEK068	Blanquette De Gastu	Algeria	Chetoui	Tunisia	Molecular variant and synonyme of Chetoui (Cimato and Attilio, 2003; El Bakkali et al., 2019)
13	MEK074(1)	Blanquette de Guelma	Algeria	Picholine marocaine	Morocco	Mislabelling
14	MEK074(2)	Blanquette de Guelma	Algeria	Picholine marocaine	Morocco	Mislabelling
15	MEK004	Bouchouk Soummam	Algeria	Azeradj	Algeria	Synonymous of Azeradji (El Bakkali et al., 2019)
16	MEK035(1)	Canino	Italy	Ascolana tenera	Italy	Mislabelling
17	MEK035(2)	Canino	Italy	Picholine marocaine	Morocco	Mislabelling
18	MEK051	Carboncella	Italy	Craputea	Italy	Mislabelling
19	MEK029	Carmelitana	Italy	Frantoio	Italy	Mislabelling
20	MEK016	Castellana	Spain	Picholine marocaine	Morocco	Mislabelling
21	MEK067	Cellina Di Nardo	Italy	Blanqueta	Spain	Mislabelling
22	MEK024(1)	Changlot Real	Spain	Changlot Real	Spain	
23	MEK024(2)	Changlot Real	Spain	Ogliarola del Bradano	Italy	Mislabelling
24	MEK071(1)	Chemlal De Kabylie	Algeria	Chetoui	Tunisia	Mislabelling
25	MEK071(2)	Chemlal De Kabylie	Algeria	Chetoui	Tunisia	Mislabelling
26	MEK0V9	Chemlal De Kabylie	Algeria	Picholine marocaine	Morocco	Mislabelling
27	MEK077	Chemlali de Nord	Tunisia	Arbequina	Spain	Synonymous of Arbequine (El Bakkali et al., 2019)
28	MEK078	Chemlali de Sfax	Tunisia	Picholine marocaine	Morocco	Mislabelling
29	MEK072	Chetoui	Tunisia	Blanqueta	Spain	Mislabelling
30	MEK076(1)	Chetoui	Tunisia	Frantoio	Italy	Mislabelling
31	MEK076(2)	Chetoui	Tunisia	Frantoio	Italy	Mislabelling
32	MEK030	Coratina	Italy	Coratina	Italy	
33	MEK002	Cordovil de Serpa	Portugal	Madural	Portugal	Synonymous of Madural (Bracci, 1937 ; Trujillo et al., 1995 and 2014 ; El Bakkali et al., 2019)
34	MEK007(1)	Cornicabra	Spain	Cornicabra	Spain	Molecular variant of Cornicabra
35	MEK007(2)	Cornicabra	Spain	Picholine marocaine	Morocco	Mislabelling
36	MEK066	Correggiolo	Italy	Correggiolo-66	Italy	Mislabelling
37	MEK023	Dolce Del Morocco	Italy	Americano	Italy	Mislabelling
38	MEK061	Dritta di Moscufo	Italy	Dritta di Moscufo	Italy	
39	MEK014	Du Tell	Algeria	Picholine marocaine	Morocco	Synonymous of Picholine

