

Molecular characterization of olive (*Olea europaea* L.) Sicilian cultivars using SSR markers



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ABSTRACT

Olive (*Olea europaea* L.) is one of the economically most important fruit crops for the Mediterranean area, with production being mainly destined to oil extraction. In Sicily, olive has been cultivated since ancient times and its germplasm is characterized by a wide genetic diversity that could be related to its domestication and spread in ancient times, and to some reproductive biological peculiarities as self-incompatibility. This analysis was conducted on 65 genotypes with the purpose of characterizing a large collection of Sicilian accessions (47 genotypes) and to compare them with varieties coming from Southern Italy and from the most important countries of the Mediterranean basin. With this aim we used 8 simple sequence repeat (SSR) markers, which detected a total of 74 alleles and identified an average of 19.5 genotypes in the population investigated. A larger variability than expected was found in the analyzed genotypes, some synonymies already reported in literature were confirmed, but also some cultivars considered as identical were discriminated such as in the case of Castriciana, Ogliarola messinese and Passalunara. The whole study revealed a wide intraspecific variability within the gene pool examined, independently from the geographical origin.

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1. Introduction

Olive (*Olea europaea* L. subsp. *europaea*) is one of the most economically important and widespread fruit crops of the Mediterranean area and is characterized by a prevalent allogamy and great longevity which have determined high levels of heterozygosity (Rallo et al., 2000) as well as the accumulation of a number of mutations (Lopes et al., 2004; Baali-Cherif and Besnard, 2005).

The lack of replacement of traditional and well-adapted cultivars and its vegetative propagation, gave rise to very rich germplasm with a large number of cultivars present in the main olive oil producing countries (Baldoni and Belaj, 2009).

However, this rich biodiversity often implies difficulties in identification of plant material due to the frequency of synonyms and homonyms (Bartolini, 2007). Among the olive growing countries, Italy has about 600 different accessions, representing a large part of the known world olive germplasm (Bartolini et al., 2005). In Sicily, olive is widely cultivated since ancient times and wild olives are important components of the Mediterranean scrublands (Zohary and Hopf, 1994). Sicily

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offers a number of favorable growing conditions for olive due to different climatical and edaphic characteristics and therefore a wide genetic patrimony has been accumulated (Bottari and Spina, 1952). Furthermore, the presence of intra-cultivar variation represented by plants with the same phenotype which display small genetic differences has been reported (Lopes et al., 2004; Muzzalupo et al., 2011; Ipek et al., 2012).

Biochemical and molecular markers offer a wide choice of analytical systems to analyze plant germplasm; in olive AFLPs (Angiolillo et al., 2006), SCARs (Hernandez et al., 2001; Bautista et al., 2003), ISSRs (Gomes et al., 2009) and SNPs (Muleo et al., 2009; Hakim et al., 2010) have been used for the characterization. In the last years, SSRs markers, widely used in the genetic identification and discrimination of cultivars, have been isolated from olive germplasm (Sefc et al., 2000; Carriero et al., 2002; Cipriani et al., 2002). Microsatellites represent a powerful tool for varietal identification and have also been used to evaluate inter and intra-cultivar variability (Belaj et al., 2008; Roubos et al., 2010; Ercisli et al., 2011).

In the current report, the genetic polymorphism level and the relationships among 47 Sicilian olive accessions and 18 varieties coming from other Italian regions and Mediterranean countries was investigated by microsatellite markers in order to provide a reliable and unambiguous tool for the characterization of genetic resources especially in germplasm collection farms.

