Evaluation of Olives Cultivated in Southern Italy by Simple Sequence Repeat Markers

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Additional index words. discrimination power, genetic similarity, molecular markers, *Olea europaea*, SSR

Abstract. Olive (*Olea europaea* L.) is a species of great economic importance in the Mediterranean basin. Italy is very important for the olive industry; in fact, olive's genetic patrimony is very rich and characterized by an abundance of cultivars. At present, the majority of ancient landraces are vegetatively propagated by farm. It is likely that the number of cultivars is underestimated because of inadequate information on minor local cultivars that are widespread in different olive-growing areas. The existence of many cultivars reinforces the need for a reliable identification method. It is important to improve the ex situ plant germplasm collection and fairly to characterize all cultivars for future breeding programs. In the present report, we used 11 loci microsatellites to characterize 211 olive cultivars of an olive collection cultivated in six regions of southern Italy. These regions represent the major area for olive cultivation in Italy and have a strategic geographical location in the Mediterranean basin. The dendrogram obtained, using the unweighted pair group method with arithmetic mean clustering algorithm, depicts the pattern of relationships between the studied cultivars. There is a clear structuring of the variability relative to the geographic origin of olive cultivars. This work, for the very high number of the Italian olive cultivars analyzed, highlights the degree and distribution of genetic diversity of this species for better exploitation of olive resources and for the design of plant breeding programs. Besides, the use of molecular markers, like simple sequence repeats, is imperative to build a database for cultivar analysis, for traceability of processed food, and for appropriate management of olive germplasm collections.

The olive tree (*Olea europaea* L. subsp. *europaea*) is one of the oldest cultivated plants, and its fruit has been used for nourishment for more than 5000 years in the Mediterranean regions where it originated. Olive is one of the Mediterranean basin's

main crops with great cultural and economic importance. From a commercial perspective, in the Mediterranean basin grow many olive tree varieties and it produces 99% and consumes 87% of the world's olive oil. Among Mediterranean countries, Italy occupies a very important place in the olive industry being the main exporter of olive oil in the world (Muzzalupo and Perri, 2008).

The genetic diversity existing in the cultivated olive trees is enormous (over 650 cultivars and over 1300 synonyms; Bartolini et al., 2005). It is also likely that this number is underestimated because of inadequate information on minor local cultivars that are widespread in different olive-growing areas. At present, 2600 different olive cultivars have been described (Bartolini et al., 2005). The olive germplasm therefore represents an important reserve of genetic diversity for the Mediterranean basin ecosystem. This undefined genetic nature gives rise to several

problems, both for olive nurseries and for the correct estimation of the platforms needed to classify and properly exploit olive products like canned olives and olive oil.

Olive tree germplasm was traditionally evaluated by morphological and phenological parameters. Polymerase chain reaction (PCR) based on DNA markers is a powerful tool for genetic analysis because of its simplicity and ease of handling (Kojima et al., 1998) providing an opportunity for direct comparison and identification of olive tree material independently from environment and/or developmental stages (Martins-Lopes et al., 2007). An ideal molecular marker technique should have the following criteria: 1) to be polymorphic and evenly distributed throughout the genome; 2) to provide adequate resolution of genetic differences; 3) to be simple, quick, and inexpensive; 4) to need small amounts of tissue and DNA samples; and 5) to have linkage to distinct phenotypes (Agarwal et al., 2008). Microsatellites are highly popular genetic markers because of their codominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing simple sequence repeat (SSR) size variation by PCR with pairs of flanking primers (Agarwal et al., 2008; Muzzalupo et al., 2006, 2008; Rekik et al., 2008). Recently, many efforts have been made to develop good and reliable molecular tools such as microsatellite markers that could shed light on olive tree genetics (Carriero et al., 2002; Cipriani et al., 2002; De la Rosa et al., 2002; Diaz et al., 2006; Rallo et al., 2000; Sabino Gil et al., 2006; Sefc et al., 2000). They have been used for cultivar identification for elucidating genetic relationships between several olive cultivars (Muzzalupo et al., 2006, 2008). Microsatellites have revealed themselves to be very useful markers for olive's progenies parentage checking from controlled crossings (Diaz et al., 2006; Rekik et al., 2008).