40	MEK065	Frantoio	Italy	Frantoio	Italy	
41	MEK010	Galega	Portugal	Galega vulgar	Portugal	Molecular variant of Galega
42	MEK019	Galega D'Elvas	Portugal	Galega vulgar	Portugal	Synonymous of Galega Vulgar
43	MEK001	Galega Grada	Portugal	Madural	Portugal	Mislabelling
44	MEK045	Ghiandaro	Italy	Ghiandaro	Italy	
45	MEK033	Grappolo	Italy	Grappolo-33	Italy	Mislabelling
46	MEK044	Grossa de Sicilia	Italy	Passulunara	Italy	Possible synonymous of Passulunara
47	MEK008	Hojiblanca	Spain	Ocal	Spain	Mislabelling
48	MEK055	Lavagnina	Italy	Frantoio	Italy	Molecular variant and possible synonymous of Frantoio
49	MEK059(2)	Leccino	Italy	Picholine marocaine	Morocco	Mislabelling
50	MEK059(1)	Leccino	Italy	Leccino	Italy	
51	MEK009	Leucocarpa	Italy	Leucocarpa	Italy	
52	MEK075	Limli	Algeria	Picholine marocaine	Morocco	Synonymous of Picholine marocaine (El Bakkali et al., 2019)
53	MEK062	Loretana	Italy	Dritta di Moscufo	Italy	Synonymous of Dritta di Moscufo (Barranco et al., 2000)
54	MEK036	Madonna Dell'Impruneta	Italy	Madonna Dell'Impruneta-	Italy	Mislabelling
55	MEK011	Madural	Portugal	Madural	Portugal	
56	MEK0V11	Manzanille de Sevilla	Spain	Villalonga	Spain	Mislabelling
57	MEK006	Marsalina	Tunisia	Picholine marocaine	Morocco	Mislabelling
58	MEK053	Maurino	Italy	Maurino	Italy	
59	MEK012	Meslala	Morocco	Meslala	Morocco	
60	MEK058	Mignolo	Italy	Americano	Italy	Mislabelling
61	MEK037(2)	Moraiolo	Italy	Picholine marocaine	Morocco	Mislabelling
62	MEK037(1)	Moraiolo	Italy	Moraiolo	Italy	
63	MEK060	Moraiolo	Italy	Moraiolo	Italy	
64	MEK056	Morellona Di Grecia	Italy	Frantoio	Italy	Mislabelling
65	MEK054	Morenillo	Italy	Frantoio	Italy	Mislabelling
66	MEK057	Nebbio	Italy	Picholine marocaine	Morocco	Mislabelling
67	MEK046	Nostrale	Italy	Frantoio	Italy	Mislabelling
68	MEK027	Oblonga	USA	Frantoio	Italy	Synonymous of Frantoio (Trujillo et al., 2014 ; Muzzalupo et al., 2014)
69	MEK047	Ogliarola	Italy	Ogliarola del Bradano	Italy	
70	MEK050	Olivella	Italy	Gremigno di Fauglia	Italy	Synonymous Gremigno di Fauglia
71	MEK022	Oliviere	France	Picholine marocaine	Morocco	Mislabelling
72	MEK031	Piangente	Italy	Piangente	Italy	
73	MEK0V4	Picholine marocaine	Morocco	Picholine marocaine	Morocco	
74	MEK017	Pigale	France	Picholine marocaine	Morocco	Mislabelling
75	MEK039	Pisciottana	Italy	Frantoio	Italy	Mislabelling
76	MEK034(1)	Racemo	Italy	Picholine marocaine	Morocco	Mislabelling
77	MEK034(2)	Racemo	Italy	Coratina	Italy	Synonymous of Coratina (Muzzalupo et al., 2014)
78	MEK032	Rama Pendula	Italy	Picholine marocaine	Morocco	Mislabelling
79	MEK064	Razzo	Italy	Frantoio	Italy	Synonumous of Frantoio (Perri et al., 1999)
80	MEK003(1)	Redondal	Portugal	Ascolana tenera	Italy	Mislabelling
81	MEK003(2)	Redondal	Portugal	Madural	Portugal	Mislabelling
82	MEK013	Ronde de la Menara	Morocco	Ronde de la Menara-13	Morocco	Mislabelling
83	MEK052	Rosciola	Italy	Dritta di Moscufo	Italy	Mislabelling
84	MEK040	Rotondella	Italy	Moraiolo	Italy	Mislabelling
85	MEK069	Rougette	Algeria	Arbequina	Spain	Mislabelling
86	MEK063	Rougette de Pignan	France	Frantoio	Italy	Mislabelling
87	MEK041	Sallela	Italy	Gremigno di Fauglia	Italy	Synonymous of Gremigno di Fauglia
88	MEK048	Serrana	Spain	Sevillenca	Spain	Synonymous of Sevillenca (Barranco et al., 2000)

89	MEK043	Serranas	Spain	Sevillenca	Spain	Synonymous of Sevillenca
90	MEK018	Tabelout	Algeria	Tabelout	Algeria	
91	MEK049	Taggiasca	Italy	Taggiasca	Italy	
92	MEK038	Teschi	Italy	Picholine marocaine	Morocco	Mislabelling
93	MEK021	Verdale	France	Picholine marocaine	Morocco	Mislabelling
94	MEK020	Verdial	Portugal	Verdial transmontana	Portugal	
95	MEK042	Vernina	Italy	Vernina	Italy	