2. Materials and methods

2.1. Plant material and DNA extraction

In this work, 47 Sicilian cultivars were analyzed and compared with 10 of the most representative Italian cultivars and 8 representatives from other Mediterranean countries (Supplementary Table S1). Twenty-four Sicilian cultivars were sampled from the Experimental Farm of Catania University (lat. 37°24'41"N; long. 15°03'15"E) in which most of the accessions described by Bottari and Spina (1952) are conserved. All the other accessions (41) were collected from the Collection Field of Olive Germplasm of "CNR-ISAFO" and "Provincia Regionale di Enna" (lat. 37°30'52"N; long. 14°17'46"E). Young leaves were sampled, 100 mg of fresh tissue were ground using Qiagen Tissuelyser II (Qiagen, Hilden, Germany) and genomic DNA was extracted using the Plant DNA Mini Kit (Bioline, London, UK). Quantity and quality of extracted DNA were checked by spectrophotometry using a Nanodrop 2000 system (Thermo Scientific, Waltham, Massachusetts, USA).

2.2. SSR analysis

Eight SSR loci, out of ten originally investigated, were selected for the analysis: *ssrOeUA-DCA3*, *ssrOeUA-DCA9*, *ssrOeUA-DCA16*, *GAPU59*, *GAPU101*, *UDO99-008*, *UDO99-012*, *UDO99-024* (Table 1). The choice was made based on the degree of polymorphism, as well as on clearness and reproducibility of amplified DNA fragments. The amplifications were carried out in 15 µl volumes containing about 30 ng genomic DNA, 1X NH₄ Reaction Buffer (Bioline), 0.2 mM dNTPs (Bioline), 1 mM MgCl₂, 0.167 nM of forward and reverse primers, 0.13 nM of M13F labeled with a fluorescent dye (6-FAM, NED and PET; MWG Operon, Ebersberg, Germany) and 1 U of Taq Polymerase (Bioline). Amplification was performed in two thermal cyclers (GeneAmp PCR 9700 and 2700 systems from Applied Biosystems, Foster City, USA) with an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, annealing temperature 53 °C for 30 s and 72 °C for 45 s, and a final extension at 72 °C for 15 min. An aliquot of 0.4–0.6 µl of PCR product (depending on the performance of amplification of each primer pair) was mixed with 13 µl of formamide and 0.3 µl of LIZ-500 size standard and denatured at 95 °C for 5 min. Up to three PCR products labeled with FAM, PET, or NED were pooled before separation in the ABI 310 Genetic Analyzer (Applied Biosystems).

Table 1

Primer sequences, repeat motif, range (bp) of amplified fragments used for olive germplasm evaluation.

Source	Locus	Primer sequence (5–3)	Repeat motif	Size range (bp)
Sefc et al. (2000)	(B) <i>ssrOeUA-DCA3</i> *	F: CCCAAGCGAGGTGTATATGTTAC R: TGCTTTTGTGCTGTTGAGATGTTG	(GT) ₁₉	227–255
	(Y) <i>ssrOeUA-DCA9</i> *	F: AATCAAAGTCTCTCTCTCATTTTCG R: GATCCTTCCAAAAGTATAACCTCTC	(GT) ₂₃	162–212
	(B) <i>ssrOeUA-DCA16</i> *	F: TTAGGTGGGATTCGTAGATGGTTG R: TTTTAGGTGAGTTCATAGAATTAGC	(GT) ₁₃ (GA) ₂₉	121–231
Carriero et al. (2002)	(R) <i>GAPU59</i>	F: CCCTGCTTTGGTCTTGCTAA R: CAAAGGTGCACCTTCTCTCG	(CT) ₉	201–219
	(Y) <i>GAPU101</i> *	F: CATGAAAGGAGGGGGACATA R: GGCACCTTGTGTGCAGATTG	(GA) ₈ (GA) ₃ (AG) ₃	182–219
Cipriani et al. (2002)	(B) <i>UDO99-008</i>	F: AAAAAACACAACCCGTGCAAT R: AAATTCCTCCAAGCCGATCT	(AC) ₁₃	155–169
	(R) <i>UDO99-012</i>	F: TCACCATTTCTTAACCTCACACCA R: TCAAGCAATTCACGCTATG	(GT) ₁₀	155–167
	(Y) <i>UDO99-024</i>	F: GGATTATTAAAGCAAAACATACAAA R: CAATAACAATGAGCATGATAAGACA	(CA) ₁₁ (TA) ₂ (CA) ₄	165–199

M13F sequence (CACGACGTTGTAACGAC) was added at 5' end of forward primers; forward primers fluorescently labeled: B, blue dye (6-FAM); Y, yellow dye (NED); R, red dye (PET).

and analyzed using the Genemapper 4.0 software. All the samples were analyzed with the same methodology and in the same conditions to make the results comparable.