The systematic collection of olive cultivars in specific catalog fields began in 1997 by CRA—Centro di Ricerca per l'olivicoltura e l'industria olearia (CRA-OLI) of Rende (CS), Italy. To date, more than 400 cultivars have been introduced into the CRA-OLI collection. The list of cultivars has already been published on FAO's web site (<http://apps3.fao.org/views/olive/oliv.jsp>). The goal of such collections is to safeguard all cultivars, and particularly the minor ones, to avoid a loss in genetic diversity and to offer an interesting genetic basis for breeding programs. Apulia, Basilicata, Calabria, Campania, Sardinia, and Sicily are the regions in Italy with the greatest areas of olive growing and have an extremely complex varietal assortment (Muzzalupo and Perri, 2008). These regions are a major area for olive cultivation in Italy and have a strategic geographical location in the Mediterranean basin. In the present report, we used 11 loci microsatellite to characterize 211 olive cultivars present in the CRA-OLI germplasm collection. The present study is an attempt, using SSR markers, to identify, characterize,

Received for publication 17 Nov. 2008. Accepted for publication 5 Feb. 2009.

This research was supported by the Italian "OLVIVA," "RGV FAO," and "RIOM" Projects granted by MIPAAF.

We thank N. Lombardo for his endless efforts in the individuation and collection of olive germplasms. We also thank M. Pellegrino, G. Godino, A. Saijad, M. A. Caravita, A. Salimonti, and R. Falabella, who helped with DNA extractions and with molecular techniques. We thank M. Pellegrino, G. Godino, A. Ciliberti, and A. Madeo for their help with collecting plant material in the CRA-OLI olive collection.

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Table 1. Codes of 211 olive cultivars growing in the CRA-OLI olive germplasm collection at Cosenza (Italy).²

Number	Cultivar name	Register number	Growing regions
1	Abunara	B-205	Sicily
2	Agrostigna	C1-319	Calabria
3	Aitana	B-206	Sicily
4	Annarea	B-262	Basilicata
5	Augellina	A-10	Basilicata
6	Barilari	C1-346	Sicily
7	Biancolella	C2-358	Campania
8	Biancolilla	C1-347	Sicily
9	Borgese	A-18	Calabria
10	Bosana	A-71	Sardinia
11	Bottone di Gallo	C1-308A	Sicily
12	Brandofino	A-78	Sicily
13	Buscionetto	A-A32	Sicily
14	Cacaredda	B-207	Sicily
15	Cacaridduni	B-208	Sicily
16	Cacazzara	C2-383	Sicily
17	Caiazzana	B-240	Campania
18	Calatina	A-124	Sicily
19	Cammarotana	C1-324	Campania
20	Carboncella Campana	C2-359	Campania
21	Cariasina	A-72	Sardinia
22	Carmelitana	A-120	Apulia
23	Carolea	A-19	Calabria
24	Carpellese	C1-325	Campania
25	Carpinella	C2-390	Basilicata
26	Cassanese	A-20	Calabria
27	Castricianella Rapparina	B-209	Sicily
28	Cavalieri	B-182	Sicily
29	Cazzinicchio	A-121	Apulia
30	Cellina di Nardò	A-55	Apulia
31	Cerasuola	A-80	Sicily
32	Ciciarello	A-115	Calabria
33	Cicinella	C1-326	Campania
34	Cima di Melfi	A-11	Basilicata
35	Cima di Mola	A-56	Apulia
36	Coratina	A-57	Apulia
37	Cornacciola	C2-391	Basilicata
38	Corneglia	B-241	Campania
39	Cornia	B-242	Campania
40	Corniola	B-192	Calabria
41	Cornula	C2-NN1	Apulia
42	Corsicana da Mensa	C1-311	Sardinia
43	Corsicana da Olio	A-73	Sardinia
44	Crastu	C1-348	Sicily
45	Curatora	C2-366	Campania
46	Demoniata	C2-384	Sicily
47	Dolce di Andria	A-196	Apulia
48	Dolce di Cassano	A-122	Apulia
49	Dolce di Cerchiara	C1-320	Calabria
50	Donna Francesca	C2-NN2	Apulia
51	Erbano	C1-349B	Sicily
52	Faresana 1	B-202	Basilicata
53	Faresana 2	C1-A1	Basilicata
54	Fasolina	C1-265	Basilicata
55	Fasolona	C1-266	Basilicata
56	Femminella di Torraca	B-243	Campania
57	Frangivento	A-58	Apulia
58	Geracese	A-21	Calabria
59	Ghiacciora	C2-NN3	Calabria
60	Ghiannara	B-263	Basilicata
61	Giarfara	B-210	Sicily
62	Giarraffa	A-81A	Sicily
63	Giusta	B-223	Basilicata
64	Grossa di Spagna	A-59	Apulia
65	Grossale	B-244	Campania
66	Iacona	C1-350A	Sicily
67	Imperiale	A-158	Calabria
68	Lezze	A-145	Apulia
69	Lumiaru	B-194	Sicily
70	Mafra	A-22	Calabria
71	Mafra di Cerchiara	C1-321	Calabria
72	Maiatica di Ferrandina	A-12A	Basilicata
73	Majorca	C2-378	Sardinia