Morphological characterization

Forty randomly chosen endocarps per tree were characterized independently by two experienced observers over two years (2016 and 2017) following the protocol described by Trujillo et al. (2014). Specifically eleven endocarp traits were used: weight, shape in position A, symmetry in positions A and B, position of maximum transverse diameter in position B, shape of apex in position A, shape of base in position A, roughness of surface, number of grooves on basal end, distribution of the grooves on basal end and presence of mucro.

Data analysis

SSR profiles of the 95 olive trees were compared to 672 distinct genotypes of the reference database of WOGB-M/C using Excel Microsatellite TOOLKIT (Park, 2001). Genetic diversity in each dataset, Meknes collection and WOGB-M/C, was estimated by calculating a set of parameters for each microsatellite locus using Excel Microsatellite TOOLKIT including; allele size, number of alleles (Na), number of unique alleles (Nu), observed (Ho) and expected heterozygosity (He; Nei, 1987) and polymorphism information content (PIC, Botstein et al., 1980). In addition, pairwise comparison among samples within Meknes collection based on endocarp traits was conducted to identify distinct morphological profiles using a binary matrix of different morphological states; 0: absent and 1: present.

Moreover, phylogenetic relationships among accessions in Meknes collection was revealed by converting SSR data into a binary matrix (0 and 1) and constructing dendrogram using Dice similarity index (Dice, 1945) and UPGMA method with NTSYS-PC V2.02 software (Rohlf, 1998). Spatial distribution of the genotypes in both Meknes collection and WOGB-M/C was described based on Principal Coordinate Analysis (PCoA) with simple matching coefficient (Sokal and Michener, 1958) using the DARWIN v.5.0.137 program (Perrier et al., 2003).

Finally, comparison between Meknes collection and WOGB-M/C was carried out based on different criteria such as: (i) accessions name, (ii) number of shared genotypes and cultivars, (iii) number of alleles (Na) and Nei diversity index (He) and (iv) allelic richness (Ar, Petit et al., 1998). Allelic richness (Ar) was computed following a generalized rarefaction approach at the standardized G value using the ADZE program (Szpiech et al., 2008).

Significant differences in rarefied Ar and He were revealed using Mann-Whitney comparison test ($p \leq 0.05$) with PAST program (Hammer et al., 2001).

Results

1. Characterization of Meknes collection

1.1. SSR polymorphism

Based on 14 SSR markers, a total of 122 alleles were observed in Meknes collection among which 15 alleles were unique (Table 2). The number of alleles ranged from 4 for DCA15 to 14 for DCA09 with a mean of 8.71 alleles/locus. Index of diversity (He) varied from 0.468 for DCA05 locus to 0.855 for DCA16 locus with an average of 0.699. Twelve markers among the 14 had a PIC value greater than 0.5. These data indicate significant genetic variability among accessions in the collection.

Table 2. Summary of genetic diversity parameters of 14 SSR markers observed in both Meknes collection and reference database.

SSR Marker	Meknes collection						Reference database				
	Size (bp)	Na	Nu	Ar[1]	He	PIC	Size (bp)	Na	Nu	Ar[1]	He
DCA01[a]	204-272	7	1	5.5	0.672	0.613	204-274	21	3	5.4	0.624
DCA03[a]	227-253	9		8.3	0.824	0.797	227-255	15		8.3	0.850
DCA04[a]	129-174	9	2	7.4	0.627	0.566	116-198	35	3	9.9	0.761
DCA05[a]	191-211	8	1	6.9	0.468	0.443	191-213	12		6.8	0.443
DCA08[a]	123-159	13	3	9.7	0.808	0.778	123-168	23	3	9.0	0.825
DCA09[a]	160-214	14	2	10.6	0.826	0.800	160-218	26	2	12.3	0.873
DCA11[a]	126-180	12	3	8.9	0.801	0.768	126-185	26	2	10.0	0.824
DCA15[a]	243-267	4		3.9	0.573	0.509	243-267	7	1	4.1	0.551
DCA16[a]	122-179	13	1	10.9	0.855	0.834	122-230	39	6	10.9	0.861
DCA18[a]	154-180	10		8.5	0.812	0.781	154-193	19	3	9.2	0.824
EMO90[b]	181-193	5		4.7	0.642	0.583	181-208	10	1	5.9	0.659
GAPU59[c]	206-226	6		5.0	0.633	0.572	194-239	13	4	5.2	0.615
GAPU71A[c]	207-240	7	2	5.7	0.475	0.441	206-256	16	3	4.2	0.452
GAPU 71B[c]	118-141	5		5.0	0.780	0.739	118-147	10		6.0	0.803
Mean		**8.71**	**1.07**	**7.2***	**0.699***	**0.659**		**19.42**	**2.8**	**7.6***	**0.711***
Total		**122**	**15**	**100.**				**272**	**31**	**106.4**	