2.3. Data analysis

Genetic distance was calculated using the simple matching dissimilarity index. From the dissimilarity matrix obtained, a weighted Neighbor-joining tree (Saitou and Nei, 1987) was computed using the Dissimilarity Analysis and Representation for Windows (DARwin5) software version 5.0. (Perrier and Jacquemoud-Collet, 2006) and the robustness of branches was tested using 1000 bootstraps. Several genetic parameters were determined by PowerMarker (Liu and Muse, 2005): major allele frequency, number of genotypes detected, number of alleles per locus, observed heterozygosities (H_o) and polymorphism information content (PIC) (Botstein et al., 1980). The PIC value ranged from 0 (monomorphic) to 1 (highly discriminative) and is an indication of discriminative power of a given marker, not only for the number of alleles at a locus, but also for the relative frequencies of those alleles in the genotypes under study (Botstein et al., 1980).

3. Results

The 8 microsatellite primer pairs produced clear and reproducible genotyping profiles in the 65 accessions analyzed (Supplementary Fig. S1). The size of the scored alleles ranged from 121 to 248 bp and a total of 74 alleles was identified in the 65 accessions, whereas the number of alleles scored in the accessions coming from Sicily was 67. The number of alleles per primer pair was on average 8.4 in Sicilian accessions and 9.3 in the total sample (Table 2). Locus UDO99-008 showed the lowest number of alleles (4) whereas *ssrOeUA-DCA9* presented the highest value (15). Major allele frequency was higher in GAPU59 and in UDO99-024 (0.585). The observed heterozygosity ranged from 0.431 for primer UDO99-008 to 0.969 for *ssrOeUA-DCA3* (mean 0.760) while PIC values ranged from 0.549 for GAPU59 to 0.853 for *ssrOeUA-DCA9* (mean 0.722). The highest discriminant power was observed for *ssrOeUA-DCA9* which identified 32 genotypes; on the other hand UDO99-008 showed the lowest number of alleles and discriminated only 9 different genotypes (Supplementary Table S2). Overall the 8 SSRs were highly polymorphic and unambiguously differentiated 58 out of 65 analyzed genotypes. The undiscriminated genotypes were Calamignara and Ogliarola messinese; Morghetana and Moresca; Nocellara Etnea, Marmorina, Virdisa and Zaituna; Verdello Grosso and Turdunazza; Busciunetto and Abunara.

The genetic distance among the selected genotypes was evaluated according to the simple matching dissimilarity index. The Neighbor-joining dendrogram (Fig. 1) showed high variability among the analyzed accessions; three main clusters (A, B and C) are present, of which two, are further divided into three and two subclusters, respectively (A1, A2 and A3; B1 and B2). Sicilian genotypes are represented in all subclusters whereas genotypes from abroad felt in only two of them. Namely, three Spanish varieties (Arbequina, Manzanilla and Picudo) and one from Morocco (Picholine Marocaine) were included in the same group of the subcluster A1, while the two Tunisian varieties (Chetoui and Chemlali) were clustered together with Koroneiki (Greek) and Arbosana (Spanish) in subcluster B2 together with three Sicilian genotypes. The other Italian varieties were generally clearly separated among them and from the Sicilian accessions. Some of the most important cultivars from Sicily, mainly grown in its east side, such as Nerba, Tonda Iblea, Moresca and Ogliarola messinese, which have large fruits and intermediate ripening period, showed a high level of similarity and were grouped together in subcluster A2, which also includes some of the accessions (Calamignana, Passalunara and Castricana) already described as synonymies of Ogliarola messinese (Seed and Plant Genetic Resources Service, FAO).