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Table 1. (Continued) Codes of 211 olive cultivars growing in the CRA-OLI olive germplasm collection at Cosenza (Italy).²

Number	Cultivar name	Register number	Growing regions
74	Mandanici	A-125	Sicily
75	Manna	C2-379	Sardinia
76	Mantonica	B-236	Sicily
77	Marinese	C2-360	Campania
78	Mele	A-60	Apulia
79	Minuta	A-83	Sicily
80	Monaca	B-212	Sicily
81	Mora	A-131	Apulia
82	Morellona di Grecia	B-178	Apulia
83	Moresca	A-84	Sicily
84	Murtiddara	C2-385	Sicily
85	Nasitana Frutto Grosso	B-195	Sicily
86	Nebba	B-259	Sicily
87	Nera di Cantinelle	A-23	Calabria
88	Nera di Gonnos	A-74	Sardinia
89	Nera di Oliena	C1-312	Sardinia
90	Nera di Villacidro	A-113	Sardinia
91	Nerba	B-258	Sicily
92	Nerba Catanese	C1-309	Sicily
93	Nocellara del Belice	A-85	Sicily
94	Nocellara Etnea	A-86	Sicily
95	Nocellara Etnea Ovale	B-183	Sicily
96	Nocellara Messinese	A-87	Sicily
97	Nocellara Nissena	C1-351A	Sicily
98	Nociara	A-61	Apulia
99	Nolca	A-62	Apulia
100	Nostrana	A-24	Calabria
101	Nostrana Campana	B-200	Campania
102	Ogliara	B-245	Campania
103	Ogliarola Barese	A-63	Apulia
104	Ogliarola del Bradano 1	C2-A13	Basilicata
105	Ogliarola del Bradano 2	A-13	Basilicata
106	Ogliarola del Murge	C2-NN4	Apulia
107	Ogliarola del Vulture	A-15	Basilicata
108	Ogliarola di Montalbano	A-14	Basilicata
109	Ogliarola Garganica	B-257	Apulia
110	Ogliarola Messinese	A-88	Sicily
111	Ogliarola Salentina	A-64	Apulia
112	Ogliastro Grande	B-246	Campania
113	Olianedda	C2-380	Sardinia
114	Oliastro	A-112	Apulia
115	Olieddu	C1-313	Sardinia
116	Oliiva Bianca	C2-361	Campania
117	Oliiva da Polpa	C2-365	Campania
118	Oliiva Rossa	C2-376	Apulia
119	Oliiva Torsa	C2-362	Campania
120	Olivella Appuntita	C1-327	Campania
121	Olivella di Cerchiara	C1-322	Calabria
122	Olivella di Grottaminarda	C2-363	Campania
123	Oliivo da Mensa	C1-267	Basilicata
124	Oliivo da Olio	B-247	Campania
125	Oliivo da Salare	B-248	Campania
126	Oliivo di Castiglione	C1-310	Sicily
127	Olivona	C2-NN5	Apulia
128	Opera Pia	C2-NN6	Sicily
129	Orazio	C2-392	Basilicata
130	Ortice	B-201	Campania
131	Ortolana	A-161	Campania
132	Ottobratica	A-25	Calabria
133	Palma	C2-381	Sardinia
134	Palmarola	C1-268	Basilicata
135	Pampagliosa	A-162	Campania
136	Paschixedda	C1-314	Sardinia
137	Pasola	A-65	Apulia
138	Passulunara	A-143	Sicily
139	Patrinotraru	C2-386	Sicily
140	Pennulara	B-225	Calabria
141	Peppino Leo	A-132	Apulia
142	Peranzana	A-66	Apulia
143	Perciasacchi	B-249	Campania
144	Pezzè quaddu	C2-382	Sardinia
145	Pidicuddara	A-153	Sicily
146	Pirunara	C1-352	Sicily

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Table 1. (Continued) Codes of 211 olive cultivars growing in the CRA-OLI olive germplasm collection at Cosenza (Italy).²