Na: Number of alleles, Nu: number of unique alleles, Ar: allelic richness, He: expected heterozygosity, Ho: observed heterozygosity, PIC: polymorphic information content.
[1]Computed at G value of 42. No significant difference between both datasets (Mann-Whitney test, *p-value* >0.05). [2]Shared alleles between both Meknes collection and reference dataset. *Index of significance at p-value < 0.05.
[a]Sefc et al. (2000), [b]De La Rosa et al. (2002), [c]Carriero et al. (2002).

1.2. Identification of cultivars using SSR markers and morphological traits

Based on SSR profiles, the 95 olive trees maintained in the Meknes collection were classified into 42 distinct genotypes (Tables 3). The best discriminative markers that allowed differentiation among all accessions are DCA04, DCA09, and DCA16. The 42 identified genotypes were represented

by 25 accessions with unique SSR profiles and 70 accessions having multiple overlapping SSR profiles that define 17 distinct genotypes.

Endocarp traits-based classification of the Meknes collection accessions yielded virtually similar patterns. In sum, a total of 38 different morphological profiles were identified. The 38 profiles were classified as 22 accessions with unique morphological profiles and 70 accessions sharing core sets of traits that determine 16 morphotypes.

Combined information from SSR markers and endocarp traits differentiates 40 different olive cultivars in Meknes collection (Figure 1; Tables 1 and 3). Only two cases of molecular variants were identified in the collection for "Frantoio" and "Chetoui" cultivars (Figure 1). Overall, these results highlight the crucial role of coupling genetic markers and endocarp characteristics in disentangling differences and similarities among olive tree accessions.