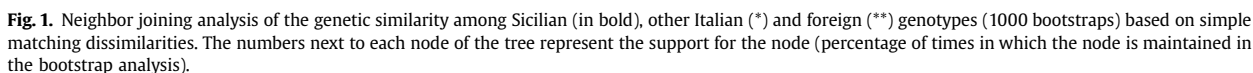
Some other well distinguished Sicilian varieties were grouped in subcluster A3 and subcluster B1 where two Nocellara varieties (Nocellara Etnea and Nocellara del Belice) are grouped together with other late ripening varieties. Cluster C included two of the most important Italian varieties for oil production, Frantoio and Leccino.

4. Discussion

The high level of polymorphism observed is consistent with results obtained by several authors that investigated olive germplasm with SSR markers (Lopes et al., 2004; Alba et al., 2009) and reflects the great diversity within olive cultivars

Table 2
Markers assayed for the characterization of the olive genotypes and parameters of diversity.

Marker	Major allele frequency	Genotype no	Allele no	Gene diversity	Heterozygosity	PIC
<i>ssrOeUA-DCA3</i>	0.246	25	9	0.841	0.969	0.821
<i>ssrOeUA-DCA9</i>	0.208	32	15	0.867	0.923	0.853
<i>ssrOeUA-DCA16</i>	0.300	30	14	0.829	0.800	0.810
GAPU59	0.585	12	6	0.594	0.585	0.549
GAPU101	0.208	22	9	0.850	0.938	0.832
UDO99-008	0.462	9	4	0.689	0.431	0.640
UDO99-012	0.431	10	5	0.690	0.800	0.635
UDO99-024	0.585	16	12	0.624	0.631	0.600
Mean of total samples	0.378	19.5	9.3	0.748	0.760	0.718
Mean of Sicilian samples	0.393	15.6	8.4	0.734	0.788	0.702



The Sicilian accessions showed a high value of genetic variability, comparable with that recorded when genotypes from other Mediterranean countries were included in the analysis. The high level of genetic variability may be due to the spread and exchange of propagation material among many cultivation areas and to the empiric selection performed after spontaneous crosses. These events are connected to the history of olive cultivation, for many centuries subjected to an intense anthropic diffusion, being Sicily a land of meeting for Mediterranean populations since ancient time (Zohary and Hopf, 2000; Rugini et al., 2011).

Some of the undiscriminated accessions have already been considered as synonyms in literature (Bottari and Spina, 1952; La Mantia et al., 2005; Caruso et al., 2007). In particular, Nocellara Etnea, the most important cultivar of the Etna volcano area, shared the same profile with Marmorina (meaning “like marble”, to underline the hardness of fruit), Virdisa (meaning “green colored” to indicate the light color of the drupe) and Zaituna. While Marmorina and Virdisa are commonly admitted as synonymous of Nocellara Etnea, in the case of Zaituna, a higher number of SSR primers or of different kind of markers, such as SNPs (Single Nucleotide Polymorphisms), could unambiguously distinguish it. In fact, Zaituna, although historically considered as synonymous of Virdisa (Bottari and Spina, 1952), is also the name of a different cultivar still cultivated in the Syracuse district, very close to Nocellara Etnea if morphological traits are considered (Bottari and Spina, 1952). Other two pairs of genotypes (Abunara and Busciunetto; Turdunazza and Verdello Grosso) resulted undistinguished; these are minor accessions stored in the collection field and that have been never characterized before. On the contrary, some accessions (Castriciana, Passalunara and Ogliarola messinese) already reported by other authors as synonyms, were discriminated in our analysis.

However, due to the poor level of polymorphisms, it's likely that these accessions have been originated for somatic mutation and may represent intra-cultivar variation, as already reported for other olive genotypes (Marra et al., 2013). On the other hand Santagatense, representing the main Sicilian cultivar spread in north coast and inland, Montonica and Rizza, which were previously considered as synonymies (La Mantia et al., 2005), were clearly separated among them and felt into three different clusters.