Number	Cultivar name	Register number	Growing regions
147	Pisciottana	A-31	Campania
148	Pizzè Carroga	A-75	Sardinia
149	Pizzo di Corvo	C1-353A	Sicily
150	Pizzutella	B-213	Sicily
151	Provenzale	B-255	Campania
152	Racemo	C1-344	Apulia
153	Racioppa Lucana	C1-317	Basilicata
154	Racioppa Campana	B-250	Campania
155	Racioppella	A-163	Campania
156	Ravece	C1-328	Campania
157	Regina	C2-NN7	Campania
158	Ritonnella	B-251	Campania
159	Rizza	A-16	Basilicata
160	Rizzitella	B-252	Campania
161	Roma	C2-393	Basilicata
162	Romanella Lucana	C2-394	Basilicata
163	Romanella Calabrese	A-129	Calabria
164	Rossanese	A-26	Calabria
165	Rotondella	C1-296	Basilicata
166	Rotondella di Foggia	C2-377	Apulia
167	Rotondella di Sanza	C1-329	Campania
168	Ruveia	C1-330	Campania
169	Salella	C2-364	Campania
170	Sammartinara	B-214	Sicily
171	Sammartinenga	B-224	Basilicata
172	San Benedetto	B-197	Apulia
173	Sanginara	C1-331	Campania
174	Santa Maria	B-253	Campania
175	Santagatese	A-89	Sicily
176	Sant'Agostino	A-67	Apulia
177	Santomauro	A-160	Calabria
178	Scarpetta	C1-318	Basilicata
179	Semidana	A-76	Sardinia
180	Sessana	B-256	Campania
181	Simona	A-68	Apulia
182	Sinopolese	A-27	Calabria
183	Sivigliana da Olio	C1-345	Sardinia
184	Spezzanese	C1-323	Calabria
185	Spinoso	C2-395	Basilicata
186	Tenacella	C1-332	Campania
187	Termite di Bitetto	A-69	Apulia
188	Terza Grande	A-133	Sardinia
189	Terza Piccola	C1-316	Sardinia
190	Tombarello	B-193	Calabria
191	Tonda di Alife	B-254	Campania
192	Tonda di Cagliari	A-77	Sardinia
193	Tonda di Filadelfia	A-28	Calabria
194	Tonda di Filogaso	A-29	Calabria
195	Tonda di Strongoli	A-116	Calabria
196	Tonda Dolce	B-226	Calabria
197	Tonda Dolce di Partanna	B-237B	Sicily
198	Tonda Iblea	A-90	Sicily
199	Tondina	A-30	Calabria
200	Toscanina	A-70	Apulia
201	Tunnulidda	B-215	Sicily
202	Turdunazza Antimosca	C2-387A	Sicily
203	Uccellara	A-17	Basilicata
204	Vaddara	C1-354	Sicily
205	Verdello	A-91	Sicily
206	Vigna della Corte	C1-333	Campania
207	Vraja	C2-396	Calabria
208	Zaituna	A-82A	Sicily
209	Zarbo	B-211	Sicily
210	Zimbimbo	A-123	Apulia
211	Zinzifarica	A-147	Calabria

²Cultivar names, register number, and regions of origin or of major cultivation.

and establish relationships between geographically related olive tree cultivars.

Materials and Methods

Plant materials. Young leaves were harvested from 211 plants growing in the CRA-

OLI olive germplasm collection in Cosenza, Italy. The olive plants analyzed are autochthonous of six regions of southern Italy: 35 cultivars from Apulia, 28 cultivars from Basilicata, 30 cultivars from Calabria, 43 cultivars from Campania, 20 cultivars from Sardinia, and 55 cultivars from Sicily (Table 1).

DNA extraction. Young olive leaves were washed by 4% sodium hypochlorite and 0.2 g of plant tissue was ground into liquid nitrogen and DNA extraction was carried out as described by Muzzalupo and Perri (2002). DNA was quantified by H33258 dye incorporation detected by a Hoefer DyNA Quant® 200 fluorometer (Amersham Pharmacia Biotech, Milan, Italy) following the method described by Muzzalupo et al. (2007a).

Microsatellite markers. Eleven published SSR markers were preselected for their high level of polymorphism and easily scorable patterns. SSRs used are reported in Table 2. SSR amplification was carried out as described by Rekik et al. (2008). PCR products were analyzed using a Bioanalyzer 2100 (Agilent Technologies, Waldbronn, Germany) on a DNA 500 LabChip (Muzzalupo et al., 2007b).