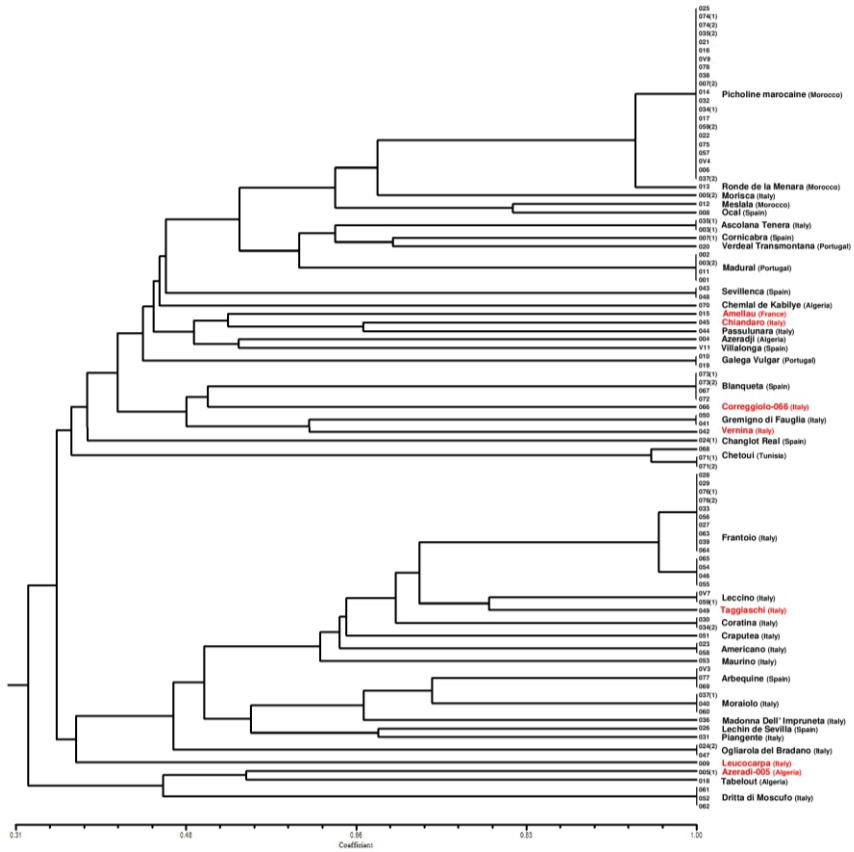

Figure 1. Dendrogram based on UPGMA method and Dice similarity index of the 40 identified cultivars in Meknes collection showing redundant accessions and molecular variants. Numbers indicate the accessions code. Cultivars that are shared with the reference database (black) and those specific to Meknes collection (red) are shown.

Table 3. Number of olive accession per origin, number of genotypes including variants and identified cultivars in Meknes collection. Number of shared genotypes and cultivars with the reference database are indicated.

	No. of trees analyzed	**No. of accessions**	**No. of denomination ()[1]**	**No. of genotypes[2]**	**No. of identified cultivars[3]**
Algeria	13	10	9 (8)	4 (3)[4]	4 (3)[4]
France	5	5	5 (2)	1 (0)	1 (0)
Italy	43	39	38 (17)	21 (15)	20 (15)
Morocco	3	3	3 (3)	3 (3)	3 (3)
Portugal	8	7	7 (7)	3 (3)	3 (3)
Spain	14	11	10 (8)	8 (8)	8 (8)
Tunisia	7	6	5 (5)	2 (2)	1 (1)
Turkey	1	1	1 (1)	0	0
USA	1	1	1 (1)	0	0
Total	**95**	**83**	**79 (52)**	**42 (34)[4]**	**40 (33)[4]**

[1]Number of similar denominations compared to El Bakkali et al. (2019) database. [2]based on 14 SSR loci only. [3]based on both SSR loci and endocarp traits. [4]nomber of shared genotypes and cultivars with the reference database.

2. Comparison between Meknes collection and WOGB-M/C

2.1. Based on accessions' denomination

Among the 83 accessions in Meknes collection, 79 accessions' names were listed versus 713 denominations among the 1,091 accessions in WOGB-M/C (Table 1). Fifty two accessions' names were listed in both datasets, whereas 27 labels such as "Canino", "Leucocarpa" and "Vernina" were specific to Meknes collection. Italian germplasm contained the greatest number of shared denomination (17; 32.7%). Otherwise, common denominations were scattered across genetic materials of different origins and ranged between 1 and 8 denominations respectively for American ("Oblanga") and Turkish ("Ayvalik") and Algerian germplasm (i.e. "Blanquette de Guelma", "Bouchouk Soummam", "Chemlal de Kabylie"…etc.; Table 1).

2.2. Based on SSR markers and morphological traits

As per SSR analysis, the combined dataset (42 genotypes from Meknes collection and 672 genotypes from WOGB-M/C) encompassed 272 alleles with a mean of 19.42 alleles/locus. The 2 datasets shared 122 alleles (46%) including the 15 unique alleles observed in Meknes collection (Table 2). No significant difference between the two datasets was observed in terms of allelic richness, computed at G value of 42, or diversity index (He; Mann-Whitney test, p-value > 0.05; Table 2).

There were 34 SSR profiles overlap between Meknes and WOGB-M/C collections and 8 were specific to the former (i.e. "Leucocarpa", "Taggiaschi", "Vernina"…etc.; Table 1; Figure 1). The 34 shared genotypes had 114

(93.4%) alleles versus 80 (65%) alleles in SSR profiles that were specific to Meknes collection (8 genotypes). Principal coordinate analysis explained 14% of the total genetic variation within the combined dataset and showed that the 42 genotypes of Meknes collection span the range of the genetic diversity contained in the 672 genotypes of WOGB-M/C dataset (Figure 2).