In conclusion, our results contributed to shed light on some aspects of the complex variability of Sicilian germplasm, allowing the identification of some varieties that were not described before and representing a useful tool for olive germplasm collection and managing.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.bse.2014.07.010>.

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Supplementary Table S1. *Olea europaea* cultivars analyzed by SSR markers

Name	Cultivar Origin	Collection Field
Abunara	Italy (Sicily)	Catania University
Aitana	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Biancolilla	Italy (Sicily)	Catania University
Bottone di Gallo	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Brandofino	Italy (Sicily)	Catania University
Buscionetto	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Calamignara	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Calatina	Italy (Sicily)	Catania University
Castriciana	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Cavaliera	Italy (Sicily)	Catania University
Cerasuola	Italy (Sicily)	Catania University
Erbano	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Giarfara	Italy (Sicily)	Catania University
Giarraffa	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Gioconda	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Lumiaro	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Mandanici	Italy (Sicily)	Catania University
Marmorina	Italy (Sicily)	Catania University
Minuta	Italy (Sicily)	Catania University
Monachella	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Montonica	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Moresca	Italy (Sicily)	Catania University
Morghetana	Italy (Sicily)	Catania University
Nasitana	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Nerba	Italy (Sicily)	Catania University
Nocellara del Belice	Italy (Sicily)	Catania University
Nocellara etnea	Italy (Sicily)	Catania University
Nocellara messinese	Italy (Sicily)	Catania University
Occhiolà	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Ogliarola messinese	Italy (Sicily)	Catania University
Olivo di Castiglione	Italy (Sicily)	Catania University
Passalunara	Italy (Sicily)	Catania University

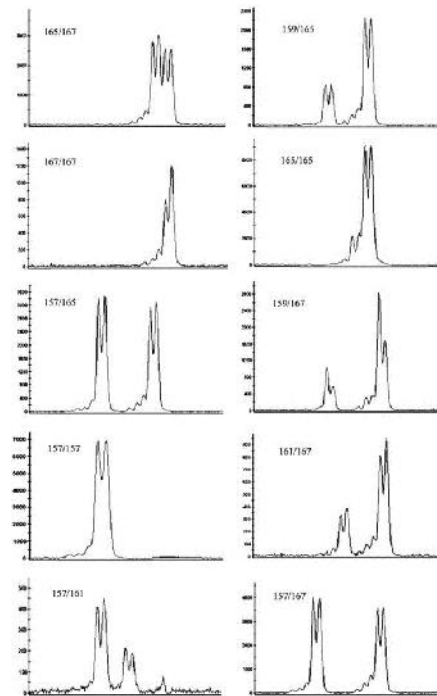
Randazzese	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Riondello	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Rizza	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
S. Benedetto	Italy (Sicily)	Catania University
Santagatese	Italy (Sicily)	Catania University
Tonda iblea	Italy (Sicily)	Catania University
Tortella	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Turdunazza	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Vaddarica	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Verdella	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Verdello grosso	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Virdisa	Italy (Sicily)	CNR-ISAFOM and Provincia Regionale di Enna
Zaituna	Italy (Sicily)	Catania University
Bella di Cerignola	Italy (Apulia)	CNR-ISAFOM and Provincia Regionale di Enna
Cellina di Nardò	Italy (Apulia)	CNR-ISAFOM and Provincia Regionale di Enna
Cipressino	Italy (Apulia)	CNR-ISAFOM and Provincia Regionale di Enna
Coratina	Italy (Apulia)	CNR-ISAFOM and Provincia Regionale di Enna
Ogliarola leccese	Italy (Apulia)	CNR-ISAFOM and Provincia Regionale di Enna
Carolea	Italy (Calabria)	CNR-ISAFOM and Provincia Regionale di Enna
Dolce di Rossano	Italy (Calabria)	CNR-ISAFOM and Provincia Regionale di Enna
Grossa di Gerace	Italy (Calabria)	CNR-ISAFOM and Provincia Regionale di Enna
Leucocarpa	Italy (Calabria)	CNR-ISAFOM and Provincia Regionale di Enna
Ottobratica	Italy (Calabria)	CNR-ISAFOM and Provincia Regionale di Enna
Frantoio	Italy (Tuscany)	CNR-ISAFOM and Provincia Regionale di Enna
Leccino	Italy (Tuscany)	CNR-ISAFOM and Provincia Regionale di Enna
Koroneiki	Greek	CNR-ISAFOM and Provincia Regionale di Enna
Picholine marocaine	Morocco	CNR-ISAFOM and Provincia Regionale di Enna
Arbequina	Spain	CNR-ISAFOM and Provincia Regionale di Enna
Arbosana	Spain	CNR-ISAFOM and Provincia Regionale di Enna
Manzanilla	Spain	Catania University
Picudo	Spain	CNR-ISAFOM and Provincia Regionale di Enna
Chemlali	Tunisia	CNR-ISAFOM and Provincia Regionale di Enna
Chetoui	Tunisia	CNR-ISAFOM and Provincia Regionale di Enna