Data analysis. Data were processed using POPGENE 32 software (Yeh and Boyle, 1997). The software allowed calculation of the number of alleles and their frequency. The heterozygosity, both observed (*Ho*) and expected (*He*), was calculated using the same software. The expected heterozygosity (*He*) of each locus was calculated according to the formula $He = n(1 - \sum p_i^2)/(n-1)$, where p_i is the frequency of the i^{th} allele and n is the number of gene copies in the sample for the given locus (Nei and Roychoudhury, 1974). Deviations of observed heterozygosity values from Hardy-Weinberg expectations were analyzed using the program Genepop 3.4 (Raymond and Rousset, 2003). To determine significance levels for this test, sequential Bonferroni adjustments were used. The probability of null alleles was estimated according to the formula of Brookfield (1996): $r = (He - Ho)/(1 + He)$. The SSR loci discrimination power was calculated according to Brenner and Morris (1990).

The alleles detected for each microsatellite were recorded into a data matrix of presence (1) and absence (0) of bands (each allele representing a band). The estimations of genetic similarity based on calculation of Jaccard's similarity coefficients among 211 olive cultivars are analyzed using NTSysPc program version 2.02 (Rohlf, 1998). Finally, a tree was inferred using the unweighted pair group method using an arithmetic average (UPGMA) clustering algorithm. The cophenetic correlation coefficient was calculated, and Mantel's test (Mantel, 1967) was performed to control the goodness of fit of a cluster analysis for the matrix on which it was based.

A likelihood-based parentage analysis was performed using FaMoz software (Di Vecchi Staraz et al., 2007; Gerber et al., 2003) to find potential parent-offspring or sibling relationships among cultivars. We calculated exclusion and identity probabilities based on the allele frequencies of the 11 microsatellites. Simulations, calculations, and tests were done considering no genotyping errors. Because genotypes represent adult trees growing in different regions, the

Table 2. Simple sequence repeat amplification products observed among the 211 olive cultivars.^z

Locus	Accession numbers	Repeat motif	Size range (bp)	Na	Ho	He	HW ^y	r
GAPU 59	EU371064	(CT) ₁₀	208 (208–222)	5	0.673	0.764	NS	–0.054
GAPU 71A	EU700281	(AG) ₆	210 (210–259)	9	0.483	0.689	NS	–0.139
GAPU 71B	EU376382	(AG) ₇ (AAG) ₈	130 (121–144)	5	0.901	0.717	NS	0.097
GAPU 103A	EU700297	(TC) ₃₂	184 (136–203)	8	0.853	0.822	NS	0.017
UDO 001	FJ268736	(CA) ₈	140 (140–150)	3	0.133	0.568	S	–0.384
UDO 003	FJ268735	(AC) ₁₄	135 (135–202)	6	0.085	0.629	S	–0.501
UDO 012	FJ268738	(GT) ₁₁	166 (164–193)	5	0.768	0.744	NS	0.014
UDO 028	FJ268737	(CA) ₁₉ (TA) ₇	154 (143–245)	9	0.630	0.819	NS	–0.116
UDO 039	FJ268741	(AT) ₉ (GT) ₁₆	185 (108–243)	12	0.379	0.828	S	–0.326
DCA09	FJ268739	(GA) ₁₂	172 (162–206)	7	0.853	0.840	NS	0.007
DCA18	FJ268740	(CA) ₄ (CT) ₁ (CA) ₃ (GA) ₁₉	177 (173–185)	6	0.730	0.733	NS	–0.002
Totals			108–259	75	—	—	—	—
Mean			—	6.82	0.590	0.741	—	–0.126
SD			—	—	0.285	0.087	—	0.194

^zFor each locus, the GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>) accession numbers for the total nucleotide sequences, the size range in base pairs, the number of alleles (*Na*), the observed heterozygosity (*Ho*), the expected heterozygosity (*He*), the probability of exact “Hardy-Weinberg” test (*HW*), and the probability of null alleles (*r*).

^yAfter Bonferroni correction; NS = nonsignificant; S = significance ($P < 0.001$).

parent–offspring relationship can be difficult to distinguish from sibship.

Results and Discussion

Molecular characterization and discriminating capacity. A total of 211 olive cultivars, chosen from the six regions of southern Italy (Apulia, Basilicata, Calabria, Campania, Sardinia, and Sicily), were genotyped with 11 SSR loci. A total of 75 alleles over 11 loci were produced, ranging from three at UDO01 to 12 alleles at UDO39, with an average of 6.82 alleles per locus (Table 2). This is comparable to the number of alleles among olive cultivars reported by Charafi et al. (2008), but somewhat lower than that published by Sarri et al. (2006), probably because it includes a large number of foreign cultivars and for a different set of SSR loci.