The use of endocarp traits alone decreased resolution in discerning differences among accessions as only 378 different morphological profiles were identified in the whole dataset compared to 680 SSR profiles. Seven olive trees in Meknes collection exhibited unique morphotypes whereas the others shared similarities with accessions of WOGB-M/C database. This resulted in the identification of 31 different morphological profiles in common.

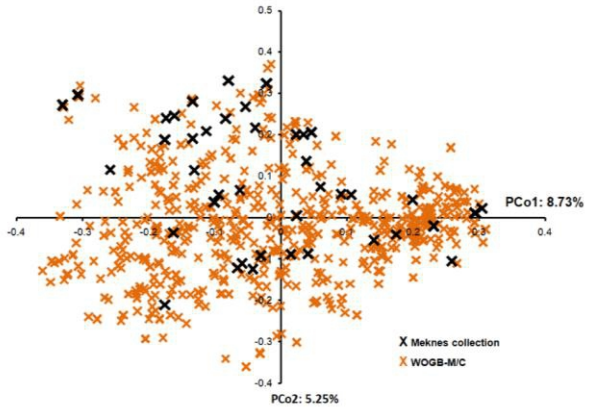

Figure 2. Principal coordinate analysis (PCoA) showing two-dimensional distribution of the genotypes in the two datasets. The first two principal axes account for 13.98% of the total genetic variation among genotypes. The 42 genotypes identified in Meknes collection span the range of all the genotypes in WOGB-M/C collections.

3. Cultivars authentication and synonymy detection in Meknes collection

3.1. Cultivars authentication

Using endocarp traits and SSR markers combined, a total of 542 cultivars were identified in the composite dataset with 33 cultivars belonging to both collections (Figure 1). Thus, only 7 cultivars such as “Leucocarpa” from Italy and “Amellau” from France were exclusive to Meknes collection (Figure 1). Though there were mismatches between accession names in Meknes collection and their corresponding putative cultivars in WOGB-M/C, we were able to authenticate the 33 cultivars shared between Meknes and WOGB-M/C as they showed similar SSR and morphological profiles with their counterparts in the latter such as “Picholine marocaine” from Morocco, “Frantoio” from Italy, “Galega Vulgar” from Portugal and “Arbequina” from Spain. However, the 7 cultivars that were specific to Meknes collection (Figure 1) remained unauthenticated and require further studies in this regard.

3.2. Plantation mislabeling and Synonymy detection

Fifty cases of mislabeled plantations were detected in Meknes collection. For instance, based on their profiles, the accessions "Olivière" (MEK022) from France and "Leccino" (MEK059-2) from Italy showed similar profiles to the well-known cultivar "Picholine marocaine". In addition, all of the mislabeled accessions were different from their correspondent cultivars in WOGB-M/C. Interestingly, most of cultivars concerned by labeling errors turned out to belong to "Picholine marocaine" (18 cases) and "Frantoio" (9 cases).

Aside from plantation mislabeling, several synonymy cases were present in Meknes collection. Specifically, there were 17 names used interchangeably for 10 cultivars. While 10 of these had been previously described, seven cases are reported for the first time in this work (Table 1). For instance, in addition to "Limli" that was reported as a synonym of "Picholine marocaine" (El Bakkali et al., 2019), we here found that "Du Tell" from Algeria is synonymous to that cultivar as well. Similarly, besides "Razzo" and "Oblonga" formerly recognized as referring to the cultivar "Frantoio" (El Bakkali et al., 2019), our work adds "Lavagnina" from Italy to this list. Furthermore, no case of homonymy was identified within Meknes collection; all accessions sharing similar name and showing different profiles were noted as mislabeling errors.

Discussion

Establishment of experimental design of olive species for phenotyping in contrasted environment is a tedious and costly task. As a perennial fruit species with a long juvenile period, the use of available germplasm collections represents an efficient alternative. However, mislabeling plantations compels management of these collections and can thus have potential negative implications on the use of genetic materials in breeding programs. Such issues have been encountered in wide spectrum of other fruit species such as cherimoya (Escribano et al., 2007), apple (Evans et al., 2011) and grape (Riaz et al., 2008). Hence, conducting phenotyping studies using several germplasm collections at a time by mining diversity maintained in different collections could be hindered by the identification and authentication process of varieties. Thus, there is urgent need for standardizing genotyping methods and protocols that lead to compatible outcomes before any use of germplasm.