Table 2 Supplementary. SSR allelic profiles of the 65 olive cultivars analyzed at 8 SSR loci.

NOME	ssrOeUA-DCA3	ssrOeUA-DCA9	ssrOeUA-DCA16	GAPU59	GAPU101	UDO99-008	UDO99-012	UDO99-024
Abunara	234/248	192/207	121/173	212/212	204/215	163/163	157/167	186/186
Aitana	240/244	162/201	148/153	212/218	188/204	155/163	165/167	184/186
Arbequina	242/244	162/204	123/146	212/212	190/215	165/165	157/157	186/186
Arbosana	227/238	192/204	121/123	212/222	182/188	163/163	159/165	202/202
Bella di Cerignola	244/248	162/180	153/177	212/212	198/215	165/165	157/157	167/186
Biancolilla	238/242	162/184	148/177	212/212	188/215	160/165	157/167	167/186
Bottone di gallo	234/248	165/192	177/177	212/222	192/198	165/165	159/167	167/186
Brandofino	227/242	190/204	121/173	212/212	204/215	165/165	157/167	186/186
Buscionetto	234/248	192/207	121/173	212/212	204/215	163/163	157/167	186/186
Calamignara	238/248	184/192	148/177	208/212	188/198	160/165	157/165	186/186
Calatina	234/242	162/162	173/173	208/222	182/188	155/155	165/165	179/186
Carolea	227/248	162/196	123/153	212/218	190/215	165/165	157/167	184/186
Castriciana	238/248	184/194	148/173	208/212	188/198	160/165	157/165	186/186
Cavaliera	227/238	171/184	148/165	208/222	188/190	160/165	159/165	181/186
Cellina di Nardò	234/240	180/204	148/155	208/212	182/196	156/160	165/167	181/186
Cerasuola	227/238	162/207	158/173	212/214	188/198	165/165	157/157	179/186
Chemlali	227/234	171/171	121/146	212/212	190/196	160/160	157/157	184/186
Chetoui	227/234	168/192	121/173	212/214	190/204	163/163	159/167	186/191
Cipressino	238/238	162/184	148/148	208/208	190/204	165/165	167/167	186/188
Coratina	234/238	184/196	148/148	212/212	196/215	160/160	157/167	167/186
Dolce di Rossano	238/240	171/184	148/148	218/218	190/204	160/160	167/167	186/188
Erbano	234/238	162/165	157/173	212/222	188/204	163/163	159/167	186/188
Frantoio	232/238	180/204	148/155	208/212	182/196	156/160	165/167	181/186
Giarfara	238/244	162/171	148/173	212/212	198/204	163/163	157/167	186/186
Giarraffa	244/248	162/192	123/173	212/212	198/215	165/165	157/157	186/186