The observed heterozygosity for the 211 olive cultivars ranged from 0.085 at UDO03 to 0.901 at GAPU71B with a mean value of 0.590. The expected heterozygosity ranged from 0.568 at UDO01 to 0.840 at DCA09 with a mean value of 0.741. The probability of occurrence of null alleles values ranged from 0 to 0.33 at UDO03. At four of the 11 loci, the observed heterozygosity was higher than the expected values under “Hardy Weinberg” equilibrium. Although loci GAPU59, GAPU71A, UDO028, and DCA18 presented a lower heterozygosity value than expected, the differences between theoretical and observed values were not significant. In contrast, the level of heterozygosity was significantly lower than expected at the UDO001, UDO003, and UDO39 loci ($P < 0.001$). A possible explanation of such a deficit is the occurrence of null alleles at these loci because the corresponding probability is highly significant (Table 2).

For each SSR locus, discrimination power (PD) values ranged from 0.941 at DCA09 to 0.638 at UDO001 (Table 3). To assess the ability of the SSR markers to discriminate among cultivars, the combined PD was calculated first of all 22 markers and then the PD of all 10 marker combinations (removing

locus of low PD, one at a time), and so on. The combined PD of all loci is 0.9999999998, which means that the probability of finding two cultivars with the same genotype combination for the 11 SSR markers is one over one billion, indicating the very high discrimination of the marker system used. To get an overall picture of discrimination power of combined loci, the PD of all the possible combinations of two and three loci were determined. The DCA09-UDO28 pairs were the most discriminating (0.9960); the other 10 best pair combinations were all higher than 0.99. The PD values of the best three-locus combinations were all higher than 0.999; DCA09-UDO28-GAPU103A was the most discriminative (0.9997).

The lowest allelic frequency (0.0024) was observed for alleles 228 bp of GAPU71a in ‘Cellina di Nardò’ (Apulia region), 259 bp of GAPU71a in ‘Bottone di Gallo’ (Sicily region), 202 bp at UDO03 in ‘Moresca’ (Sicily region), and 243 bp at UDO39 in ‘Cornula’ (Apulia region) (Table 4). These four alleles were observed in one copy in the whole set of studied cultivars analyzed. Alleles 143 bp of UDO01 showed the relatively highest frequency (0.5877). The shortest allele among the 11 polymorphic loci was allele 108 bp at UDO39, whereas the longest was 259 bp at GAPU71A.

Identifying molecular profiles and genetic diversity. Using 11 microsatellite loci, four cases of homonymy were identified. Olive trees under the ‘Faresana 1’ and ‘Faresana 2’ denomination (Basilicata region) were classified into two molecular profiles, which were differentiated by six SSR alleles. ‘Ogliarola del Bradano 1’ and ‘Ogliarola del Bradano 2’ (Basilicata region) were differentiated by 10 SSR alleles; ‘Racioppa 1’ and ‘Racioppa 2’ (Basilicata and Campania regions, respectively) were classified into two molecular profiles, which were differentiated by 13 SSR alleles; finally, ‘Romanella 1’ and ‘Romanella 2’ (Basilicata and Calabria regions, respectively) were differentiated by 15 SSR alleles.

Synonyms included cultivars with the same profile for all SSR examined and cultivar pairs different from each other for one or two alleles (La Mantia et al., 2005). In the case of slightly different molecular patterns, we considered that such differences were too few to have originated through sexual reproduction, olive being an outcrossing species with a highly heterozygous genome. Given the high number of cultivars (211 cultivars) and high number of molecular markers (75 allele SSRs), we decided to treat the genotype with highest values of similarity (≥ 0.941 , different for one allele) as possible cases of synonyms. Parent–offspring relations were found for ‘Giarraffa’ and ‘Pizzo di Corvo’, ‘Nera di Oliena’ and ‘Paschixedda’, and ‘Ogliarola Messinese’ and ‘Iacona’; and these three cultivar pairs differ by only one allele (Table 5). However, given the high polymorphism of our markers and the reproductive biology of the olive tree, rather than parent–offspring, it is far more probable that those individuals are clones that have recently diverged by mutation or that the divergent allele is the result of genotyping error.

Ten different olive cultivar pairs are genetically indistinguishable from one another (Table 5), potentially representing cases of synonymy. Many cultivars were identified as possible cases of synonyms and are reported to Table 5; the remainder are clearly separated from each other. These possible cases of synonymy are in agreement with previously studies based on morphological descriptors and molecular marker systems (Lombardo et al., 2004; Muzzalupo et al., 2006, 2007a, 2008; Sarri et al., 2006). Consequently, it is possible to say that the SSRs used in this study are able to distinguish 199 unique genotypes (94% of the cultivars analyzed). Therefore, synonyms characterization is very important to avoid genotype redundancy to maximize genetic diversity in the Italian olive germplasm collection.