Efforts have been devoted to genotyping many olive collections all over the world (Khadari et al., 2003; Koehmstedt et al., 2011; Trentacoste et al., 2011; Muzzalupo et al., 2014; Xanthopoulou et al., 2014). However, though virtually the same set of SSR markers were used in these studies, full reproducibility of their results has not always been achieved due to differences in genotyping laboratory conditions (Baldoni et al., 2009). Significant

international efforts have been made to gather olive germplasm in a single and commun database. The 2008 web-based edition (http://www.oleadb.it/) is currently the largest database with information extracted from almost 1,520 publications and concerned about 1,250 cultivars conserved in over 100 collections in 54 countries (Bartolini, 2008). However, this generated database represents an underestimate level of olive tree diversity, since many minor local cultivars that are specific to olive growing areas such as Morocco, Cyprus, Egypt and Syria, are not included. Taking advantage from the study of Trujillo et al. (2014), an attempt was conducted to establish a large database by gathering and aligning profiles of almost total accessions in the two Worldwide Olive Germplasm Banks of Marrakech, Morocco, and Cordoba, Spain, using 20 common SSR markers and 11 endocarps traits (El Bakkali et al., 2019). The open-access database represents the first most exhaustive genotyping analysis on olive cultivars germplasm conducted so far and considered an efficient tool for the identification of cultivars and management of the olive accessions in local and regional collections throughout the world.

In addition to the WOGBs of Marrakech and Cordoba, other regional and local collections exist which constitute valuable platforms for preserving genetic diversity and use in breeding programs. Regardless of their sizes and locations, effective management of these collections and their exploitation in breeding programs have been constrained by the lack of precise identification of their genetic resources. In this work, we addressed this challenge and showed that the use of a combination of genetic and morphological traits can play a paramount role in the process of olive tree authentication in the collection of Meknes-Morocco. We were able to identify many cases of synonymous and mislabeling errors using both SSR markers and endocarp traits. These findings demonstrate the discriminative power of coupling advanced genetic techniques and precise morphological features in characterizing unauthenticated olive germplasm.

We propose a strategy based on aligning germplasm olive collections with the open-access dataset (WOGB-M/C) to establish a single consensus database. Core cultivars, such as “Picholine marocaine”, “Frantoio”, “Leccino”, “Arbequina” and “Picual” …etc., largely present in most collections and cultivated around the world could play a capital role in the characterization and varietal identification processes by using them as an anchor to align the true size of alleles while taking advantage of the resolution power and high sensitivity provided by the 6 SSR markers and 11 endocarp traits used in the investigation (El Bakkali et al., 2019).

The identification and authentication of varieties within a given germplasm collection is a prerequisite step before conducting further breeding studies. Taking advantage from the previous study of El Bakkali et al. (2019), we were able to authenticate 33 cultivars, from a total of 40 identified cultivars

in which 32 are maintained in the WOGB of Marrakech (except "Villalonga" cultivar). The 32 identified cultivars represent an efficient experimental design to study traits of agronomical interest in two contrasted Moroccan sites such as Meknes and Marrakech. This finding is supported by the high genetic diversity detected within these cultivars regarding the similarity index, the insignificant difference of allelic richness, and their spatial distribution that span the range of the 672 genotypes identified in both WOGB of Marrakech and Cordoba as observed by the Principal Coordinate Analysis (Figure 2). Such identified cultivars could be used as experimental design to evaluate agronomical traits in complement to other contrasted environmental conditions and therefore to conduct further studies such as genetic association mapping.

Conclusion

Conservation of olive genetic resources comes often with the challenge of effective management of *ex-situ* collections. This may put at stake the ultimate objective of creating these collections. Indeed, synonymous and homonymous cases coupled with mislabeling errors can have downstream tremendous negative impacts on olive production traceability. The study case we present in this work sheds light on this issue and provides strong evidence that comparative genetics and morphologic features with well-characterized referential databases has the potential to unlock the secrets of unauthenticated olive plant materials in *ex-situ* collections throughout the world.

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