Gioconda	234/238	180/192	148/171	212/212	196/215	160/160	157/167	167/186
Grossa di Gerace	227/238	184/204	121/153	204/218	188/188	160/165	157/165	167/186
Koroneiki	234/234	180/204	148/148	208/212	190/204	163/163	159/167	186/188
Leccino	238/248	162/204	148/173	208/212	196/198	155/155	157/167	183/183
Leucocarpa	238/248	184/194	148/148	208/212	188/198	160/165	157/165	186/186
Lumiaro	238/248	165/171	121/123	212/212	188/198	160/165	157/165	186/186
Mandanici	227/234	165/199	121/144	222/222	188/204	160/160	159/167	186/195
Manzanilla	240/248	162/204	153/173	212/212	196/217	165/165	157/157	167/186
Marmorina	238/244	171/192	123/148	212/212	204/215	155/165	157/167	167/186
Minuta	234/240	180/201	121/177	208/208	188/192	155/155	159/165	167/179
Monachella	227/244	162/184	146/173	212/222	190/215	165/165	157/161	167/186
Montonica	238/248	192/199	121/173	212/218	198/198	165/165	157/157	186/186
Moresca	240/244	184/192	148/173	208/212	188/198	155/165	157/165	186/186
Morghetana	240/244	184/192	148/173	208/212	188/198	155/165	157/165	186/186
Nasitana	234/248	162/201	148/153	208/214	192/198	165/165	157/165	181/186
Nerba	238/244	162/171	121/123	208/212	188/198	160/165	157/165	186/186
Nocellara del Belice	238/244	162/171	148/173	212/212	198/204	163/165	157/167	186/186
Nocellara Etna	238/244	171/192	123/148	212/212	204/215	155/165	157/167	167/186
Nocellara Messinese	227/232	184/192	153/153	208/212	182/215	160/165	157/165	171/181
Occhiolà	232/244	171/184	173/173	208/222	198/198	155/165	157/165	179/179
Ogliarola leccese	238/251	171/171	148/148	212/212	182/196	163/163	159/167	167/186
Ogliarola Messinese	238/248	184/192	148/177	208/212	188/198	160/165	157/165	186/186
Olivo di Castiglione	240/248	192/192	146/148	208/212	188/190	160/165	165/167	179/186
Ottobratica	227/234	184/201	155/155	208/222	190/196	163/163	157/157	179/179
Passalunara	238/248	184/192	148/173	208/212	188/198	160/165	157/165	186/186
Picholine marocaine	234/248	192/204	153/171	212/222	196/215	165/165	157/157	167/186
Picudo	240/248	182/192	153/173	212/222	196/215	165/165	157/157	167/186
Randazzese	227/244	192/204	121/175	212/212	204/215	165/165	157/167	186/186

Riondello	227/232	180/201	148/153	208/212	188/196	163/163	165/167	173/181
Rizza	227/238	162/192	148/148	212/218	188/198	163/163	157/167	184/186
S. Benedetto	234/248	162/201	123/177	208/212	192/198	165/165	157/165	179/186
Santagatese	227/240	192/192	121/153	208/218	188/190	155/165	165/167	167/184
Tonda Iblea	238/244	162/171	148/173	208/212	188/198	155/165	157/165	186/186
Tortella	227/244	192/204	121/173	212/212	204/215	165/165	157/167	186/186
Turdunazza	238/248	162/171	148/173	212/212	196/198	160/165	157/167	167/186
Vaddarica	232/240	171/180	121/153	208/222	196/196	155/163	161/167	181/184
Verdella	234/248	162/201	177/177	208/212	192/198	165/165	157/165	179/186
Verdello grosso	238/248	162/171	148/173	212/212	196/198	160/165	157/167	167/186
Virdisa	238/244	171/192	123/148	212/212	204/215	155/165	157/167	167/186
Zaituna	238/244	171/192	123/148	212/212	204/215	155/165	157/167	167/186



Supplementary Fig. S1 Capillary electrophoresis electropherogram showing the different haplotypes of the microsatellite locus UDO99-012.

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