Phenetic analysis of the genetic polymorphism carried out by means of the UPGMA clustering algorithm helped us to clarify the

Table 3. The combined discriminating power (PD) of all loci analyzed and for their best combinations.

Simple sequence repeat loci	PD									
DCA09										0.9406304530
UDO028										0.9330529885
GAPU103A									0.9960174075	0.9285115002
UDO039								0.9997150455		0.9046868444
GAPU59										0.8905036769
DCA18										0.8780501471
UDO012										0.8459812471
GAPU71A										0.8439163541
GAPU71B										0.8034234354
UDO003										0.6667280079
UDO001										0.6380359830

Table 4. Allele size (bp) and frequencies (*in italics*) for each simple sequence repeat locus in 211 olive genotypes.

Locus	No. of alleles	Allele length (bp)									
GAPU 59	5	208	212	214	218	222					
<i>frequency</i>		<i>0.211</i>	<i>0.360</i>	<i>0.147</i>	<i>0.107</i>	<i>0.176</i>					
GAPU 71a	9	210	212	214	218	221	224	228	250	259	
<i>frequency</i>		<i>0.157</i>	<i>0.104</i>	<i>0.507</i>	<i>0.114</i>	<i>0.031</i>	<i>0.076</i>	<i>0.002</i>	<i>0.007</i>	<i>0.002</i>	
GAPU 71b	5	121	124	126	130	144					
<i>frequency</i>		<i>0.009</i>	<i>0.341</i>	<i>0.197</i>	<i>0.109</i>	<i>0.344</i>					
GAPU 103a	8	136	150	157	159	170	184	197	203		
<i>frequency</i>		<i>0.109</i>	<i>0.128</i>	<i>0.137</i>	<i>0.277</i>	<i>0.163</i>	<i>0.171</i>	<i>0.005</i>	<i>0.009</i>		
UDO 001	3	140	143	150							
<i>frequency</i>		<i>0.244</i>	<i>0.588</i>	<i>0.168</i>							
UDO 003	6	135	143	150	166	182	202				
<i>frequency</i>		<i>0.126</i>	<i>0.491</i>	<i>0.339</i>	<i>0.036</i>	<i>0.007</i>	<i>0.002</i>				
UDO 012	5	164	166	177	182	193					
<i>frequency</i>		<i>0.090</i>	<i>0.318</i>	<i>0.308</i>	<i>0.059</i>	<i>0.225</i>					
UDO 028	9	143	150	154	156	161	182	205	210	245	
<i>frequency</i>		<i>0.232</i>	<i>0.092</i>	<i>0.130</i>	<i>0.005</i>	<i>0.088</i>	<i>0.284</i>	<i>0.109</i>	<i>0.056</i>	<i>0.005</i>	
UDO 039	12	108	125	164	170	185	200	205	209	213	243
<i>frequency</i>		<i>0.102</i>	<i>0.007</i>	<i>0.045</i>	<i>0.545</i>	<i>0.040</i>	<i>0.095</i>	<i>0.315</i>	<i>0.024</i>	<i>0.204</i>	<i>0.002</i>
DCA09	7	162	172	176	182	194	198	206			
<i>frequency</i>		<i>0.173</i>	<i>0.156</i>	<i>0.078</i>	<i>0.173</i>	<i>0.078</i>	<i>0.230</i>	<i>0.111</i>			
DCA18	6	173	174	177	179	181	185				
<i>frequency</i>		<i>0.014</i>	<i>0.005</i>	<i>0.173</i>	<i>0.310</i>	<i>0.344</i>	<i>0.154</i>				

Table 5. Synonyms identified by simple sequence repeat fingerprinting.

Main cultivars	Synonyms ^a
Coratina (Apulia)	Racemo (1.000—Apulia)
Giarruffa (Sicily)	Pizzo di Corvo (0.941—Sicily)
Majorca (Sardinia)	Manna (1.000—Sardinia)
Nera di Gonnos (Sardinia)	Tonda di Cagliari (1.000—Sardinia)
Nera di Oliena (Sardinia)	Terza piccola (1.000—Sardinia); Paschixedda (0.941—Sardinia)
Nera di Villacidro (Sardinia)	Terza grande (1.000—Sardinia)
Nolca (Apulia)	Mele (1.000—Apulia)
Ogliarola Barese (Apulia)	Ogliarola del Bradano 1 (1.000—Basilicata); Ogliarola di Moltalbano (1.000—Basilicata); Ogliarola Garganica (1.000—Apulia)
Ogliarola Salentina (Apulia)	Cima di Mola (1.000—Apulia)
Ogliarola Messinese (Sicily)	Iacona (0.941—Sicily)

^aValues in parentheses are the values of similarity from the main cultivars and regions of origin.

Fig. 1. Unweighted pair group method with arithmetic mean dendrogram obtained from simple sequence repeat data for 211 olive genotypes.

genetic relationships among 211 olive cultivars. The phenogram reported in Figure 1 depicts the pattern of relationships between the studied cultivars. The first cluster includes 46 olive cultivars and it is composed of: 38 cultivars from the Sicily region, three from the Apulia region, three from the Basilicata region, one from the Calabria region, and one from the Campania region. The second cluster of 16 olive cultivars did not show a clear structure for the Italian geographic origin and was composed of: eight from the Apulia region, six from the Sicily region, and one from the Calabria region. The third cluster includes 26 olive cultivars and it is composed of: 11 from the Basilicata region, seven from the Sicily region, three from the Calabria region, three from the Campania region, one from the Apulia region, and one from the Sardinia region. The fourth cluster includes 20 olive cultivars and it is composed of: 15 from the Apulia region, four from the Basilicata region, and one from the Sicily region. The fifth cluster includes eight olive cultivars, it does not show a clear structure for the Italian geographic origin; it is composed of: three from the Basilicata region, one from the Apulia region, one from the Calabria region, one from the Campania region, one from the Sardinia region and finally, one from the Sicily region. The sixth cluster includes 31 olive cultivars prevalently from the Calabria region. In fact, it is composed of: 24 from the Calabria region, three from the Basilicata region, two from the Apulia region, and two from the Sicily region. The seventh cluster containing 10 olive cultivars is composed of: five from the Apulia region, four from the Campania region, and one from the Basilicata region. The eighth cluster includes 39 olive cultivars prevalently from the Campania region. In fact, it is composed of: 29 from the Campania region, seven from the Sardinia region, and three from the Basilicata region. The ninth cluster containing 16 olive cultivars is composed of: 11 from the Sardinia region and five from the Campania region.

There is a clear structuring of the variability relative to the geographic origin of olive cultivars. Nine distinct clusters of olive cultivars were clearly recognizable and seven of these were clearly link to a geographically defined area. In fact, 38 (69%) cultivars of the 55 total autochthonous of the Sicily region were contained in Cluster 1; 24 of 30 (80%)

olive cultivars autochthonous of the Calabria region were contained in Cluster 5; 29 of 43 (67%) cultivars from the Campania region were contained in Cluster 8; 22 of 35 (63%) cultivars from the Apulia region were contained in Clusters 4 and 7; 11 of 20 (55%) cultivars from the Sardinia region were contained in Cluster 9; and finally, 11 (39%) cultivars of the 28 total autochthonous of the the Basilicata region were contained in Cluster 3. The two clusters of cultivars that clustered independently (Clusters 2 and 6) did not show a clear structure for the Italian geographic origin.

Conclusions

This study shows that the use of molecular markers like SSRs is a very useful tool to build a database available for cultivar analysis and for olive germplasm collection management. An important question affecting the management of the cultivated olive germplasm is related to the possibility of distinguishing the area of origin of each cultivar. Such information could help safeguard local cultivars and could be used for tracing purposes to select genotypes that are better adapted to specific environmental conditions. Thus, the obtained data allow us, for the first time, a proper evaluation of the 211 olive cultivated in six different regions of southern Italy by 11 loci SSRs and attempt to assign a correlation between the olive cultivars and their geographic origin. The presence of such cases of identity among cultivars might be attributable to their ancient diffusion into different cultivation areas where growers might have given them different local names. The dendrogram based on UPGMA cluster analysis depicts the pattern of relationships between the studied cultivars based on SSR markers.

Analysis of SSRs in cultivars of the Italian germplasm collection allowed us to construct a molecular catalog that can be used to compare the molecular pattern of the various cultivars as well as other samples of unknown origin, avoiding the collection of redundant genetic entities.

This work, for the very high number of the Italian olive cultivars analyzed, highlights the degree and distribution of genetic diversity for a better exploitation of olive resources and for the design of plant breeding programs. The use of molecular markers like SSRs, in addition to other information, is imperative to build a database for cultivar analysis, for traceability of processed food (olive oil), and for appropriate management of olive germplasm collections.

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